38° CONGRESSO NAZIONALE
Società Italiana di Endocrinologia

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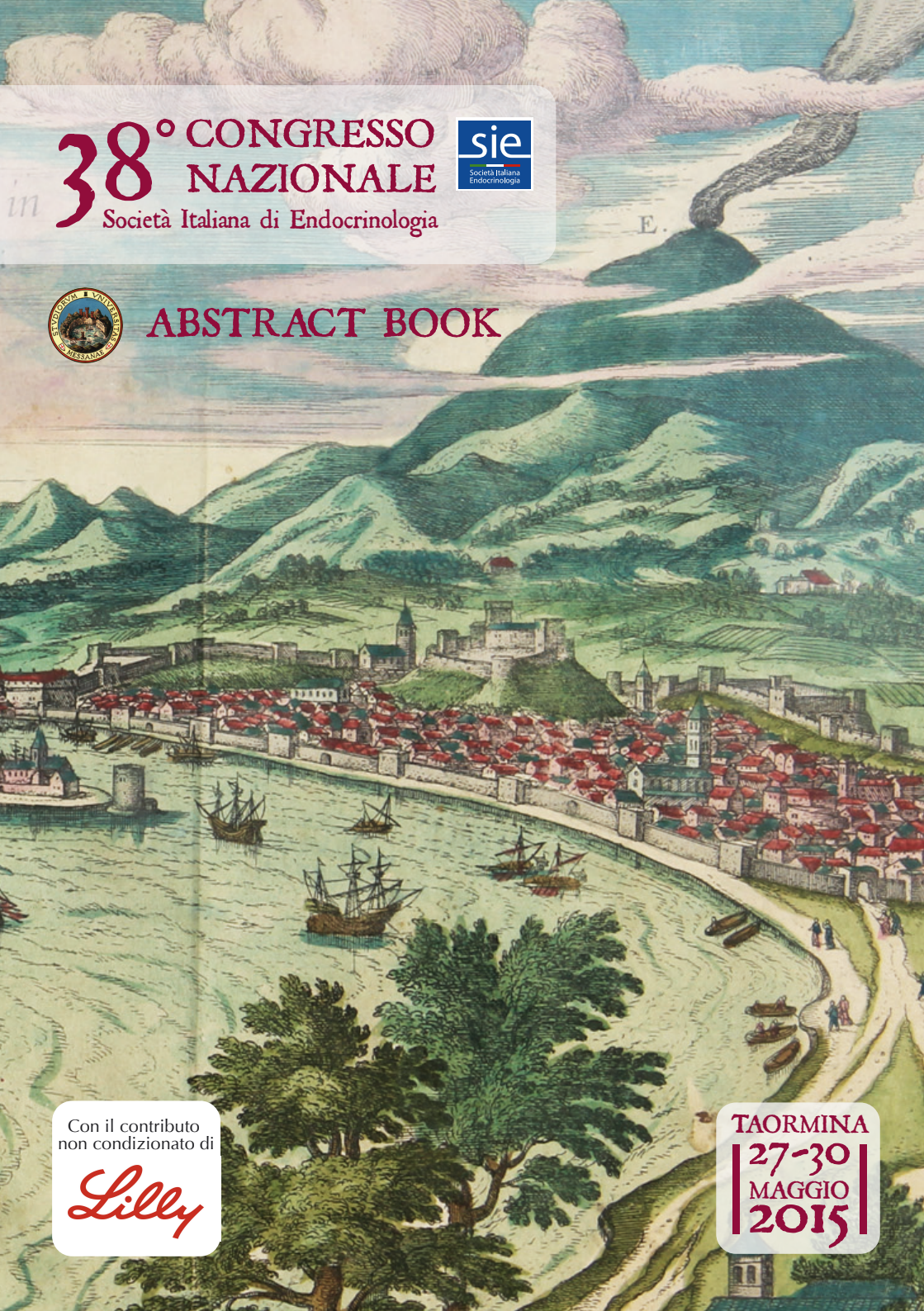
ABSTRACT BOOK



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TAORMINA
27-30 MAGGIO
2015

INDICE LAVORI

SELECTIVE THYROID HORMONE RECEPTOR ALPHA KNOCKDOWN IN ZEBRAFISH

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Introduction: This work is dedicated to the study of Resistance to Thyroid Hormone due to defective hormone receptor alpha (RTHα), an inherited thyroid disease that was discovered in 2012. RTHα is due to heterozygous mutations in the *THRA* gene and is characterized by dramatic manifestations similar to those of untreated congenital hypothyroidism (short stature, skeletal dysplasia, learning disability and bradicardia), without the typical biochemical signature of CH. The early treatment with high L-T4 doses could overcome the resistance in some target tissues (eg, brain), but untoward thyrotoxic manifestations in others (eg, liver). There are currently 6 known *THRA* mutations (E406X, F397fs406X, D211G, A263V, A382PfsX7 and N359Y), localized in the ligand-binding domain (LBD) of *THRA* that inhibit the function of its WT counterpart in a dominant negative (DN) manner. The occurrence of clinical features in RTHα is extremely variable, perhaps depending on the severity or site of the mutations. Since the mechanisms of TH action are well conserved in zebrafish, we generated an *in vivo* DN-model to deeper understand the mechanisms of thyroid hormone action and the effects of its disruption underlying the RTHα pathology. **Methods and Results:** By the microinjection into 1-4 cells embryos of a specific morpholino (splMO) we obtained heterozygous embryos (*thraa*_MOs) carrying a deletion at the LBD of *thraa* (homologous of the human *THRA*) mRNA. By luciferase assays we found that the deleted_*thraa* maintained the ability to bind the DNA and dimerize, but have a reduced ability to bind T3 and strongly inhibited the transcriptional activation of the WT counterpart in a DN manner. Morphologically, *thraa*_MOs presented stunted growth and altered maturation of cartilages. Biochemically, *thraa*_MOs showed normal thyroid size and *tshβ* expression, but an increased T3/T4 ratio. The *thraa*_MOs also displayed alterations in TH metabolism (low *dio3a*/*dio3b* and high *dio2* expression), thus explaining the altered TH levels found in *thraa*_MOs. Phenotypically, *thraa*_MOs showed heart malformations, low heart rate and cardiac edema. Additionally, *thraa*_MOs developed a smaller brain and hydrocephalie. Moreover, injected embryos displayed an altered CNS and PNS development. Finally, we performed rescue experiments by the coinjection of splMO and human *THRA*-wild type or -mutant mRNAs. The wild type mRNA was able to revert with significant percentage the pathological traits of *thraa*_MOs. Conversely, the mutant mRNAs failed to rescue, and in some cases (F397fs406X and A382PfsX7), their injection resulted in a more severe embryonic delay exacerbating both cardiac and CNS phenotype. In conclusion, our DN-model recapitulates most of the biochemical (normal TSH, high T3/T4 ratio) and clinical (delayed embryonic growth and skeletal maturation, bradicardia and psychomotor retardation) signs described in patients with RTHα and in mouse models. Zebrafish thus represents an emerging *biotools* to uncover human disease associated with mutation in the *THRA* gene.

ADIPOCYTE MINERALOCORTICOID RECEPTOR ACTIVATION LEADS TO METABOLIC SYNDROME AND INDUCTION OF PROSTAGLANDIN D2 SYNTHASE.

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Metabolic syndrome (MetS) is a major risk factor for the development of diabetes and cardiovascular diseases. Adipose tissue plays a central role in the obesity-related metabolic abnormalities and it's known that mineralocorticoid receptor (MR) blockade could ameliorate MetS condition in preclinical models. However, mechanistic studies are lacking to delineate the role of MR activation within the adipocyte.

In the present study we showed that MR expression is increased in visceral adipose tissue (VAT) in a preclinical mouse model of MetS and in obese patients (respectively 1-2 and 2-3 fold increased vs control, $p<0.01$). Thus, we generated a double transgenic mouse model with a conditional overexpression of MR in adipocyte (2-3 fold increased vs control, $p<0.01$) that develops, even under regular diet, multiple metabolic abnormalities. The metabolic profile include body weight gain (Δ body weight of 4g after 12 week, $p<0.01$), visceral obesity (2 fold increased in VAT mass, $p<0.05$), glucose intolerance and insulin resistance (as shown by differences in ITT and GTT curves, $p<0.01$), as well as dyslipidaemia (1-2 fold increase in plasma total cholesterol and triglycerides, $p<0.05$). Moreover, we identified prostaglandin D2 synthase (PTGDS) as a novel MR target gene in adipocytes (3-5 fold increased, $p<0.01$) and we found that AT56, a specific inhibitor of PTGDS enzymatic activity, blunted adipogenic aldosterone effect in 3T3-L1 adipocytes. Finally, translational studies showed that expression of MR and PTGDS are strongly correlated in adipose tissues from obese patients ($r_S=0.7$, $p<0.002$).

Our data support the concept that MR over activation in adipocyte participates to the development of metabolic syndrome. The identification of PTGDS as a novel MR target involved in the aldosterone effects on adipogenesis should stimulate further studies analyzing the therapeutic impact of PTGDS inhibitors in this context.

CHRONIC, LONG TERM ADMINISTRATION OF VARDENAFIL IMPROVES ENDOTHELIAL FUNCTION AND CORRECTS HYPOGONADISM IN PATIENTS WITH TYPE 2 DIABETES MELLITUS. A LONGITUDINAL, PROSPECTIVE, RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE BLIND, CLINICAL TRIAL.

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Background: Endothelial dysfunction leads to cardiovascular complications in type 2 diabetes mellitus (T2DM), through a reduction of nitric oxide (NO)-mediated relaxation. Phosphodiesterase-5 inhibitors (PDE5i) have hemodynamic effects, improving NO levels. Aim: To investigate if long term, chronic treatment with the PDE5i Vardenafil improves systemic endothelial function in men with T2DM. Methods: A longitudinal, prospective, investigator-started, randomized, placebo-controlled, double-blind, clinical-trial was carried out. 54 male patients affected by T2DM diagnosed within the last 5 years were enrolled. 26 and 28 patients were assigned by permuted block randomization to the verum and placebo-group, respectively. The study consisted of an enrollment phase, a treatment phase (24 weeks) (Vardenafil/placebo 10mg twice-daily), and a follow-up phase (24 weeks). Parameters evaluated: International Index of Erectile Function (IIEF)-15, flow mediated dilation (FMD), intima media thickness (IMT), routine hematologic analyses. Serum testosterone (T) and its precursors were measured by liquid-chromatography tandem mass-spectrometry (LC-MS/MS). Gonadotropins were evaluated by ELISA. Results: Only one serious adverse event was registered in the placebo group. The erectile function domain of IIEF-15 (p=0.049) improved after treatment. At the end of the treatment phase FMD significantly increased (p=0.002) while IMT (p=0.003), fibrinogen (p=0.005), white blood cells count (p=0.018) and Red cells Distribution Width (p=0.028) significantly decreased. FMD was significantly related to T serum levels (p=0.002), which significantly improved after Vardenafil treatment only in hypogonadal men (T<10.4 nmol/L) (p=0.023), without changes in gonadotropin serum levels. Smoking-habits, hypertension and glycemic control influenced the hemodynamic and inflammatory parameters. Conclusions: This is the first double-blind, placebo-controlled clinical-trial in which T2DM men are chronically treated with Vardenafil for 6 months, and followed-up for 6 months after therapy-withdrawal. Chronically administered Vardenafil is safe and effective in T2DM patients and improves both tissue oxygenation and inflammatory markers, but this effect is lost after therapy withdrawal. For the first time, we demonstrate that chronic Vardenafil therapy improves T (measured by LC-MS/MS) in diabetic, hypogonadal men, an effect possibly due to improved microcirculation in the testis.

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CO01 - IMPACT OF POST-OPERATIVE RADIOIODINE REMNANT ABLATION IN DIFFERENTIATED THYROID CANCER ACCORDING TO RISK CATEGORIES

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Introduction: Radioiodine remnant ablation (RRA) is largely used in differentiated thyroid cancer (DTC) after total thyroidectomy because it allows more accurate post-operative staging and facilitates post-surgical follow-up. Patients who will really benefit from RRA are not accurately identified.

Aims: To evaluate, in a continuous series of DTC patients, all followed-up in our Thyroid Clinic, the long-term outcome comparing patients either treated or not with RRA.

Patients and methods: A consecutive series of 4,284 DTC patients, 3,418 F and 866 M (F/M=3.9/1) with mean age at diagnosis of 46.3 yrs (median 46 yrs, range 8-86) and mean follow-up of 6.3 yrs (median 4.8 yrs, range 1-37.5), was studied in the 1974-2014 period. Histotype was papillary in 3,822 (89.2%) and follicular in 462 (10.8%) cases. According to TNM categories, patients were subdivided into a low and high risk groups: low risk= T1-T2 with N0 or Nx; high risk= T3, T4 or N1. Post-surgical RRA (30-100 mCi of 131-I) was given to 2,658/4,284 (62%) patients while 1,626/4,284 (38%) were not ablated. All patients were periodically followed with Tg and AAT measurements and also with neck ultrasonography under L-T4 therapy; in addition to this, RRA patients were evaluated after one year of treatment with TSH stimulation. If the patient did not result cured, further I-131 treatment or surgery was carried out.

Results: At the last control, patients in the RRA group more frequently had persistent/recurrent disease in comparison with patients who did not have RRA: 486/2,658 (18.3%) vs 203/1,626 (12.5%) (p=0.0001). Clinical outcome was not different when each group was analyzed according to the risk categories: a) Low-risk: recurrence in 133/937 (14.2%) in ablated and 173/1,487 (11.6%) in not ablated patients, p=ns; b) High-risk: recurrence in 353/1,721 (20.5%) in ablated and 29/139 (20.8%) in not ablated patients, p=ns. In non-ablated low-risk patients, persistent/recurrent disease occurred more frequently in Nx (13.3%) vs N0 patients (6.8%) (p= 0.0004). Also in the T1 category, recurrence was also statistically more frequent in T1aNx (13.0%) vs T1aN0 (6.6%) (p=0.009). Similar results were observed also in the T1b category (15.5% vs 6.5%, p= 0.03).

Conclusions: The clinical outcome of DTC patients is related to the risk category, independently of RRA. Furthermore, in low-risk not ablated patients, persistence/recurrence is less frequent when lymph nodes are negative (N0 vs Nx). Further studies are needed to clarify who really benefits from RRA.

CO02 - A REAPPRAISAL OF SECOND LINE TESTS IN THE DIFFERENTIAL DIAGNOSIS OF ACTH-DEPENDENT CUSHING'S SYNDROME: THE ROLE OF THREE DINAMIC TESTS

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Introduction: The diagnosis of Cushing's syndrome (CS) might be challenging especially in ACTH-dependent CS when it comes to detect the origin of ACTH secretion. Since the non-invasive tests have proved poor accuracy for the identification of the ACTH source, the performance of bilateral inferior sinus sampling is recommended in all patients with a pituitary lesion of less than 6 millimeters. The aim of this study is to assess the value of the second line tests in the differential diagnosis between Cushing's disease (CD) and ectopic ACTH secretion (EAS).

Materials and methods: We retrospectively recorded data about 170 consecutive patients (f/m=133/37, mean age 43.24 ± 14.51 years) with ACTH-dependent CS (149 CD, 21 EAS) referring to two Endocrinology Units between 2001 and 2013. We focused especially on the performance of 3 dynamic tests: the dexamethasone 8 mg overnight challenging (HDDST), the CRH and the desmopressin (DDAVP) test.

Results: Patients with EAS were slightly older and had higher 8 a.m. ACTH levels, 8 a.m. and after 1 mg dexamethasone cortisol levels, and UFC than patients with CD (p<0.01). On the contrary CD patients had a greater ACTH and cortisol response after CRH injection (p<0.0001) and also a more pronounced decreased of cortisol after HDDST (p<0.0001). A threshold for ACTH increase after CRH stimulation of 72% was able to identify CD with a sensitivity (SE) of 76% (95%CI: 68-83) and a specificity (SP) of 100% (95%CI: 83-100). Regarding HDDST, a cortisol reduction >53% suggested a pituitary origin with SE of 88% (95%CI: 81-93) and SP of 90% (95%CI: 68-99); the latter could be increased to 100% by moving the cut-off to 75% of decrease. The AUC of CRH test and HDDST was 0.93 (95%CI: 0.89-0.97) in both cases (p=ns). There were no EAS with both positive responses to these 2 tests. ACTH and cortisol increases after DDAVP test were also higher in CD than in EAS (p<0.01), but they added very little to the power of the other dynamic tests.

Conclusions: Patients with CD showed an increased response to both HDSST and CRH; the cut-offs found had good SE and SP in discriminating patients with CD from those with EAS. Lack of response of both tests was highly suggesting of EAS. On the contrary when both tests gave positive responses it indicated CD. We also confirmed the limited role of DDAVP test in this diagnostic phase. In conclusions, dynamic tests may play an important tool in the differential diagnosis of ACTH-dependent CS.

CO03 - PROGRESSION/REGRESSION OF ALBUMINURIA IN TYPE 2 DIABETES: THE RENAL INSUFFICIENCY AND CARDIOVASCULAR EVENTS (RIACE) ITALIAN MULTICENTER STUDY

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In patients with type 2 diabetes (T2D), increased albumin excretion rate (AER) is a major risk factor for development of both end-stage renal disease (ESRD) and cardiovascular disease (CVD), independent of GFR. However, progression from normoalbuminuria (NA) to macroalbuminuria (MA) does not invariably occur and both microalbuminuria (μA) and MA may regress. We analyzed AER progression / regression in T2D subjects from the Renal Insufficiency an Cardiovascular Events (RIACE) Italian Multicenter Study, an observational, prospective cohort study on the impact of estimated GFR (eGFR) on morbidity and mortality from CVD over a 5-year follow-up. The RIACE enrolled 15,773 Caucasian patients in 19 Diabetes Clinics throughout Italy in years 2007-2008. Baseline and follow-up data were available from 11,959 patients, after excluding those who deceased or dropped out before the follow-up visit. At baseline, 8,892 (74.4%) patients had NA, 2,567 (21.5%) had μA, and 498 (4.2%) had MA, whereas at follow-up, 8,612 (72.0%) patients had NA, 2,738 (22.9%) had μA, and 607 (5.08%) had MA. Of subjects with NA at baseline, 7,613 (85.6%) remained with NA, 1,201 (13.5%) progressed to μA, and 78 (0.9%) to MA. Of subjects with μA at baseline, 1,375 (53.6%) remained with μA, 252 (9.8%) progressed to MA, and 940 (36.6%) regressed to NA. Of subjects with MA at baseline, 277 (55.6%) remained with MA, 192 (32.5%) regressed to μA, and 59 (11.9%) to NA. Loss of eGFR was higher (>10 ml/min/1.73 m^2) in patients who had MA at baseline (even those who regressed to μA or NA) or developed it during the follow-up. Progression to ESRD occurred only in patients who remained MA or μA or progressed to MA. Cases of major acute CVD events (myocardial infarction, stroke, ulcer/gangrene/amputation, and revascularization) and hospitalization for heart failure were less frequent (P<0.001) in NA subjects who remained with NA (769, 10.1%) than in those who progressed to μA (171, 14.2%) and particularly MA (17, 21.8%). Likewise, cases were lower (P<0.001) in μA patients who regressed to NA (164, 17.5%) than in those who remained with μA (291, 21.2%) or progressed to MA (87, 34.5%). Conversely, 30% of MA subjects had such events independent on whether they remained with MA or regressed to μA or NA. Longitudinal data from the RIACE Study indicate that AER may either progress or regress in T2D patients and that baseline levels and, to a lesser extent, changes in AER may predict subsequent loss of eGFR with progression to ESRD as well as development of CVD.

CO04 - THE ABILITY OF A SINGLE BMD AND FRACTURE HISTORY ASSESSMENT TO PREDICT FRACTURE OVER 20 YEARS IN POST-MENOPAUSAL WOMEN: THE STUDY OF OSTEOPOROTIC FRACTURES.

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~~Background. The value of risk factors including bone mineral density (BMD) and history of fractures to predict fracture risk is well established. However, existing risk prediction models are based on extrapolation of data from short-term studies to predict 5 or 10 year fracture risk and their accuracy has not been directly validated. Furthermore, current guidelines for treatment are unclear as to the optimal interval to repeat BMD in untreated individuals.

Objective. To investigate how well a single assessment of BMD and fracture history can predict fracture risk in postmenopausal women over 20 years.

Methods. The Study of Osteoporotic Fractures (SOF) recruited 9704 women >= 65 years and assessed DXA and risk factors in 1986-88 and have continued to follow them for over 20 years. We used the follow-up from initial assessment to calculate 20 year risk of any non-vertebral, wrist and hip fracture as a function of BMD, fracture history and age at the single initial assessment.

Results. The estimated cumulative incidence over 20 years was of 23% for hip fractures, 13% for wrist fractures and 55% for any non-vertebral fractures. Both femoral neck (FN) BMD and fracture history remained independently predictive for all three fracture types for 20 years. After 20 years, the cumulative risk of hip fracture was 26.4% in those in the lowest (age-adjusted) BMD quartile compared to only 6.5% in those highest quartile. History of hip fracture predicted hip fractures slightly better than history of non-vertebral fracture (RR=1.7 (95%CI:1.2,2.3) vs 1.4 (95%CI:1.3,1.6), respectively) but prediction was the same (RR=1.2) after BMD adjustment.

Conclusions. Our results suggest a single assessment including BMD and fracture history can predict fracture risk for at least 20 years with little degradation of prediction in post menopausal women. History of any fracture is as predictive as site-specific history. Our results support the value of a single BMD and fracture history assessment for long-term fracture risk prediction.

CO05 - MICRORNAS EXPRESSION SIGNATURE OF IN-VITRO DE-DIFFERENTIATED HUMAN PANCREATIC ISLETS

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Beta-cell de-differentiation has recently been identified as a mechanism of beta-cell dysfunction both in type-1 and type-2 diabetes. Previously, we have set up a model of in vitro de-differentiation of human islets (HI) that, when cultured in appropriate conditions, undergo an epithelial-mesenchymal-like transition process (EMT), generating human pancreatic islet-derived mesenchymal cells (hPIDM). MicroRNAs are small endogenous RNAs, which regulate gene expression. They have been demonstrated to control several biological processes including stemness and cell-differentiation. Here, we aimed at characterizing the microRNAs expression profile during human pancreatic islet cell de-differentiation process. Human pancreatic islets were collected from 3 multiorgan donors and cultured for 15 days. De-differentiated hPIDM cells were collected and total RNA was extracted from HI and from hPIDM cells. Expression of 768 microRNAs was evaluated using Taqman microRNAs arrays. MicroRNAs target genes followed by gene ontology analysis was performed using Targetscan6.2 and David6.7 algorithms. MicroRNA target genes expression levels on 3 HI and 3 hPIDM cells samples were evaluated using Real-Time PCR. Following HI de-differentiation, 110 miRNAs resulted significantly decreased and 13 increased in hPIDM cells vs HI. Upregulated microRNAs included miR-100, miR-337-3p, miR-214, miR-199a-3p and -5p, miR-137, miR-708, miR-99a and miR-302s. To gain insights into the biological pathways potentially controlled by these microRNAs, we looked at the their target genes. Using Targetscan6.2, we extrapolated a list of 196 predicted target genes which were then functionally classified. Most genes belonged to cell-cell adhesion mechanisms and to differentiation process. By analyzing the expression levels of a selection of these predicted target genes in HI and hPIDM, we observed the downregulation of 44 genes during de-differentiation, in line with the inverse expression levels of targeting microRNAs. In conclusion, we detected a specific microRNAs signature of in-vitro de-differentiated islet cells which may function as regulators of those genes controlling de-differentiated phenotype acquisition

CO06 - GPCR6A AS COMMON RECEPTOR FOR HUMAN OSTEOCALCIN AND SHBG: A COMPUTATIONAL AND EXPERIMENTAL STUDY

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Testosterone (T) acts both through classical binding to its nuclear receptor, regulating steroid response elements in gene promoters, and by exerting rapid non-genomic effects. The identity of the putative G-protein coupled receptor (GPCR) that mediates the non-genomic effects of androgens has been poorly investigated. Recently, it has been demonstrated that *Gprc6a* ablation in animal models results in loss of testosterone-dependent rapid signaling in target tissues, consistent with a physiological role of this receptor in mediating non-genomic androgen responses. Moreover, *in vitro* studies showed that anabolic steroids, including testosterone, can activate GPRC6A, suggesting that GPRC6A may mediate the non-genomic effects of testosterone and other anabolic steroids.

GPRC6A is also considered as the putative receptor for osteocalcin (OC), a small protein of osteoblast origin recently shown to influence a number of metabolic/hormonal processes. By a computational approach, we studied the structure of OC in relationship to its degree of g-carboxylation. We found that calcium ions have a profound influence on the stability of the protein since, in the absence of Ca^{2+}, both g-carboxylated (cOC) and uncarboxylated (ucOC) forms of OC were predicted to explore a larger conformational space compared to the Ca^{+2}-bound form. These data were confirmed by circular dichroism experiments. Furthermore, in terms of Kd, cOC had 30 fold higher affinity for Ca^{2+} compared to the ucOC. Translated in a physiological scenario, this evidence suggests that cOC and ucOC experience a different conformational status that could subtend the different hormonal activities of the two forms of the protein on GPRC6A.

We hypothesize that non-genomic effects of androgens on GPRC6A could be actually mediated by the complex between T and SHBG, the soluble globulin mediating the transport of T through the bloodstream. In a computational analysis, SHBG and ucOC were docked to GPRC6A to determine the preferred binding site on the protein. Among the candidate SHBG-derived peptides, only the sequence corresponding to aminoacids 145-161 showed an OC-like protein structure, suggesting the possible binding of both ucOC and SHBG on the same receptor domain. This hypothesis was confirmed experimentally on HEK-293 cell line. In a set of displacement assays by flow cytometry, we observed a relative decrement of the mean signal for ucOC and cOC respectively of 85% and 37% when cells were incubated with 100-fold equimolar excess of SHBG. Correspondingly, the Erk activation due to ucOC stimulation underwent to stronger blunting, compared to cOC, after SHBG displacement. These results open new cues about the modulation of androgens l·ŏúλØλűè ōè l· y2yπăűŜ2ℕλŘl·f l·LJLJℕ2l·ŏKφ

CO07 - GALECTIN-3, GLUCOSE HOMEOSTASIS AND ADIPOGENIC DIFFERENTIATION

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Adipose tissue (AT) is involved in the pathogenesis of insulin resistance and type 2 diabetes (T2D). Galectin-3 (Gal-3) knockout (KO) mice are more susceptible to high fat diet-induced T2D and show specific structural and molecular alterations of the AT when overfed. However, they also show diet-independent dysregulation of glucose metabolism. The aim of this study was to investigate the role of Gal-3 in the regulation of glucose homeostasis and in AT differentiation and activity. Wild type (WT) and Gal-3 KO mice were analyzed for glucose and insulin levels between 1 and 5 months of age. Insulin sensitivity was assessed by the homeostatic model assessment (HOMA)-IR index and an hyperinsulinemic euglycemic clamp test. Glucose tolerance was evaluated by intraperitoneal glucose tolerance test (IPGTT). Visceral AT (VAT) was analyzed by morphometry and qRT-PCR. Finally, adipocytes and stromal precursor cells were isolated from VAT and subcutaneous AT (SAT) and characterized by molecular analysis and staining with Oil red O. Growth curves were similar in the two genotypes. In contrast, fasting glucose levels were increased in Gal-3 KO mice, starting at 3 months (139.4±8.7 vs. 121.5±7.1 mg/dl; $P<0.01$). Insulin levels were higher in KO mice at 1 month (30.6±5.3 vs. 14.7±1.6 μU/L; $P<0.001$) and fell below the level of WT at 5 months (10.8±1.1 vs. 14.9±1.4 μU/L; $P<0,05$). As a result, the HOMA-IR index was increased in KO mice only at 1 month (4.3±0.8 vs. 2.1±0.3; $P<0.01$). However, the hyperinsulinemic euglycemic clamp performed in 5-month old mice revealed reduced insulin sensitivity in KO mice (41.4±28.1 vs. 86.9±20.8 mg/Kg/min; $P<0.05$), which also showed reduced β-cell function compared to WT (HOMA-%β 43.2±5.3 vs. 64.3±14.3; $P<0.05$) and impaired glucose tolerance at IPGTT at both 1 and 5 months. At end-of-study, both absolute and relative weight of VAT was higher in KO vs WT mice (12.2±2.3 vs. 9.1±0.9 mg/g body weight; $P<0.05$). In contrast, the adipocyte size (1993±28 vs. 2169±69 μm^2; $P<0.05$) and the expression of adipogenic genes (PPAR-γ, ACACA, FASN and SREBP1c) were reduced, whereas expression of inflammation and fibrosis markers (IL-1β e coll1α1) were increased in VAT from KO mice. Finally, stromal precursor cells from Gal-3 KO mice showed defective differentiation, as revealed by an increased expression of Pref-1, a preadipocyte marker and inhibitor of adipogenesis, a decreased expression of adipogenic markers (C/EBPβ e PPARγ), and a blunted (halved) expression of adiponectin. In conclusion, Gal-3 ablation is associated with reduced insulin secretion and sensitivity as well as with a defective adipogenic differentiation and a proinflammatory phenotype of the AT which could play a role in the impairment of glucose homeostasis. These data point to the need for more research on the role of Gal-3 in AT, which might be of potential relevance for the treatment of T2D.

CO08 - TRIIODOTHYRONINE (T3) PREVENTS FASTING-INDUCED SKELETAL MUSCLE ATROPHY IN VITRO AND IN VIVO

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Muscle atrophy may ensue from several pathological conditions including prolonged muscle disuse, cancer cachexia, anorexia etc. Skeletal muscle represents a major target for thyroid hormones (THs) action. Hence, adequate intracellular T3 concentrations warrant healthy muscle homeostasis, since both hyper- and hypothyroidism lead to muscle weakness, hypotrophy and atrophy. All atrophic conditions feature an imbalance between protein synthesys and degradation. AKT is a key regulator of this homeostatic process and has been recently recognized as T3 target. However, whether T3 may play a role to protect muscle from progressive wasting is, as yet, unknown. Aim of the study was to analyze the effects of T3 treatment on muscle atrophy *in vitro* and *in vivo* and to analyze the T3-related intracellular signaling involved. To this end, we induced muscle atrophy in myotubes derived from non neoplastic C2C12 myoblasts cell line either by removing serum from the media (starvation) or by adding TNFa [100 ng/ml] every 24 hours, for two days, in the presence or the absence of T3 [100 nM]. While both starved- and TNF-treated myotubes showed a strong reduction in diameters, as measured on May Grunwald Giemsa stained samples, no such reduction has been observed in T3-treated myotubes. Moreover, following starvation of myotubes, T3-treatment prevented the induction of Atrogin1 expression, a marker gene of atrophy. These *in vitro* data on myotubes suggest a novel role for T3 on regulating skeletal muscle homeostasis and prompted us to test its effect *in vivo*, on a starvation-induced muscle atrophy model. So far, adult male BALB/c mice were used as an *in vivo* model. Muscle atrophy was induced by food-deprivation for 48 hours in half of mice (starved). At the same time, starved and fed mice were treated with daily, intraperitoneal injections of T3 [100 µg/kg BW] or vehicle [NaCl 0.95%] as a control. Starvation led to a 20% drop of body weight, independently from T3 treatment. Similarly, when muscle mass was measured at the level of *Tibialis anterior (TA),* it has been observed a significant weight reduction of TA in untreated starved mice as compared to untreated fed mice. However, in mice treated with T3 no muscle mass weight reduction was observed. Morphometric analyses confirmed a variation in myofibers size consistent with TA weights. In summary, our data indicate that while fasting induced a reduction of total weight, muscle mass and myofiber size in untreated mice, in T3-treated mice muscle mass and myofibers size were comparable to fed controls. So far, in this model, the overall effect of T3 treatment is a protective one on starvation- induced muscle atrophy.

CO09 - CIRCULATING MIRNA-190 AND -95 ARE NOVEL AND NON-INVASIVE BIOMARKERS IN THE DIFFERENTIAL DIAGNOSIS OF THYROID NODULES: PRELIMINARY DATA OF A PROSPECTIVE SERIES.

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Background. MicroRNA (miRNAs) are small (approximately 22 nt), non-protein encoding RNAs that post-transcriptionally regulate gene expression by suppressing specific target mRNAs involved also in cancer initiation and progression. Tissue miRNA profiles may be useful to distinguish benign from malignant lesions. Cells are able to release miRNAs into the circulation and miRNA profiles in serum have been found to be altered in tumors. Recently, we have identified in the serum of a retrospective series of patients, with benign nodular goiter (n=80) and papillary thyroid cancer (PTC: n=79), 2 miRNAs (-190 and -95) that in combination (providing the risk of malignancy defined as pmiRNA) allow the differential diagnosis of thyroid nodules with great accuracy. This study was aimed to confirm the diagnostic accuracy of miRNA-190 and -95 in a prospective series of patients.

Methods. Blood samples were collected from 121 consecutive patients undergoing fine needle aspiration cytology at our Institute. MiRNAs were extracted from serum using the miRNeasy Serum/Plasma kit (Qiagen), and reverse-transcribed using Megaplex Human microRNA RT primers pools v2.1 (Life Technologies). Relative expression quantification was evaluated by the comparative cycle threshold (CT) method ($2^{-\Delta\Delta Ct}$) (Rotor-gene Q, Qiagen). Endogenous miRNA16 and miRNA451 were selected as reference to normalize miRNA expression values.

Results. According to cytology, following BTA guidelines, 85/121 (70.2%) cases were benign (Thy2), 14/121 (11.6%) were suspicious for malignancy (Thy4/5), 16/121 (13.2%) were indeterminate (Thy3) and 6/121 (5%) were non diagnostic (Thy1). A subgroup of 48 patients underwent surgery and the lesions at histology were benign in 30/48 (62.5%) cases and malignant in 18/48 (37.5%). PmiRNA showed a high sensitivity (84%), specificity (93.5%) and diagnostic accuracy (90%) in PTC diagnosis. Moreover, by applying pmiRNA, we could have avoided 2 out of 3 unnecessary surgeries in patients with Thy3 cytology.

Conclusions. These preliminary data strengthen the utility of pmiRNA as a non-invasive diagnostic tool for the differential diagnosis of thyroid nodules especially in case of indeterminate lesions. Further studies with larger series are needed.

CO10 - PATHOGENIC PHENOTYPES OF TYPE 2 DIABETES AT THE TIME OF DIAGNOSIS. THE VERONA NEWLY DIAGNOSED TYPE 2 DIABETES STUDY (VNDS).

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Presence and frequency of beta cell (BC) dysfunction (BCD) and insulin resistance (IR) in patients with newly diagnosed type 2 diabetes mellitus (NDT2D) are imperfectly known, because previous studies used small cohorts and/or only surrogate indexes of BC function and IR. We assessed BC function and IR with state-of-art methods in the VNDS. In 712 GADA-negative, drug naïve, consecutive Italian NDT2D patients we assessed: 1. standard parameters; 2. insulin sensitivity (IS) by the euglycaemic insulin clamp); 3. BC function by state-of-art modeling of prolonged (5 hours) OGTT-derived glucose/C-peptide curves. Thresholds for BCD and IR were the 25^{th} percentiles of BC function and IS assessed with the same methods of the VNDS in Italian subjects with normal glucose regulation of the GENFIEV (n=340) and GISIR (n=386) studies, respectively. In the VNDS, 89.8% [95% C.I.: 87.6 – 92.0%] and 87.8% [85.4 – 90.2] patients had BCD and IR, respectively. Patients with only one defect were 19.7% [16.8 – 22.6]. Isolated BCD and isolated IR were present in 10.9% [8.6 – 13.2] and 8.9% [6.8 – 11.0] patients, respectively. Coexistence of BCD and IR was observed in 78.9% [75.9 – 81.9] of the patients. 1.4% [0.5 – 2.3] of the patients had no detectable alterations in BC function and IS. Patients (19.7%) with only one metabolic defect had lower BMI, fasting glucose, HbA1c, triglycerides and BC function, and higher HDL-cholesterol and IS than patients with both BCD and IR ($p<0.01$ or less after Bonferroni's correction). In conclusion, in NDT2DM patients: 1. at least 75.9% have both BCD and IR; 2. At least 87.6% and 85.4% have BCD and IR, respectively; 3. At least 16.8% have only one defect and a significantly different (milder) metabolic phenotype compared to patients with both defects. These findings may be relevant to therapeutic strategies centered on the metabolic phenotype of the patient. ClinicalTrial.gov Identifiers: NCT00879801, NCT01526720

CO11 - THE FIRST EXOME STUDY IN SECRETORY AZOOSPERMIA

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Introduction: Azoospermia is a heterogeneous condition from a histological point of view and in about 50% of cases the etiology is unknown.

Aim: to perform the first systematic mutational *screening* of candidate genes involved in the development/differentiation of PGCs/spermatogonia, and in meiosis, in highly selected patients affected by Sertoli Cell Only Sdr (SCOS) and Spermatogenic Arrest (SGA).

Material and Methods: Exome Sequencing by using the Nextera Rapid Capture Enrichment Kit (Illumina). This design covers 62 Mb of the human genome that correspond to the exons of about 30,000 genes and microRNA. The sequencing has been performed in i) 2 brothers with SGA and their father (consanguineous parents; ii) 2 unrelated SCOS patients (consanguineous parents); iii) 2 sporadic SCOS cases. Bioinformatic analysis: standard filtering procedure followed by i) recessive model analysis in consanguineous cases (discovery driven) ; ii) prioritization based on 193 candidate genes in sporadic cases (hypothesis driven).

Results: 1) Familial case of SGA: the most promising homozygous mutation has occurred in the *RNF212* gene (exon2:c.111_112insT).This variation provokes a premature truncation of the protein at amino acid 40 (p.K38_K39delinsX), thus its loss of function. RNF212 is required for chiasm formation and assembly of crossover-specific recombination complexes and its mutation is compatible with spermatocytic arrest observed in the brothers. Meiotic studies on testis tissue slides are currently ongoing to further demonstrate the pathogenic effect of the mutation. 2) **SCOS patients** from consanguineous parents: 114 homozygous variants for patient 04-170 and 73 variants for patient 12-056 have been found. After prioritization based on testis expression, deleterious mutations in homozygosis have been identified in 5 genes. Only one of them (*FANCA*) has been previously studied in human, allowing us to discover 4 novel SCOS candidate genes. 3) **sporadic SCOS cases**: after filtering for 193 candidate genes, 3 deleterious mutations were found in heterozygosis, except a mutation in *MAGEA3* on the X chromosome.

Conclusions: WES approach in consanguineous cases is highly efficient and allowed us to identify homozygous mutations in genes with potential role in the early phases of spermatogenesis and in meiosis. We were able to detect the first mutation in human in the RNF212 gene and in 4 other novel candidate genes. In the 2 sporadic SCOS cases we observed more than one "deleterious" variants which would fit with the digenic/oligogenic model observed in central hypogonadism.

CO12 - MONITORING LH AND FSH ACTION ON MOUSE SPERMATOGENESIS IN A 3D CULTURE SYSTEM: EVIDENCE OF A PROTECTIVE ROLE OF BOTH GONADOTROPINS ON CISPLATIN INDUCED GERM CELL DEATH.

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~~Cisplatin, a chemotherapy drug employed in several types of solid malignancies included testicular tumors, has been shown to severely impair germ cell survival in the testis. We then aimed to study the potentially beneficial effects of gonadotropins on cisplatin induced germ cell apoptosis in an in vitro culture system of testicular fragments. To this end we took advantage of the recently reported culture system of mouse prepuberal testes, which fully supports spermatogenesis. By using a gas-liquid interface culture method of neonatal testis fragments, which only contain gonocytes as germ cells, fully developed sperm can be obtained in a serum free culture medium (Sato et al., 2011). Testicular fragments obtained from 5 days post partum (dpp) transgenic mice, expressing the fluorescent probe EGFP under the control of c-kit regulatory regions, were cultured onto agar blocks in the presence or absence of 10M cisplatin for 24 h and then transferred for 1 or 2 weeks in cisplatin free medium. To evaluate the role of gonadotropins in protecting germ cells from cisplatin induced apoptosis, parallel experiments were also performed. At the end of the culture period, testicular fragments were fixed and observed by fluorescence microscopy to evaluate the presence and the number of EGFP positive germ cells. We found that 24 h cisplatin treatment induced massive germ cell death and tubules were found empty by one week of additional culture. FSH addition to the control cultures stimulated the spermatogenetic wave showing an increase of EGFP positive germ cell numbers along the seminiferous tubules. In the presence of cisplatin, FSH was able to partly protect germ cells from cell death, as suggested by the presence of about 15% of EGFP positive tubules, compared to the FSH treated samples. LH addition did not modify the numbers of EGFP positive tubules in control cultures, however EGFP positivity within the tubules was significantly preserved (about 30% EGFP positive tubules, with respect to LH alone) in the presence of LH and cisplatin. LH and FSH treated cultures were similar to FSH only cultures, however the presence of both gonadotropins significantly increased the numbers of EGFP positive tubules (40% of EGFP positive tubules, with respect to FSH and LH alone) . The effect of LH and FSH concomitant addition was mimicked by forskolin, an activator of adenylyl cyclase, suggesting that the protective effects of LH and FSH on cisplatin-induced germ cell death was mediated by cAMP. Altogether our result suggest a positive role of gonadotropins on cisplatin-arrested spermatogenesis.

Sato et al. 2011, Nature 471, 504.

CO13 - TRANSMEMBRANE MCT8-MEDIATED T3 TRANSPORT IS INHIBITED BY SOME COMMONLY USED DRUGS.

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Thyroid hormone cell membrane transporters are essential for thyroid hormone (TH) metabolism and action. MCT8 is the most specific TH transporter identified to date that has been linked to human disease. Mutations in the *MCT8* gene are associated with a form of mental retardation and severe neurological impairment, suggestive of TH deficiency in brain and with a characteristic combination of thyroid function tests (TFTs) abnormalities. Aside from brain, MCT8 is also widely expressed in many other tissues and likely plays an essential role in TH delivery for regulation of developmental and metabolic functions throughout the body. It is conceivable that besides genetic alterations other factors can impair MCT8 activity.The aim of the study was to investigate whether some commonly used drugs having a structural similarity with TH and/or whose treatment is associated with TFTs abnormalities are able to inhibit MCT8-mediated TH uptake into cells. COS-7 cells were transiently transfected with human MCT8 (hMCT8) or pcDNA3 and then incubated with [^{125}I] T_3. To study the effects of our selected drugs on MCT8 activity, transfected cells were exposed to increasing concentrations of glucocorticoids (hydrocortisone, dexamethasone, prednisone and prednisolone), amiodarone, desethylamiodarone, dronedarone, buspirone, carbamzepine and valproic acid and [^{125}I] T_3 uptake was measured at 15'. Exposure to each glucocorticoids gave different results: hydrocortisone dose-dependently inhibited T_3 uptake, which was significantly reduced at the highest concentration (45% of inhibition at 1000 µM); dexamethasone significantly inhibited T_3 uptake even at the lowest concentration and showed at the highest (100 µM) a 79 % of inhibition; conversely, prednisone and prednisolone were entirely devoid of inhibitory potential in the competition assay. With respect to the antiarrhythmic agents, amiodarone caused a significant reduction of MCT8-mediated T_3 transport at the highest concentration (40% of inhibition at 100 µM), this effect was weaker than that produced by desethylamiodarone and dronedarone at the same concentrations; at 100 µM they showed a 52% and 87% of inhibition, respectively ; buspirone resulted a potent inhibitor, significantly reducing the uptake of T_3 even at low concentrations (% of inhibition ranging from 75 to 87%). Carbamzepine and valproic acid had no effect on MCT8-mediated T_3 uptake. In conclusions this study shows a novel effect of some commonly used drugs, that is inhibition of T_3 transport into cells mediated by MCT8. Specifically, hydrocortisone and amiodarone modestly inhibit T_3 uptake whereas dexamethasone, desethylamiodarone and dronedarone behave as potent inhibitors. Treatment with these drugs may interfere with T_3 delivery and action in the tissues where MCT8 represents the main mediator of transmembrane passage of TH.

CO14 - SIMULTANEOUS MEDULLARY AND PAPILLARY THYROID CARCINOMAS. PRELIMINARY DATA OF AN OBSERVATIONAL MULTICENTER RETROSPECTIVE STUDY

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The primary objective of this multicenter study was to collect a large number of cases with simultaneous MTC/PTC to examine the epidemiological characteristics and pathological conditions and to evaluate the clinical outcome. The secondary objective will be to collect molecular data in order to characterize their genetic profile.To date we collected epidemiological, pathological and follow-up data of 98 patients MTC/PTC diagnosed between 1992 and 2014 in 14 Italian centers. There were 39 males and 59 females (M: F = 0.66). The median age at diagnosis was 59 years (range 19-84): 28% > 65 years, 30% <50 years and 42% between 51 and 65 years. The median follow-up was 40 months (range 3-261). Diagnosis of these cancers was mostly based on increased serum calcitonin and / or the presence of suspicious nodules. At histology the PTC variants were distributed as follows: 71 cases (72%) were classic, 16 (16%) were follicular varaints while the remaining 11 cases (11%) had other less common variants. As for the staging of PTC, there were 79 cases pT1, cases 3 pT2, 8 cases pT3-4, 8 cases pTx; 65 cases were N0, 11 cases N1and 22 cases Nx; 60 cases were M0 and 38 Mx. As for the MTC, 51 cases were pT1, 11 pT2 and 23 pT3-4; 45 cases were N0, 30 N1 and 23 Nx; 55 cases were M0, 6 M1 and 37 Mx.Information on RET gene mutation was available in 59 MTC pts (60%), 39% of them showed the presence of the mutation either at germinal or somatic level. Regarding the outcome, 89/98 (89.8%) MTC/PTC patients are in clinical remission both for PTC and MTC. In conclusion, the analysis of the data collected so far in this big series of MTC/PTC shows an equal gender distribution that was expected for MTC but not for PTC, a not evident age-dependent prevalence although the median age is shifted to older age as compared to either tumor taken separately, a tumor size of both PTC and MTC predominantly pT1 ($\leq$ 2cm) with a prevalence of these small tumors (80% of PTC and 52% of MTC) higher than in isolated cases. At the present the majority of patients are cured showing a clinical behavior substantially similar to that of the two types of cancer considered separately

especially when compared with cases with similar stage.

CO15 - TYPE OF FISH CONSUMED AND THYROID AUTOIMMUNITY (TAB) IN PREGNANCY AND EARLY POSTPARTUM (PP)

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Fish consumption or supplementation with omega–3 fatty acids (ω-3 FA) were reported to cure and/or prevent autoimmune and nonautoimmune disorders. Serum positivity for TAb is a predictive marker of PP thyroiditis and PP depression. We hypothesized that stable consumption of the ω-3–rich oily fish (OF) was associated with a more favorable profile of thyroglobulin antibodies (TgAb) and thyroperoxidase antibodies (TPOAb) throughout pregnancy and early PP compared with stable consumption of swordfish (SwF), a predator that concentrates pollutants. Certain pollutants may trigger autoimmunity.

We prospectively measured serum TgAb and TPOAb in pregnancy [1st, 2nd trimester (TRIM)] and PP (day 4), in 236 thyroid disease–free, non–smoker women with stable dietary habits. Based on fish consumption, women were divided in groups A (n=48; SwF), B (n=52; OF), C (n=68; SwF+other fish, not necessarily OF), D (n=68; fish other than SwF and OF). Major endpoints were positivity rates and serum levels of the 2 TAb.

Both during gestation and at day 4 PP, positivity rates were the greatest in group A (25%, 16.7%, 12.5% for TgAb; 25%, 12.5%, 12.5% for TPOAb) and the lowest in group B (0%, 0%, 0%; P<0.001, P<0.01, P<0.01 *vs.* group A). These differences paralleled those of serum levels of either TAb (P<0.05 to P<0.001). Relationship between monthly fish consumption and serum levels of either TAb was direct in group A (TgAb 2nd TRIM, r=0.45, P<0.001; TPOAb 1st TRIM, r=0.35, P<0.01; 2nd TRIM, r=0.35, P<0.01; PP, r=0.4, P<0.01) but inverse in group B (TgAb 1st TRIM, r=-0.65, P<0.001; 2nd TRIM, r=-0.50, P<0.001; PP,r=-0.65, P<0.001; TPOAb 1st TRIM, r=-0.65, P<0.001; 2nd TRIM, r=-0.45, P<0.01; PP, r=-0.75, P<0.001). The estimated monthly content (EMC) of ω-3 FA on fish consumed was the greatest in group B (13.2±5.4 g), the lowest in group D (5.1±3.8), and intermediate in the groups A (6.3±2.1) and C (6.0±2.8, B *vs.* any other group P<0.001). Concerning the EMC of heavy metals, for mercury this was the highest or lowest in groups A (1000±300 µg) or B (26±12, P<0.001) with an almost 40-fold difference. Groups A and B also differed, though at a lower extent, for the EMC of lead (65±23 µg *vs.* 46±21, P<0.001).

We validated our hypothesis that consumption of ω-3 rich OF impacts favorably on serum TAb. As TAb are markers of autoimmune–related PP problems, our data suggest a dietary prophylaxis of such problems. Our data reinforce recommendations that pregnant women should avoid consuming SwF, and indicate consumption of OF as a proper alternative.

CO16 - CHANGES IN EYE MUSCLE DUCTIONS AS A QUANTITATIVE PARAMETER OF RESPONSE TO I.V. METHYLPREDNISOLONE (IVMP) IN PATIENTS TREATED FOR ACTIVE MODERATE-SEVERE GRAVES' ORBITOPATHY (GO).

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Parameters assessing changes of eye muscle function are of utmost importance for the assessment of the therapeutic outcome in GO patients. To date, the assessment of motility changes has relied mostly on qualitative parameters (Gorman score). We studied the changes of eye motility by measuring the ductions and calculating a total motility score (TMS), in an attempt to quantify the response to immunosuppression.

Thirty patients with active GO treated with i.v. methylprednisolone (cumulative dose: 7.5 g), were retrospectively studied.

We calculated a reference range for TMS, in a group of 100 normal controls by determining the mean±2 SD TMS (212±20).

Before therapy, 20 of 30 patients had diplopia, eight intermittent (TMS 190.8±5.9), five inconstant (TMS 149±7.3) and seven constant diplopia (TMS 133.2±16.8). In patients with inconstant and costant diplopia, TMS was significantly lower compared to absent/intermittent diplopia (P=0.017). Mean TMS was significantly higher in controls when compared to GO patients, regardless the degree of diplopia (Kruskal-Wallis test p<0.0001). Of the seven patients with constant diplopia, six were submitted to squint surgery.

At 24 weeks improvement of the Gorman score was observed in 33% of patients, while worsening or stabilization was found in 30% and 36% of patients, respectively. TMS significantly increased at 12 and 24 weeks after treatment, when compared to baseline (164.3±9 vs 180.2±7 and 180.4±7; repeated measures Anova P<0.0001), while no differences were found between TMS at 24 when compared to TMS at 12 weeks. Baseline TMS was 166.1±6.3 and improved at 24 weeks (185.3±5; Wilcoxon P<0.0001) in patients who responded to i.v. methylprednisolone, but not in patients submitted to squint surgery (156.4±10.4 vs 168.2±8.6; Wilcoxon P=NS).

In conclusion, by calculating TMS on patients' ductions we were able to quantify the changes of eye muscle function after immunosuppression and identify those who required squint surgery.

CO17 - WHOLE EXOME SEQUENCING: A POWERFUL AND UNBIASED APPROACH TO MAP THE GENETIC BACKGROUND OF FAMILIAL NONMEDULLARY THYROID CANCER.

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Familial thyroid cancer is now a well known, distinctive, clinical entity in subjects with thyroid cancer deriving from follicular cells, that is, nonmedullary thyroid carcinoma (FNMTC). FNMTC may be associated with more aggressive behavior than sporadic cases and preventing screening will permit earlier detection, faster intervention, and hopefully improved outcomes for patients and their families. Even if, in the last years, an important number of genetic studies on FNMTC have been published, single gene variants involved in FNMTC susceptibility have not yet been identified. Interestingly, some susceptibility loci of familial forms have been associated in selected kindred, but not replicated in further studies. Our aim has been a comprehensive study of coding gene variants shared in familial FNMTC, by a whole exome approach.

Patient selection: On a total of five families affected with FNMTC, we have selected two families with two affected brothers (DG1, DG2) and two affected sisters (DP1 e DP2), respectively. All patients underwent total thyroidectomy, radio metabolic treatment and levothyroxine suppressive therapy. The median follow up period was 78±8 months and no signs of recurrence were detected in all patients.

Material and Methods: We studied affected sibpairs by whole exome sequencing (WES) by the selection of exomic sequences with Haloplex enrichment procedure and 100x2 bp next generation sequencing (NGS) on Illumina HiSeq 1000 platform.

Results: The medium coverage was >100x in all patients studied. No variations of known candidate genes involved in the pathogenesis of sporadic and familial nonmedullary thyroid tumors were found among 28,000 variations/subjects. We focused on the heterozygous calls and considering the frequency of FNMTC, we eliminated all variants present with a frequency higher than 1%. The resulting filtered list of variants was composed of a total of 165 stopgain of which three were shared between the four patients. Validation studies are currently underway.

Conclusions: Our preliminary results have proved the usefulness of newly advanced molecular technology for the molecular characterization of familial tumors. WES [illegible]

CO18 - PREVALENCE OF THYROID CANCER AFTER EXTERNAL BEAM RADIOTHERAPY (EBR) FOR CANCER IN CHILDHOOD: CLINICAL AND HISTOPATHOLOGICAL FEATURES AND LONG-TERM OUTCOME

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Introduction: Patients treated for tumors arising in childhood have a high incidence of secondary cancers of the thyroid.

Objectives: Assessment of the prevalence and analysis of clinical and histopathological features and long-term outcome of secondary thyroid cancer(TC) in patients treated with EBR or EBR plus chemotherapy for a variety of childhood cancers.

Patients and Methods: In the 1999-2014 period 135 consecutive patients(71 F, 64 M) previously treated for childhood cancers(131 with EBR plus chemotherapy and 4 with only EBR) underwent clinical examination to our Thyroid Clinic. TSH, thyroid hormones and antibody measurements thyroid and neck ultrasound were carried out in all patients; FNAb only when suspicious thyroid nodules were present. Patients with TC were treated in accordance to current guidelines.

Results: Thyroid nodules were found in 32/135(23.7%) cancer survivors, 17 F and 15 M, mean age at the time of first anti-cancer treatment 9.6 yrs(median 8.3, range 1-18); mean age at the time of diagnosis of thyroid nodules 22.2 yrs, median 22.1, range 12.2-33.4. Among these patients with thyroid nodules, TC was diagnosed in 16/32(50%),13 F and 3 M, 4 in patients treated with only EBR and 12 in patients treated with EBR plus chemotherapy; mean age at diagnosis of first malignancy was 9.6 yrs(median 10.4, range 1-18) and mean age at the time of our study 35.5 yrs, median 35, range 21-68, with a mean interval between of diagnosis of first malignancy and diagnosis of TC of 19.7 years(range 4.5-43).All underwent total thyroidectomy with neck lymphadenectomy in 10.In all cases TC histotype was: classic variant papillary carcinoma in 10 and follicular variant in 6 patients; mean tumors size was 12.8 mm(range 4-30); loco-regional lymph node metastases were found in 8/16(50%) and extrathyroidal invasion in 7/16(43.7%). Post-surgical ablative radioiodine treatment was given to 12 patients.At last control 13 cases(81%) were disease free while 3 had persistent disease(1 with a lung metastases).

Conclusions: Patients treated with EBR and chemotherapy during childhood have a high risk of secondary papillary TC(more frequent in females) with features of aggressiveness. Accurate screening and follow-up of these patients is required to avoid delay of diagnosis and therapy.

CO19 - PURIFICATION AND PARTIAL CHARACTERIZATION OF PROTEASES FOR THE INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN-3 (IGFBP-3). ROLE OF THE KIDNEY IN THE REGULATION OF IGFBP-3

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The finding that in chronic end-stage renal failure (CRF) circulating IGFBP-3 is considerably increased has led to the hypothesis that both the elevated serum GH values and the growth failure characteristic of CRF are due to an inhibition of IGF action by an increase in IGFBP-3. As proteolysis may be a general mechanism regulating the release of IGF from the IGF-IGFBP complex, we wanted to verify the ability of the kidney to produce IGFBP-3 specific proteases. For this reason, kidneys from 2 months old male Wistar rats were collected, homogenized and subjected to sequential differential centrifugations in order to purify four subcellular fractions (membranal, lysosomal, cytosolic, and microsomal fractions). The microsomal fraction (containing neo-synthetized proteins) was solubilized by treatment with KCI 0.2 M, followed by 3 cycles of freezing and thawing and subsequent centrifugation at 200,000 x g to separate microsomal membrane from the soluble compartment. To assess the specificity of kidney to produce IGFBP-3 specific proteases, the same purification procedure was carried out on rat livers. To evaluate the presence of IGFBP-3 protease activity (IGFBP-3-PA) in subcellular fractions, radio-iodinated recombinant human lGFBP-3 (rhIGFBP-3) was incubated in a 37°C water bath for 1 to 5 hours with kidney- or liver-derived material and then analyzed by 15% SDS-PAGE. The results demonstrated high levels of IGFBP-3-PA in the microsomal fraction of kidney, virtually absent in the other subcellular fractions. Conversely, rat liver IGFBP-3-PA was mainly detected in the lysosomal fraction, but undetectable in the specific microsomal fraction. The kidney soluble microsomal fraction containing IGFBP-3-PA was further purified by DEAE Sephadex A-50 ion exchange chromatography and two peaks of activity were found. The IGFBP-3 PA contained in both peaks were able to proteolyze rhlGFBP-3 in a dose-dependent manner producing predominantly a 18 kDa fragment (peak C) and a 14 kDa fragment (peak D). IGFBP-3 PA of peak C was inhibited by leupeptin and was insensitive to chelating agents and to serine protease inhibitors, whereas that of peak D was partially sensitive to both chelating agents and leupeptin. In conclusion, these results established that rat kidney microsomes contain at least two protease activities capable of degrading rhlGFBP-3, distinguishable on their affinity for basic resins and on their sensitivity to protease inhibitors. The subcellular localization, and particularly the localization in the soluble fraction obtained by microsomes, suggests that these proteins are "secretory proteins". The kidney, therefore, produces proteases which may be involved, once released into circulation, in the removal of IGFBP-3, regulating IGF bioactivity.

CO20 - ACRO SCORE: A NEW AND SIMPLE TOOL FOR DIAGNOSIS OF ACROMEGALY, A RARE AND UNDER-DIAGNOSED DISEASE.

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Objective: acromegaly is a rare disease due to growth hormone (GH)/IGF-I hypersecretion. It's associated to higher mortality and duration of disease is one of most important factor. It is characterized by slowly and insidious progressive course; clinical features, though very peculiar, are generally under-recognized as well as comorbidities (which overlap with common disorders) so that the diagnosis is invariably preceded by about 10 years of active but unrecognized disease. Early recognition is necessary to improve rate of treatment success and to avoid comorbidities. At present it is common opinion that, in order to increase early diagnosis, the best strategy is likely to be to increase the awareness about the disease of general practitioners and specialists who generally take care of acromegalic comorbidities. Aim of our study is to develop a risk of acromegaly score (ACROSCORE) focusing on some cardinal clinical symptoms and signs, that physician could use to identify early acromegaly. This was an Italian multicentre cross-sectional, observational study conducted in 3 tertiary referral university centres (Torino, Ancora, Messina). Methods: we enrolled 194 acromegaly patients (117 F, age mean ± SD; 47.2 ± 14.2 years old) and 243 patients (131 F, 45.8 15.8 years old) affected by non GH-secreting pituitary tumor. In all patients signs, symptoms and comorbidities were recorded by data in medical records and by a specifically designed questionnaire. The differences in the distribution of selected variables between cases and controls were evaluated by the Pearson's chi-square test, whereas their association with the diagnosis of acromegaly was estimated by computing diagnostic odds ratios (ORs) and their confidence intervals at 95% (95% CIs) from both univariate and multivariate logistic regression models. Results: strong association was observed for diabetes mellitus (OR = 3.7), hyperhidrosis (OR = 6.1), thyroid hyperplasia (OR = 13.9), colorectal polyps, carpal tunnel syndrome (OR = 4.3), spaced teeth (OR = 25.4). Based on these information, a multivariable logistic model was built and a 14 points score was produced. Scores = 0 exclude risk of acromegaly (VP^+ = 0.6%), between 1 - 5 is a gray area; for scores > 5 diagnosis of acromegaly can be taken into consideration (VP^+ = 46.1%). **Conclusions**: ACROSCORE is likely to represent a new tool for a clinical screening of a rare disease such as acromegaly upon general practitioners and specialists other that in endocrinology. This study firstly suggests the usefulness of ACROSCORE. These positive findings

need to be further validated in general population.

CO21 - PREVALENCE OF GROWTH HORMONE DEFICIENCY (GHD) IN A COHORT OF ADULT PATIENTS (PTS) WITH CYSTIC FIBROSIS (CF)

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Introduction: Cystic fibrosis (CF) is an autosomal recessive disorder caused by a variety of different mutations in the CFTR gene. Life expectancy has recently improved, however, patients (pts) with CF complain symptoms that overlap with the adult growth hormone deficiency syndrome (GHD): reduced muscle mass, bone fragility, cardiovascular complications and lower tolerance stress. The GH-IGF1 axis has been previously investigated only in small cohorts of children and adolescents with CF, while no large adult cohort studies are available. Aim: To investigate abnormalities of the GH-IGF-1 axis in adults with CF. Methods: From over 300 CF adults followed-up in our referral center, we excluded pts with liver or lung transplantation, age<18years, subjects with FEV1(% predicted) <30% or on oxygen therapy. The remaining were consecutively enrolled in a prospective study with recording of anthropometric parameters, glyco-metabolic status, pituitary, thyroid, parathyroid, gonadal and adrenal function, arginine+GHRH stimulation test for GH, spirometry, dual-energy X-ray absorptiometry, thyroid and gonadal ultrasonography and questionnaires. BMI-adjusted criteria were used for the diagnosis of severe GHD (8 or 11.5 ng/ml) and partial GHD (16 ng/ml). Data are shown as mean±SD (range), if normally distributed, or median (interquartile). Results: The first 41 enrolled pts (30 men; age 36.9 ± 10.7 yrs) had the following spirometric values: FEV1 %: 61.9 ± 18.3 (31-101), FVC%:78.7±19.5 (35-109), FEF25-75 %:39±23.7 (24-53), confirming a good CF control. All pts had two severe (class 1 and 2) CTFR mutations. Testing of GH-IGF1 axis revealed a defective response in 34.2% (14/41): severe in 12.2% (5/41) and partial in 22% (9/41). In severe GHD, median GH peak was 8.5±1.3 (8.1-10.6) and IGF1 185.4±51.5 ng/ml (137-273); in partial GHD, GH peak was 12.8±1.5 (12.4-14.6) and IGF1 208±39.7 (146-283), the remaining had a GH peak of 38.9±38.6 (25.4-58.4). All pts with severe GHD had at least one ΔF508 mutation (100%, 5/5) [homozygous in 60%], compared to controls in whom ΔF508 was less frequent [(homozygous in 33.3% (9/27)]. Compared to non-GHD adults with CF, GHD pts had a worst fasting glucose (115.9±29.1 vs 87.7±13.7) and higher BMI (23.4±2.4vs 20.9±2.7) (both $p<0.05$) which were inversely correlated to GH peak (fasting glucose: r=-0.31, BMI: r= -0.33; both $p<0.05$). No significant differences were observed in spirometry, bone density and H-QLS questionnaire. Discussion: Very few data on GHD in CF are available. Our study is the first prospectively performed in well-controlled subjects aged 22 to 60 years showing a high GHD prevalence, a possible association with genetic background and an impact on metabolic status. Given these findings and the increased life expectancy of pts with CF, a larger screening and clinical follow-up of the impact of GHD in affected subjects is warranted.

CO22 - PROGENITOR/STEM-LIKE CELLS IN HUMAN NON FUNCTIONING PITUITARY ADENOMAS: CHARACTERIZATION AND CORRELATION WITH CLINICAL TUMOR BEHAVIOR

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Several studies support the existence of multipotent stem/progenitor cells in the rodent and human pituitary, but their role in pituitary tumorigenesis is still matter of debate. Aim was to identify and characterize stem/progenitor cells in human non-functioning pituitary adenomas (NFPAs) and to investigate their role on resistance to dopaminergic agents and tumor behavior. Cells from 44 NFPAs were cultured in conditions favoring stem cell growth. The expression of stem-cell markers and pituitary specific transcription factors was evaluated by different techniques (FACS analysis, reverse transcription-PCR, immunofluorescence and immunohistochemistry). The effect of dopamine analogs was tested in primary cultures and corresponding progenitor cells. The ability to form round cell clusters (pituispheres) was correlated with clinical tumor behavior. About 70% of NFPAs formed pituispheres expressing stem-cell markers (such as SOX2, OCT4, NANOG), transcription factors involved in gonadotroph differentiation (DAX1, SF1, ERG1) and gonadotropins. Consistent with dopamine receptor 2 (DRD2) expression by pituispheres, the reduction of cell proliferation by DRD2 agonists in primary cells was maintained in the corresponding pituispheres (31±17% and 45±24% inhibition, $p<0.01$ vs basal, respectively). Analysis of tumor behavior showed that pituispheres formation was positively associated with Ki67 (OR= 10.287; P = 0.03) and inversely associated with cavernous sinus invasion (R= 0.06; P = 0.03), whereas no correlation with sex, age, tumor size and extrasellar extension was observed. Our data demonstrate the existence of progenitor/stem-like cells that form spheres in culture, express stem cell markers and pituitary embryonic factors and maintain responsiveness to dopaminergic drugs in the majority of NFPAs, particularly in those with high proliferative activity.

CO23 - VISFATIN AS A MARKER OF ADIPOSE DYSFUNCTION AND METABOLIC IMPAIRMENT IN ACTIVE ACROMEGALY AND ITS POSSIBLE USE DURING THE FOLLOW-UP

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Background Visfatin is an adipokine with insulin-mimetic and adipogenic effect related to insulin resistance (IR) and visceral adipose mass. Although acromegaly is characterized by both adipose dysfunction and IR, data on visfatin levels in acromegaly are very scarce and in the few existing studies no difference was reported between acromegalic patients and controls.

Aim To evaluate the visfatin levels in acromegaly in relation to disease activity and type of treatment.

Subjects and Methods Data of 56 patients (31 M, age 59 ± 12 yrs) were analyzed. Sixteen patients were newly diagnosed (ND), while the remaining were in therapy with somatostatin analogues (SA, 21), pegvisomant (PE, 12) and after 6 months of surgery (SU,7). Among all, 33 resulted not controlled (16 ND, 10 SA, 5 PE, 2 SU) and 23 controlled (11 SA, 7 PE, 5 SU). In addition to routine anthropometric, hormonal and metabolic parameters, visfatin, leptin and adiponectin levels were evaluated.

Results Uncontrolled patients showed higher levels of visfatin (1.51 ± 0.44 vs 0.21± 0.22 ng/ml; $p<0.001$). and lower levels of leptin (0.89 ± 0.55 vs 3.49 ± 1.28 ng/ml; $p<0.001$) in concomitant with higher insulin ($p=0.034$), Homa-IR ($p<0.001$), visceral adiposity index ($p<0.001$), triglycerides ($p<0.001$) and lower ISI Matsuda ($p<0.001$), HDL cholesterol ($p<0.001$) than controlled, with no significant difference in adiponectin levels. When we compared visfatin levels between untreated and treated patients, we found higher concentrations in the first group (1.54 ± 0.58 ng/ml) than in patients treated with SA (0.86 ± 0.62 ng/ml; $p=0.002$), PE (0.32 ± 0.51 ng/ml; $p=0.002$) or SU (0.48 ± 0.65 ng/ml; $p=0.004$), while no difference was found among different treatments. A strong correlation was found between visfatin and GH (nadir, AUC), IGF-1 and ISI Matsuda (all $p<0.001$).

Conclusions In active acromegaly visfatin could be considered a useful tool for the evaluation of metabolic alterations, such as IR and adipose dysfunction, and a marker of the disease control regardless of type of treatment.

CO24 - FIRST REPORT ON POSSIBLE PROTECTIVE ROLE OF METFORMIN THERAPY ON COLONIC POLYPS IN ACROMEGALY

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Acromegalic patients have an increased risk of cancer that constitutes their third cause of increased mortality. Adenomatous polyps may occur in up-to 30-40% of patients, with a consequent higher risk of colon carcinoma development. Although the role of metformin in cancer is controversial, there is increasing evidence that it may play a protective role in diabetic and non-diabetic patients with colonic polyps. Moreover, clinical trials have been already designed for its potential use in chemoprevention. The aim of this retrospective study is to evaluate for the first time the prevalence of colonic polyps in acromegalic patients, treated or not with metformin, in order to explore the possible protective role of metformin on the development of polyps. Of the 74 patients from two major Italian referral centers, 58 patients (age range 36-82 yrs; f 33), who performed at least one screening colonoscopy were included in the study. In this cohort, disease duration, previous or concomitant use of metformin, therapies for acromegaly, smoking, use of cardioaspirin, family history or others risk factors for colonic polyps/cancers, presence of colonic polyps and cancer, hormonal and metabolic parameters were evaluated. In line with the literature, a 36% (21/58) overall prevalence of polyps have been found. According to the presence of polyps, we identified two study-groups, comparable for age (p 0.7), BMI (p 0.5), disease duration (p 0.8), glucose (p 0.3), insulin (p 0.8), HbA1c (p 0.2) levels, as well as GH (mean 8.6±3.8 µg/L, p 0.5) and IGF-I (mean 403±44 µg/L, p 0.8) levels. Among the group with polyps (including 3 patients with carcinoma) only 5/21 (24%) patients were treated with metformin versus 21/37 (57%) in the group without polyps. The mean dose of metformin was 1020±180 mg in the group of patients without polyps vs. 262±118 mg in the group of patients with polyps (p 0.0008). Appropriate statistical analysis confirmed a significant negative association between the use of metformin and the prevalence of colonic polyps (OD 0.238, 95% CI 0.072-0.788, p=0.019), whereas no association was found between the use of cardioaspirin and polyps. In line with literature, there was no correlation between IGF-I levels and presence of polyps, while, in this cohort, no significant association between insulin levels and prevalence of polyps was found, as previously reported. In conclusion, although these are only preliminary data that need to be confirmed in a larger population, the results of the current study suggest a protective role of metformin on colonic polyps in acromegaly.

CO25 - COPY NUMBER VARIATIONS (CNV) IN THE X CHROMOSOME OF KLINEFELTER SYNDROME

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Introduction

Klinefelter syndrome (KS) is characterized by the presence of at least one extra X chromosome and represents the most common chromosomal aberration in men. Apart from infertility, the clinical spectrum of KS is variable and often not directly related to hypogonadism, whose expression is also not unpredictable. Several genetic mechanisms may explain the clinical features and variability of the phenotype in KS. In particular, gene-dosage effects and the parental origin of the supernumerary X chromosome in conjunction with (possibly skewed) X-chromosome inactivation may play significant roles. Here we investigated, for the first time, the genetic property of the X chromosomes by analysis of Copy Number Variations (CNV).

Materials and methods

We studied 93 non-mosaic (47,XXY) KS and 85 controls (46,XY men and 46,XX women) by SNP array using thel HumanOmniexpress Bead Chip 700K (Illumina), which includes more than 700,000 SNP markers. Data generated on the Illumina Infinium platform have been analysed by the GenomeStudio software v2011.1 (cnvpartition 3.1.6) and PennCNV.

Results

In KS the total number of CNV was 6.5/patient, significantly higher than 46,XY males (3.9 CNVs/patient) and 46,XX females (1.5 CNVs/patient). These CNV included duplications/patient and 12 deletions/patient, both significantly higher with respect to normal males and females. Also the total length of duplications and deletions, and the length of duplications and deletions per patient were significantly different from controls. After exclusion of CNV mapping to the PAR1 (69 CNVs) and PAR2 (6 CNVs), regions of X-Y homology (401 CNVs), centromere (72 CNVs), and CNVs not containing genes (7 CNVs), we identified 94 CNVs in KS, 10 in 46,XY men and 37 in 46,XX women. The number of deletion/patient and the length of deletion/patient were higher in KS with respect to controls. Furthermore, we identified 12 CNVs in genic regions that were patient-specific.

Conclusion

This is the first study showed the spectrum of CNVs in the X chromosomes in KS and might be important in better understanding the biology of the X chromosome in this

syndrome and the role of CNVs in the genotype-phenotype relation.

CO26 - IN VITRO DIFFERENTIATION OF MOUSE PRIMORDIAL GERM CELL LIKE CELLS (PGCLC) FROM ES CELLS THROUGH EPILSC.

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~~It has been recently shown that PGCLCs can be generated in vitro from embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) passing through epiblast-like cells (EpiLCs), a cellular state highly similar to pregastrulating epiblasts but distinct from epiblast stem cells (EpiSCs), both in mice and humans. Briefly, to obtain PGCLC, we differentiated female ES cells obtained from a transgenic mouse line in which the regulatory regions of c-Kit (a marker of PGCs) drive the expression of into EpiLCs in presence of Activin A or Nodal (both belonging to TGFβ/activin/nodal signaling pathway) and bFGF and subsequently we converted them into PGCLCs like cells in presence of BMP4, BMP8b, EGF and LIF. Interestingly, we found that Nodal can replace Activin A in the induction of PGCLCs and it is also more potent. Indeed, we found that Nodal was able to induce the presence of EGFP positive cells, presumptive of PGCLC, with a frequency three times higher than Activin A. To confirm that these cells actually converted to PGC-like cells we re-aggregated them with 13.5 dpc gonadal somatic cells from female embryos and cultured them for 4 weeks onto agarose blocks. Ovarian structures rapidly formed after one week of culture and were able to increase their size during the following three weeks of culture.

At the end of the culture period, the chimaeric ovaries were fixed and frozen sections were obtained. By fluorescence microscopy we found EGFP positive cells within the chimaeric ovary surrounded by somatic cells. By using antibodies against meiotic markers we found that some EGFP positive cells were stained for SCP3, a synaptonemal complex component specific for meiotic chromosomes. We did not find differences in the numbers of meiotic cells between Activin A and Nodal treated cells, suggesting that Nodal is able to increase the recruitment of PGCLC from EpiLSC but not their meiotic commitment. Our culture system represents the first model an in vitro only method to derive mature female gametes.

Hayashi et al. (2011) Reconstitution of the mouse Germ cell specification pathway in culture by pluripotent Stem cells. Cell 146; 1-14

Cairns LA et al. (2003) Kit regulatory elements required for expression in developing hematopoietic and germ cell lineages. Blood 1;102(12):3954-62

CO27 - DEVELOPMENT OF AND RECOVERY FROM SECONDARY HYPOGONADISM IN AGING MEN: PREDISPOSING FACTORS AND CLINICAL FEATURES - PROSPECTIVE RESULTS FROM THE EUROPEAN MALE AGING STUDY (EMAS)

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Context: Secondary hypogonadism (sHG) is common in ageing men; its natural history and predisposing factors are unclear. **Objectives:** 1) To identify factors which predispose eugonadal men (EUG) (testosterone (T)≥10.5 nmol/L) to develop sHG (T<10.5 nmol/L, LH≤9.4 U/L) and sHG men to recover to EUG. 2) To characterize clinical features associated with these transitions. **Design:** Prospective observational general population cohort survey. **Setting:** Clinical research centers. **Participants:** 3369 community-dwelling men aged 40-79 yr in eight European countries. **Intervention:** Observational follow-up of 4.3 years. **Main Outcome Measure:** Subjects were categorised according to change/no change in gonadal status during follow-up into persistently (p) EUG (n=1946), incident (i) sHG (n=145), psHG (n=133) and recovered (r) from sHG to EUG (n=97). Baseline predictors and changes in clinical features associated with isHG and rsHG were explored. **Results:** The incidence of isHG was 158.2 per 10000 per year, while 39.9% of men with sHG recovered to EUG. Overweight [BMI≥25-<30 kg/m2: odds ratio (OR) 1.96 (95% confidence interval 1.12-3.45);p<0.05], obesity [BMI≥30kg/m2: OR 3.97(2.18-7.23);p<0.0001], weight gain [OR 2.17(1.39-3.41);p=0.001] and lack of a stable relationship [OR 1.97(1.05-3.71);p<0.05] predicted isHG. isHG men developed new or experienced worsening of sexual symptoms [low libido, erectile dysfunction and infrequent spontaneous erections]. Non-obesity [OR 2.01(1.09-3.71);p<0.05], weight loss [OR 2.26(1.06-4.82);p<0.05], younger (<60yr) age [OR 2.24(1.12-4.48);p<0.05], higher education [OR 2.10(1.06-4.15);p<0.05] predicted rsHG, but these men did not show significant improvement of symptoms. **Conclusion:** Obesity-related metabolic and lifestyle factors predispose older men to the increasingly prevalent T decline compatible with sHG, which is frequently reversible with weight loss.

CO28 - SEMINAL PLASMA MIRNAS AS POTENTIAL MARKERS FOR TESTICULAR TUMOURS

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Testicular germ cell tumours (GCTs) are one of the most common tumours in men aged 20 to 35 years. They are classified as seminoma and non-seminoma. The clinical management of GCTs is based on the monitoring of the serum biomarkers αfetoprotein (AFP), β-human chorionic gonadotropin (β-HCG) and lactate dehydrogenase (LDH), which are not always highly specific. The identification of new biomarkers with high sensitivity and specificity is therefore needed to facilitate the large-scale diagnosis and follow-up of GCT patients.

Various microRNAs (miRNAs) from the clusters miR-371-3 and miR-302/367 have recently been suggested as biomarkers for GCT. These have been found to be highly expressed in both the tissue and serum of patients with seminoma and embryonal carcinoma. MiRNAs are small non-coding single-strand RNA molecules containing about 22 nucleotides which regulate various biological processes, such as cell proliferation, stem cell regulation and tumour differentiation, apoptosis and growth. Their presence in various fluids, including serum, plasma, saliva and urine, is proving to be of ever greater diagnostic importance for numerous pathological conditions.

Given that there is a lack of literature evidence on the presence of miRNAs in the seminal plasma of testicular tumour patients, in this study we analysed and compared the miRNA expression profiles in the seminal plasma of 20 pre-orchiectomy GCT patients (16 seminoma and 4 non-), 20 post-orchiectomy GCT patients and 30 cancer-free controls, using the platform TaqMan Array A+B Card 3.0. This system is based on a highly sensitive and specific quantitative PCR (q PCR) analysis and enables investigation of the more than 700 currently known human miRNAs. The results of this array were validated by performing individual assays for the expression of any miRNAs that appeared to be deregulated.

In total, differential expression was observed for 55 miRNAs in pre-orchiectomy patients, including 52 up-regulated and 3 down-regulated miRNAs, in comparison with post-orchiectomy patients and the controls. We selected miR-27a, miR-195, miR-590-3p, miR-142-3p and miR-374, which showed the greatest up-regulation (fold change >10), for real time validation with qRT-PCR. In conclusion, this preliminary study enabled the identification for the first time of new miRNAs in seminal plasma as potential biomarkers for GCT.

CO29 - EXPRESSION OF NICOTINIC RECEPTOR SUBUNITS IN HUMAN SEMEN

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Introduction. The nicotinic receptors (nAChR) are cholinergic and ionotropic consisting of five subunits. In mammals, 16 subunits, named α1, α2, α3, α4, α5, α6, α7, α9, α10, β1, β2, β3, β4, γ, δ, and ε have been identified. The results of a dose-response study, conducted recently by our group, showed that nicotine damages conventional and nonconventional sperm parameters. Subsequently, we evaluated the mechanism through which nicotine damages spermatozoa. The results of this study showed that the effects of nicotine on sperm motility and nonconventional sperm parameters *in vitro* are mediated by the interaction with a specific receptor. In particular, hexamethonium (a competitive antagonist of nicotine) fully antagonized the detrimental effects of nicotine on these parameters. On this basis, the aim of the present study was to evaluate the presence of nAChR in the semen. To accomplish this, we evaluated the expression of its subunits by RT-qPCR. **Methods**. The study was conducted on 3 normozoospermic subjects (age 32.2±5.5 years), non-smokers, without urogenital infection, systemic diseases, microorchidism, cryptorchidism, varicocele, drugs and/or alcohol use-abuse and recent hormonal treatment. We obtained 3 samples for each patient: A: pellet of raw semen after having discarded the seminal plasma; B: pellet after swim-up (total immotile cells) and C: motile sperm. Total RNA (1 μg) was analyzed by RT-qPCR to identify which of the mammalian nAChR subunits were expressed in the three preparations. Total RNA was isolated using TRIzol (Invitrogen, Carlsbad, CA, USA). First strand cDNA was synthesized using High Capacity cDNA Reverse Transcription Kit (Life Technologies). The RT-qPCR was performed with the SYBR® Green PCR Master Mix (Life Technologies) on a StepOne™ according to the manufacturer's recommended protocol (Life Technologies, USA). Each reaction was run in triplicate. Primers were specifically designed to measure the following nAChR subunits (α1, α2, α3, α4, α5, α6, α7, α9, α10, β1, β2, β3, β4, γ, δ, ε). The reference gene (housekeeping) was tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta polypeptide (YWHAZ). **Results.** All samples showed mRNA expression of the 8 following nAChR subunits: α1, α3, α4, α6, α7, β2, β4, and δ. **Conclusion.** This study showed, for the first time, that nAChR subunits are expressed in human spermatozoa. This suggests that nAChR plays a yet unknown role in sperm function.

CO30 - SERUM TESTOSTERONE REDUCTION DURING TARGETED THERAPIES FOR METASTATIC RENAL CELL CARCINOMA.

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Background: Several antiangiogenic therapies have been recently approved for treatment of metastatic renal cell carcinoma (mRCC), targeting the vascular endothelial growth factor axis or the mammalian target of rapamycin pathway. Efficacy of these agents is largely demonstrated, but toxicity profile may lack of exhaustive clinical data. Fatigue is experienced up to 77% of patients (pts) receiving antiangiogenic agents. The primary objective of this study was to assess the variations of serum testosterone levels (TST) and sexual hormone-binding globulin (SHBG) during targeted therapies in mRCC male pts. The secondary objective was to observe androgen, estrogen and progesterone receptor expression in tissue specimens derived from radical nephrectomies (RN).

Methods: We prospectively evaluated serum levels of TST and SHBG, at baseline and after 1 and 3 months of therapy, in 43 eugonadic male pts with mRCC (89% clear cell, 9% papillary, 2% mixed), submitted to Sunitinib (63%), Pazopanib (7%), Sorafenib (16%) and Everolimus (14%), as I line (81%), II line (14%) and III line (5%) treatment. Furthermore, we retrospectively assessed androgen, estrogen and progesterone receptors in 64 tissue microarray specimens of pts submitted to RN.

Results: TST significantly decreased during therapy, (mean values: baseline 316.83 ng/dl; after 1 month 250.82 ng/dl; after 3 months 262.81 ng/dl; p=0.017); SHBG significantly increased (mean values: baseline 30.88 mmol/l; after 1 month 37.41 mmol/l; after 3 months 40.61 mmol/l; p=0.0007). Immunoistochemical analysis of RN revealed androgen receptors in 12/64 (19%) of specimens, progesterone receptors in 1/64 (0.6%) of tissues and no expression of estrogen receptors (0%).

Conclusion: Sunitinib, Pazopanib, Sorafenib, Everolimus caused a statistical significant decrease in TST levels, with simultaneous increase of SHBG, in male pts treated for mRCC. The reduction of TST levels secondary to antiangiogenic treatment may contribute to all those symptoms, including fatigue anorexia, depression and sexual disfunction, often described by pts as a serious adverse event. Moreover androgen receptors expression in RN has to be carefully evaluated when TST replacement therapy is considered.

CO31 - EFFECT OF LIRAGLUTIDE ON PROLIFERATION AND DIFFERETIATION OF HUMAN ADIPOSE CELLS

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The expansion and dysfunction of white adipose tissue (WAT) underlie the development of obesity and other metabolic and cardiovascular complications. Most of the drugs used for the treatment of obesity has not led satisfactory results in terms of stable weight loss. Moreover consistent side effects are associated with some of these drugs, preventing their clinical usage. Using drugs targeting specifically adipose tissue could represent an interesting strategy for the treatment of obesity.

Liraglutide is an analogue of the incretin hormone GLP-1 and is currently used for the treatment of type 2 diabetes, with limited side effects. Weight loss observed with liraglutide in placebo-controlled studies is generally attributed to the anorexant effect of the drug, due to the stimulation of the hypothalamic GLP-1 receptors and to the inhibition of gastric emptying. Therefore, this drug has been recent approved by EMEA with indication for obesity treatment.

In the present study the in vitro effect of liraglutide on a model of adult adipose stem cells (S-ASC) isolated from human subcutaneous adipose tissue (SAT) has been investigated. Liraglutide (10-100nM) significantly inhibited ASC proliferation and viability, with a maximum effect at 6 days of culture (45% and 50% for liraglutide 10 and 100nM respectively). This effect was completely reverted by the GLP1-R inhibitor exendin 9-39. Glucose uptake was significantly reduced by liraglutide in a dose dependent manner. Moreover stimulation with liraglutide reduced intracellular lipid accumulation during in vitro adipose-differentiation. Taqman analysis showed a reduced FABP4 mRNA expression (-18%,-23%,-46% for 1nM, 10nM and 100nM, respectively) whereas an increased adiponectin mRNA expression (1.86-,2.64-,2.28-fold increase, for 1nM,10nM and 100nM, respectively) was shown.

Our study demonstates that liraglutide exerts effects on human adipose cell precursors, suggesting an inhibitory action on proliferation and differentiation and a stimulatory activity in the expression of the insulin-sensitizing adipokine APN. These effects could contribute to the actions of GLP-1 receptor agonists on body weight and insulin sensitivity.

This study was supported by Novo Nordisk, PRIN 2011 and Fondazione Ente Cassa di Risparmio di Pistoia e Pescia

CO32 - MYO-INOISITOL AND D-CHIRO-INOSITOL PREVENT PALMITATE-INDUCED AUTOPHAGY AND SENESCENCE IN HUMAN CARDIAC PROGENITOR CELLS

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Supplementation with myo-inositol and D-chiro-inositol has been shown to induce insulin-mimetic effects in humans and to improve cardiac performance in experimental heart failure. The aim of this study was to evaluate the effects of myo-inositol and D-chiro-inositol on lipotoxicity-induced autophagy and senescence in human cardiac progenitor cells (CPC) isolated from right auricle biopsies of individuals undergoing elective heart surgery. Incubation of CPC with 0.25 mmol/L palmitate for 16 h increased cell autophagy, evidenced by monodansyl cadaverine staining and confirmed by increased levels of microtubule-associated protein 1 light chain 3-II (LC3-II) and beclin-1, respectively ($p<0.05$). Palmitate also induced cellular senescence, demonstrated by increased senescence-associated β-galactosidase activity. Cellular senescence was confirmed by the detection of increased p21(WAF1/Cip1) gene and protein expression ($p<0.05$), whereas the p16(Ink4a) pathway appeared to be unaffected. Chemical inhibition of autophagy with 3-methyladenine resulted in reduced β-galactosidase staining, suggesting that increased autophagy mediates palmitate-induced cellular senescence in human CPC. When cells were pretreated with myo-inositol or D-chiro-inositol (1-1000 micromol/L), both the palmitate-induced autophagosome formation and increase in LC3-II were abrogated ($p<0.05$), and so was the increase in p21 protein levels ($p<0.05$). Increased Akt and p42/p44 MAPK phosphorylation ($p<0.05$) was observed in CPC exposed to myo-inositol or D-chiro-inositol for up to 30 min. In conclusion, palmitate increases both autophagy and senescence in human CPC. Both myo-inositol and D-chiro-inositol activate survival kinases and counteract the effects of palmitate. Inositols may thus protect the myocardium from lipotoxic damage in type 2 diabetic and/or obese subjects with elevated free fatty acid levels by preserving the CPC pool.

CO33 - IS THE TIMING OF CALORIC INTAKE ASSOCIATED WITH VARIATION IN DIET-INDUCED THERMOGENESIS AND IN METABOLIC PATTERN? A RANDOMIZED CROSS-OVER STUDY.

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Background. An increasing number of studies have shown that timing of food intake influences energy regulation and risk of weight gain, independently from total daily caloric intake. Thermic effect of food, or diet-induced thermogenesis (DIT), is the increase in resting metabolic rate (RMR) after a meal, and seems implicated in the development and persistence of obesity. DIT has been hypothesized to be lower after the evening meal, as a consequence of nocturnal insulin resistance. Objectives. We compared calorimetric and metabolic responses to identical meals consumed in the morning (8:00 am) and in the evening (8:00 pm) in healthy volunteers, after standardizing diet, physical activity level, duration of fast and resting. Design. Randomized cross-over trial. Methods. Twenty healthy volunteers (10 , 10) randomly received a standard meal at 8:00 am and after a week at 8:00 pm or vice versa. Eight hours before the meal, volunteers received also the same standard meal at home. From 7:30 to 8:00 am (or pm) a 30' basal calorimetric exam was performed. At 8:00 am (or pm), participants consumed the meal, and from 8:30 am (or pm) they rested in a supine position for 90 min. Then they underwent to a second 60' calorimetric evaluation. We collected blood samples every 30' from 8:00 am (or pm) for Glucose, Insulin, Free Fatty Acids (FFA) and Triglycerides. Results. RMR didn't change from morning to evening (1548.5±260.7 vs 1518.5±236.7 Kcal, p=0.61). After-meal RMR was higher after the morning meal (1916.0±266.1 vs 1755.5±229.7 Kcal, p<0.001); similarly, DIT was higher in the morning (median and interquartile ranges: 315.0 (155.0) vs 260.0 (110.0) Kcal, p=0.004). Basal and post-meal values of respiratory quotients (RQs) were higher in the morning (respectively: 0.87±0.04 vs 0.80±0.05, p<0.001; 0.90±0.03 vs 0.85±0.07, p=0.002). Both before- and after-meal, carbohydrates oxidation was higher (respectively: 0.12 (0.07) vs 0.05 (0.08) g/min, p<0.001; 0.19 (0.04) vs 0.16 (0.16) g/min, p<0.001) and fat oxidation lower (0.02 (0.02) vs 0.05 (0.03), p<0.001; 0.008 (0.02) vs 0.02 (0.03) g/min, p=0.01) in the morning than the evening values. Glucose area-under-the curve (AUC) 0-180' was lower after morning meal (15383.2±2045.3 vs 17183.3±2290.4 mg/dl; p<0.001), as Insulin AUC 0-180' (6912.8 (3185.3) vs 7146.8 (5757.0) µU/ml; p=0.005). FFA concentrations were increased in the evening at time 0, 30 and 60'; FFA AUCs 0-180' values were higher after evening meal (38.7 (24.5) vs 52.6 (22.5) mmol/l; p=0.025). Conclusions. DIT was significantly lower in the evening; contemporarily we found larger and delayed glycemic and insulinemic responses after evening meals, suggesting an increased insulin resistance which could have reduced the thermic

effect of meals. Time of food intake may affect both thermogenic response and metabolic pattern. Timing of meals should be considered when planning a healthy diet.

CO34 - A NOVEL COMBINED GLUCOCORTICOID/MINERALOCORTICOID RECEPTOR SELECTIVE MODULATOR PREVENTS FAT MASS EXPANSION IN MICE FED A HIGH-FAT DIET

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We have previously shown that antagonism of the mineralocorticoid receptor (MR) results in a potent antiadipogenic activity, *in vitro* and *in vivo.* Excessive glucocorticoid exposure is associated with obesity and related disorders in men and mice. In this study responses to a novel combined Glucocorticoid Receptor (GR)/MR antagonist were investigated in a model of diet-induced obesity. Female 10-week-old C57bl6 mice were fed with normal chow or a high fat diet (HFD) for 9 weeks. Mice fed a HFD were concomitantly treated for 9 weeks with the classical GR antagonist mifepristone (80mg/kg/day) or the novel combined GR/MR antagonist CORT-118335 (80mg/kg/day). Mice fed a HFD showed a significant increase in total body weight, white fat mass, mean adipocyte size, expression of white adipose tissue (WAT) markers and showed impaired glucose tolerance. Treatment with CORT-118335 dramatically prevented the HFD-induced weight gain, whereas mifepristone showed neutral effects on body weight and fat mass. Interestingly, food intake was not significantly affected by any treatment, whereas caloric efficiency was dramatically lower in CORT-118335 treated mice, when compared to the HFD group. Both compounds markedly improved glucose tolerance, decreased leptin and increased adiponectin plasma levels compared to the HFD group. Furthermore, both compounds significantly reduced mean adipocyte size and liver steatosis index. When tested *in vitro*, both compounds markedly reduced 3T3-L1 preadipocyte differentiation under steroid-deprived conditions. We then aimed to dissect the mixed GR/MR antagonist activity of CORT-118335 in this cellular model. Interestingly, only the MR-mediated pro-adipogenic effects of aldosterone were antagonized by CORT-118335, whereas GR-mediated effects of dexamethasone were not affected by the compound. This suggests that CORT-118335 mostly acts as an antagonist of MR, rather than GR, in murine cultured preadipocytes. In conclusion, combined pharmacological antagonism of GR and MR markedly reduced HFD-driven fat mass expansion *in vivo*, as well as adipose differentiation *in vitro*, suggesting that both receptors represent strategic targets to fight obesity. However, the effects of CORT-118335 at adipose tissue level are mostly mediated by MR antagonism.

CO35 - IGF-I RECEPTOR EXPRESSION, TRAFFICKING, AND DOWNSTREAM SIGNALING IS REGULATED BY DISCOIDIN DOMAIN RECEPTOR 1

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Background: The type 1 insulin-like growth factor receptor (IGF-IR) is a receptor tyrosine kinase activated by binding of its ligands, IGF1 and IGF2. It is expressed in almost every cell and plays an important role in normal organogenesis and differentiation of many tissues. Disruption of the *Igf1r* gene in mice causes growth retardation and results in perinatal lethality due to organ hypoplasia. Discoidin domain receptor 1 (DDR1) is a transmembrane tyrosine kinase, which is activated by collagen binding. It is widely expressed during development and in adult tissues where it controls cell adhesion, migration, proliferation and remodelling of the extracellular matrix. In spite of observations indicating that IGF-IR and DDRs are important regulators of growth, cell adhesion and migration, a cross-talk between DDR1 and IGF-IR has not been previously reported. **Aims:** To investigate the possibility that DDR1 functionally interacts with IGF-IR and modulates IGF-I biological effects. **Materials and methods:** we employed 3T3-like mouse fibroblasts, R^- cells, which express low levels of DDR1 and lack endogenous IGF-IR. Cells were transfected with IGF-IR and DDR1 constructs to create stable clones. DDR1/IGF-IR association was investigated by immunoprecipitation and proximity ligation assay which allows quantification and localization of protein-to-protein interactions with single molecule resolution in cells. IGF-IR protein and mRNA expression was studied by western blot and real-time PCR, respectively. The functional relevance of DDR1/IGF-IR crosstalk was evaluated by signaling studies (western blot) and by the measurement of biological effects in response to IGF-I. **Results:** We found that DDR1 associates with IGF-IR in basal conditions and that this interaction increases after IGF-I stimulation with a maximum at 5 min. IGF-I induced DDR1 internalization and tyrosine phosphorylation. Overexpression of DDR1 increased IGF-IR protein expression at a post-translational level and both basal and ligand-activated IGF-IR downstream signaling. As a consequence, DDR1 enhanced IGF-I – dependent biological effects, including cell proliferation and migration. These effects were independent of the presence of collagen, but required a functional IGF-IR. **Conclusions:** These results indicate that DDR1 exerts a scaffolding role for IGF-IR signalsome that may play a role in a variety of physiological and pathological conditions.

CO36 - REDUCED SIRT1 AND SIRT2 EXPRESSION IN VISCERAL ADIPOSE STEM CELLS MAY PROMOTE VISCERAL FAT EXPANSION IN HUMAN OBESITY.

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Adipose tissue expands as a consequence of hypertrophy of preexisting fat cells and hyperplasia by recruitment of adipose stem cells (ASC) into the adipogenic program. SIRT1 and SIRT2 have been involved in regulation of adipocyte differentiation in rodent cells. In this study, we investigated the role of SIRT1 and SIRT2 in human adipocyte differentiation in obesity. Subcutaneous (Sc)- and visceral (V)-ASC were isolated from fat biopsies of 30 obese (Ob) and 30 non-obese (n-Ob) donors and differentiated in vitro. Cell cultures were analyzed by immunofluorescence with Nile Red/DAPI to quantify triglyceride-containing cells (adipogenesis) and Oil-Red-O staining followed by spectrophotometry to measure accumulated triglycerides (lipogenesis). No differences in either adipogenesis or lipogenesis were found in Sc-ASC from Ob vs. n-Ob donors. By contrast, both the number of triglyceride-containing cells and extent of triglyceride accumulation were ~2.0-fold higher in V-ASC from Ob compared to n-Ob donors ($p<0.05$). These cellular phenotypes correlated with changes in SIRT1 and SIRT2 mRNA and protein levels, which were not different in Sc-ASC from n-Ob and Ob, and 50% lower in V-ASC from Ob compared to n-Ob ($p<0.05$) subjects. Noteworthy, the selective overexpression of SIRT1 or SIRT2 in Ob V-ASC by adenoviral-mediated gene transfer resulted in downregulation of PPARγ, SREBP1c and C/EBPalpha ($p<0.05$), and genes marking terminal adipocytes differentiation, including FAS and adiponectin ($p<0.05$), and in a significant 45% decrease in both lipogenesis and adipogenesis ($p<0.05$). Finally, assessment of SIRT1 and SIRT2 mRNA levels in human Sc and V fat biopsies from 149 donors with a wide BMI range showed a significant negative relationship between SIRT1/SIRT2 expression in V fat and BMI, both in males ($R = -0.448$, $p<0.05$) and females ($R = -0.535$, $p<0.05$), while no correlation was found between SIRT1/SIRT2 expression in Sc fat and BMI. Thus, reduced SIRT1 and SIRT2 expression in V-ASC in human obesity may promote enhanced lipogenesis and adipogenesis leading to expansion of V fat.

CO37 - PREVALENCE OF HYPOPHYSITIS IN A COHORT OF PATIENTS TREATED WITH IPILIMUMAB

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Ipilimumab is a humanized monoclonal antibody directed against CTLA-4 an inibitory receptor expressed on the membrane of activated T cells and regulatory T lymphocytes,that is approved for the treatment of metastatic melanoma patients.Blocking CTLA-4 by ipilimumab sustains T cell activation thereby enhancing anti-tumor immune response, but possibly resulting in the development of autoreactive phenomena such as autoimmune thyroiditis and lymphocytic hypophysitis. Ipilimumab-induced hypophysitis is an inflammatory disease of the pituitary similar to the autoimmune form,with an incidence up to 17%.

Aim of the study was to characterize all cases of hypophysitis occurred in a cohort of patients treated with Ipilimumab from 2006 to 2014 at our University Hospital

The study group included 220 patients who received ipilimumab as part of clinical trials (at doses of 3 mg/Kg or 10 mg/Kg) or within an Expanded Access Program at 3 mg/Kg: 196 patients had metastatic melanoma, 17 metastatic prostate cancer and 7 metastatic non-small cell lung cancer. Of the 220 patients treated, to date 174 received ipilimumab as proven by the exit from the blind phase, 6 for metastatic prostate cancer and 168 for metastatic melanoma.

Hypophysitis was diagnosed in 6/174 patients (3%; 3 females; 3 pts at dose of 3 mg/kg and 3 at dose of 10 mg/Kg) after a median of 14 weeks (range 6-19 weeks); 5 patients had metastatic melanoma and one metastatic prostate cancer. The most common presenting symptoms were sudden onset of fatigue, headache and general discomfort. Biochemical tests showed the presence of secondary hypocorticism in 5 patients, gonadotropin deficiency in 4 and TSH deficiency in 4. During follow-up TSH and gonadropin deficiency recovered in all but one patient with a permanent secondary hypothyroidism. Adrenal insufficiency did not recover. All but one patients performed pituitary MRI that showed signs consistent with hypophysitis in 3 patients and no abnormalities in 2 patients. Two patients died: one for unknown reasons and one for disease progression.

Hypophysitis represents a rare but potentially life-threatining complication of ipilimumab treatment, therefore a multidisciplinary management is crucial to prevent severe complications. In light of this, the endocrine evaluation should be routinely done in each patient during treatment with ipilimumab.

CO38 - CAMP ANALOGUES AS A POTENTIAL ANTITUMOR STRATEGY FOR MEDULLARY THYROID CANCER

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The oncogenic activation of the RET protooncogene has a main role in the pathogenesis of medullary thyroid cancer (MTC), a neuroendocrine tumor arising from the calcitonin (CT)-producing parafollicular C cells of the thyroid and highly resistant to chemo- and radiotherapy. Several lines of evidence suggest that RET function could be influenced by cyclic AMP (cAMP)-dependent protein kinase A (PKA) activity. We evaluated the in vitro antitumor activity of 8-Cl-cAMP and PKA type I selective cAMP analogues (equimolar combination of the 8-PIP-cAMP and 8-HA-cAMP) in MTC cell lines (TT and MZ-CRC-1). After 6 days of incubation, both 8-Cl-cAMP and PKA type I selective analogues (8-PIP-cAMP plus 8-HA-cAMP) significantly inhibited the growth of TT and MZ-CRC-1 cell lines in a dose-dependent manner. This antiproliferative effect appeared to be due to cell cycle modulation and, only for 8-Cl-cAMP, induction of apoptosis in both cell lines. In addition we evaluated if the antitumor activity of these compounds could be mediated by the modulation of PKA and mitogenic ERK-dependent pathways, two key signalling cascades involved in MTC cell growth. After 6 days of incubation 8-Cl-cAMP did not modify the expressions of type I regulatory subunits of PKA in both TT and MZ-CRC-1 cells, while PKA type I selective cAMP analogues induced a statistically significant but weak increase of RIIb subunit of PKA only in TT cells (evaluated by Western blot analysis using isoform-specific antibodies). On the other hand, the exposure of MTC cells to either 8-Cl-cAMP or PKA type I selective cAMP analogues for 6 days had no effects on the intracellular activation of ERK1-2 in TT cells, while we found a significant and strong decrease of the activity of ERK in MZ-CRC-1 cells after the treatment with cAMP analogues (evaluated by the immunodetection of the phosphorylated isoforms of ERK1-2). In conclusion, we provide for the first time experimental evidence that cAMP signalling cascades seem to play a critical role in the control of MTC cell growth. On these bases, 8-Cl-cAMP and the PKA I-selective cAMP analogues may have a potential antitumoral role in the treatment of MTC.

CO39 - PREVALENCE AND DEGREE OF LIVER STEATOSIS IN CUSHING'S DISEASE: ROLE OF METABOLIC SYNDROME AND CORTISOL OVEREXPOSURE

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Cushing's disease (CD) is an endocrine disorder characterized by endogenous hypercortisolism with metabolic and cardiovascular complications. Liver steatosis (LS), a clinical feature of disease, represents a probable consequence of the metabolic syndrome. The direct role of cortisol excess in determining LS has never been definitely established. The aim of the current study was to evaluate the prevalence and degree of LS in patients with CD during active disease and after disease remission and to correlate them with disease severity and duration. 32 female patients (pts), 25-65 yrs, with CD [16 with active CD (ACD, disease duration: 1-10 years) and 16 after disease remission (RCD, remission duration: 1-15 years) entered the study. LS was evaluated by two different methods: ultrasound visualization of fatty liver degree (0-3 grade on the basis of liver brightness compared with renal brightness) and biochemical method represented by the calculation of fatty liver index (FLI), through an algorithm including body mass index (BMI), waist circumference (WC), triglycerides (TG), liver enzyme (GGT). Clinical [BMI, WC, blood pressure and heart rate], metabolic [fasting glucose, insulin, HbA1c, total (TC), HDL (HDL-C), LDL (LDL-C) cholesterol and liver enzymes], and hormonal [plasma ACTH, serum cortisol and urinary free cortisol (UFC)] parameters were evaluated in all pts, together with VAI, an indirect index of visceral adipose function, proposed as a useful tool for early detection of cardiometabolic risk [WC/36.58+(1.89xBMI)] x (trigl/0.81) x (1.52/HDL), and HOMA-IR (HI), index of insulin resistance (fasting glucose (mg/dl) x insulin (mU/L)/405). LS prevalence was 87.5% in ACD and 43.8% in RCD [grade 1 (37,5% ACD; 25% RCD); grade 2 (25% ACD, 12,5% RCD); grade 3 (25% ACD, 6,2% RCD]. ACD had increased BMI ($p=0.008$), WC ($p=0.023$), fasting insulin ($p=0.018$), and HI ($p=0.047$), FLI ($p=0.012$) as well as greater LS prevalence ($p=0.012$) and score ($p=0.005$), compared with RCD. A significant correlation has been found between FLI and HI or VAI in all pts with CD ($p<0.001$; $p=0.001$)), as well as in ACD ($p=0.032$, $p=0.034$) and RCD ($p<0.001$, $p=0.025$); HI is the most predictive parameter of FLI ($t=3,48$; $p<0.001$). Conversely, no significant correlation was found between FLI and cortisol levels, disease or remission duration. Moreover, a trend to a significant correlation was registered between LS grade and HI ($p=0.067$) in ACD, and between LS grade and VAI ($p=0.03$) in RCD. In conclusion, CD is associated with high prevalence of LS, which significantly reduced after disease remission together with an improvement of metabolic syndrome. Moreover, LS seems to be more a indirect consequence of metabolic syndrome with a negligible direct role of cortisol overexposure.

CO40 - EFFECTIVENESS OF PASIREOTIDE TREATMENT IN PATIENTS WITH CUSHING'S DISEASE: A NATIONAL EXPERIENCE BASED ON CLINICAL PRACTICE

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A recent phase III clinical trial has demonstrated that the treatment with the somatostatin analogue pasireotide normalizes cortisol secretion in 15-28% of patients with Cushing's disease (CD). No data are presently available on the outcome of pasireotide treatment when used in the daily clinical practice. The aim of the current study was to evaluate the effectiveness of 6-months pasireotide treatment on clinical and hormonal profiles in a group of CD patients with mild to moderate disease. Twenty-seven patients with CD unsuccessfully treated by surgery and with persistently increased urinary cortisol (UC) levels started treatment with pasireotide at the dose of 600 mg bid. UC, plasma ACTH and serum cortisol levels were measured every three months together with clinical and metabolic parameters. Three patients discontinued pasireotide treatment after 2-4 weeks for gastrointestinal disturbances (2), or death (1, unrelated to the drug); among the remaining 24 patients, 15 with mild (12) or moderate (3) UC increase reached 6-months follow-up, and were considered for the study. After 6-months pasireotide treatment, UC levels were normalized or nearly normalized in 10 out of 15 (66.7%) patients. A significant decrease of UC ($p=0.01$) and a trend to a significant decrease in plasma ACTH levels was demonstrated in the entire cohort of CD patients. The decrease of UC levels was accompanied by a significant decrease in weight ($p=0.002$) and body mass index ($p=0.005$) and a trend to a significant decrease in waist circumference ($p=0.09$). Fasting plasma glucose ($p=0.004$) and glycosylated haemoglobin ($p=0.004$) levels increased significantly. Hyperglycaemia or deterioration of diabetes was documented in 41% whereas gastrointestinal disturbances, mainly diarrhoea, were documented in 30% of patients during the period of pasireotide treatment. In conclusion, the use of pasireotide in the management of mild or moderate CD during clinical practice induces normalization of UC in nearly 70% of patients, with consequent improvement in the clinical picture but with occurrence or deterioration of diabetes, or gastrointestinal disturbances in 30-40% of cases. These results confirmed the usefulness of pasireotide in controlling CD especially in patients with mild disease.

CO41 - GROWTH HORMONE-RELEASING HORMONE (GHRH) INHIBITS TNF-ALFA-INDUCED APOPTOSIS AND ATROPHY IN C2C12 MYOTUBES

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Skeletal muscle atrophy, or wasting, is a debilitating consequence of different chronic diseases, including cancer, heart failure and diabetes, and also occurs in aging, starvation and genetic myopathies. Muscle atrophy results from an imbalance between anabolic and catabolic processes and it is primarily caused by hyperactivation of the main cellular degradation pathways. Cytokines, such as tumor necrosis factor-alpha (TNF-α), are implicated in the pathogenesis of muscle wasting and have been found to be elevated in such condition. The hypothalamic hormone growth hormone-releasing hormone (GHRH), besides stimulating GH secretion from the pituitary, exerts survival and proliferative effects in different cell types. We and others have recently shown that GHRH displays prevent apoptosis in isolated cardiac myocytes and protects the isolated heart from ischemia/reperfusion injury and from myocardial infarction in vivo. In the present study, we investigated GHRH effect on survival and apoptosis of TNF-α-treated C2C12 myotubes and the underlying signaling pathways. GHRH increased survival and prevented apoptosis of TNF-α-treated cells through GHRH receptor-mediated mechanisms. These effects involved activation of PI3K/Akt and inactivation of GSK-3β, whereas mTOR signaling was unchanged. GHRH also increased the expression of myogenin heavy chain, a major functional protein of adult skeletal muscle, that was reduced by the cytokine. Importantly, GHRH inhibited TNF-α-induced expression of NF-kB, calpain and MuRF1, which are all involved in muscle protein degradation. Collectively, these results indicate that GHRH counteracts TNF-α-induced muscle atrophy, by promoting survival and inhibiting apoptosis of myotubes through induction of anabolic routes and inhibition of proteolytic pathways. Thus, GHRH may represent as a novel therapeutic candidate in chronic diseases and/or pathological conditions that lead to muscle atrophy.

CO42 - LONG-TERM ECHOCARDIOGRAPHIC AND CARDIOSCINTIGRAPHIC EFFECTS OF GH TREATMENT IN ADULTS WITH PRADER-WILLI SYNDROME.

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Prader-Willi Syndrome (PWS) is a genetic disorder characterized by severe obesity, multiple complications and altered GH secretion leading to cardiovascular abnormalities and premature death. GH therapy acts favorably on many dysfunctions of PWS, yet its long-term cardiovascular outcome is unknown. To address these key issue, 9 obese adults with PWS (3F/6M, age 26.2±3.1 yr, BMI 44.9±4.7 kg/m2) underwent an open-label prospective study of metabolic parameters, body composition and cardiac function by echocardiography and radionuclide angiography during a 4 yr GH treatment. At baseline, PWS were matched with 13 obese controls (6F/7M, age 26.2±3.1 yr, BMI 43.0±4.9 kg/m2).

GH treatment increased IGF-I ($p<0.0001$), decreased CRP ($p<0.05$), did not modify glucose or lipid homeostasis while decreasing visceral fat ($p<0.05$), and near-significantly improved fat and fat-free body mass in PWS patients. By echocardiography, left ventricle (LV) mass indexed by fat mass was lower than in controls and increased significantly after GH therapy ($p<0.05$), although LV ejection fraction showed a trend toward reduction during the study. Radionuclide angiography revealed stable values of LV and RV ejection fraction under GH therapy, yet LV filling rate tended to decrease during the intervention time-frame. A positive association between lean body mass and LV ejection fraction, and a negative association between BMI and diastolic function were evident ($p<0.05$ for both).

In this first study ever performed on long-term cardio-metabolic effects of GH therapy in PWS adults, we found that GH therapy increased cardiac mass without causing overt abnormalities of systolic and diastolic function. The observed subtle longitudinal modifications of functional parameters advocate appropriate cardiac monitoring in the long-term therapeutic strategy for PWS.

CO43 - HIGH PREVALENCE OF SERUM ANTI-THYROID HORMONE ANTIBODIES (THAB) AND THAB-ASSOCIATED RISK FOR THYROXINE TREATMENT IN PATIENTS WITH POLYGLANDULAR AUTOIMMUNE SYNDROME (PAS-III)

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THAb are autoantibodies directed against epitopes containing the iodinated tyrosines of thyroglobulin (Tg), and that bind T3, T4 or both T3 and T4. The prevalence of THAb is 0-2% in the general population, but up to 30% in autoimmune thyroid diseases (AITD) and/or in autoimmune nonthyroidal diseases (AINTD). The coexistence of AITD with AINTD is widely recognized, and it is provisionally classified as PAS-III. There are no data on THAb prevalence in PAS-III. Serum THAb of the IgM or IgG class were assayed using a radioimmunoprecipitation method employing ^{125}I-T4 or ^{125}I-T3 and anti-human IgM or IgG antisera. We analyzed 103 sera of patients (87F/16M;15-82years) presenting Hashimoto's thyroiditis plus one of these AINTD: chronic atrophic gastritis [CAG,n=64(group 1), non-segmental vitiligo [NSV,n=24(group 2)], celiac disease [CD,n=15(group 3)]. The presence of at least one THAb among the four possible types (T3-IgM, T3-IgG, T4-IgM, T4-IgG) was detected in 45/103 patients (43.7%), namely 45.3% (29/64), 45.8% (11/24) or 33.3% (5/15) of group 1, 2 or 3 patients, respectively. Of these 45, 27 (60.0%), 15 (33.3%) and 3 (6.7%) had selective T3, selective T4 or dual T3+T4 binding. Based on hormone binding the total number of THAb was 52, and in 39 cases just one THAb was detected. Indeed, THAb of both the IgM and IgG class were found in three patients and were restricted to T3; in another three, dual hormone specificity was observed. Peculiar observations were: a) group-3 patients had only T4-THAb (T4-IgM, n=4; T4-IgG, n=1); b) almost half of the group-1 THAb$^+$ patients (13/29= 44.8%) had an additional associated AINTD; c) in these last 13 patients, no case of T3 IgG positivity was detected; d) the rate of patients who required L-T4 treatment was greater in the THAb$^+$-patients (34/45) as compared with the remaining THAb$^-$-patients (31/58)(75.6% vs 53.5%; p=0.025); however, no difference in the daily T4 dose was recorded among these two subgroups of patients. We conclude that at least one type of serum THAb is detectable in almost half of patients with PAS-III, being more prevalent in those with CAG or NSV. Hormone specificity and Ig class of THAb are variably distributed in the three groups of PAS-III patients, with a peculiar antiT4-restricted pattern in the CD group only. Prospectively, THAb positivity may be helpful to predict a greater likelihood of: (i.) developing an additional AINTD in patients with

HT+CAG; (ii.) requiring L-T4 treatment in PAS-III patients.

CO44 - DIFFERENTAL TYPE 2 AND 3 DEIODINASES EXPRESSION IN NORMAL INTESTINE AND COLON CANCERS.

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~~Intestinal homeostasis results from complex cross-regulation of signaling pathways; their alteration induces intestinal tumorigenesis. The Wnt pathway plays a critical role in tumor development, by regulating cell proliferation, differentiation, survival and steamness. Nearly all colon tumors present a deregulated beta-catenin signalling pathway by mutation of either APC or beta-catenin, which leads to the blockade of phosphorylation by GSK-3beta, resulting in beta-catenin stabilization. Previously, we found that the deiodinase D3 is positively regulated by the Wnt pathway, modulating via thyroid hormone inactivation, the proliferation of the crypt cells and promoting tumorigenesis. Vice versa, D2 is inhibited by the Wnt pathway and by increasing thyroid hormone action, induces cell differentiation. In the present study we investigated on the D2-D3 expression in gut tumorigenesis and in normal intestine. We dissected the roles of deiodinases D2 and D3 in colon homeostasis, with particular emphasis on the stem cells compartment, and the colorectal carcinogenesis. To address the localization of D2 protein, we took advantage of a D2-3xFlag knock-in mouse line recently generated in our lab. In normal intestine, D2 was expressed mostly in the intestine villi, and also in the few stem cells localized in the basal part of the crypt. Conversely, D3 was expressed in the transit amplifyng proliferative intestinal cells migrating toward the villi. Furthermore, by using the spontaneously forming colon carcinomas APCmin mice, we were able to discover that D3 is highly expressed in the tumoral epithelial and its localization coincides with the upper layer of the tumors. Vice versa, D2 is mostly expressed in the central portion of the tumors, indicating a differential function of the two deiodinases in the colon carcinogenesis.

In conclusion, our observations strongly suggest that i) D3 expression correlates with sustained β-catenin transcriptional activity in normal intestine, while D2 is localized in the terminal differentiating intestinal cells, and, ii) D2 and D3 are both expressed in colon carcinomas, but their expression is restricted to different areas of the tumors, indicating a likely differential function in colon carcinogenesis. Together, these data suggest a novel mechanism for the tumor-promoting activity of the thyroid hormone metabolism.

This new concept may be extended to other organs and have biological relevance in therapeutic approaches aimed to target stem cells such as tissue engineering and cancer.

CO45 - CARBOHYDRATE ANTIGEN 19.9 (CA 19.9): A PROGNOSTIC FACTOR FOR MORTALITY IN PATIENTS WITH PERSISTENT/RECURRENT STRUCTURAL MEDULLARY THYROID CANCER (MTC).

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Important information about the outcome of MTC patients can derived from serum calcitonin (CT) and Carcinoembryonic Antigen (CEA) levels and, in particular, from their doubling time (Dt). Recently, we described an aggressive MTC in a man, who rapidly died from the disease and had serum Ca19.9 positivity without any gastrointestinal malignancy. This case arose the question of whether Ca19.9 can be a prognostic marker for mortality in MTC.

In order to evaluate the prognostic significance for mortality of serum Ca19.9 positivity in MTC patients, we measured serum Ca19.9, CT and CEA in a series of 100 MTC patients with persistent/recurrent structural disease. We calculated the CT Dt and we assessed the correlation between Ca 19.9 positivity and CT levels, CEA levels and the CT Dt. As control, Ca 19.9 was measured in 66 MTC patients without structural disease (cured or with biochemical disease patients).

Sixteen/100 (16%) MTC patients showed Ca19.9 positivity (mean: 988 U/ml); the mean serum CT and CEA values were 7,466 pg/ml and 1,534 U/ml, respectively. Positive linear correlations between Ca 19.9 and CT (Spearman's rho= 0.21; p= 0.04) and CEA (rho= 0.245; p= 0.01) were found. In the group of 84 Ca19.9 negative patients, the mean serum CT and CEA values were 1,934 pg/ml and 160 U/ml, respectively: both this mean values were significantly lower than the values found in Ca 19.9 positive patients ($p<0.0001$ for both CT and CEA). Moreover, in the Ca 19.9 negative group, the patients (n=20; 23.8%) who died were significantly less than those in the group with Ca 19.9 positivity (n= 11; p=0.0004). A logistic regression analysis demonstrated that Ca 19.9 positivity, when present, is an important predictor for mortality (OR=3.78, p=0.04), independent from CT Dt. All 66 patients in MTC control group without structural disease were Ca 19.9 negative.

In conclusion: 1) a subgroup of MTC patients with persistent/recurrent structural disease has a serum Ca 19.9 positivity; 2) Ca 19.9 positivity is related to higher CT e CEA levels; 3) positive linear correlations between CT and CEA values with Ca 19.9 values were found; 4) in Ca 19.9 positive patients, the mortality rate was higher; 5) Ca 19.9 positivity seems to be a significant predictor for mortality, independent from CT Dt; 6) Ca 19.9 was not found in MTC patients without structural disease.

CO46 - AGE AND DOSE ARE MAJOR RISK FACTORS FOR LIVER DAMAGE ASSOCIATED WITH INTRAVENOUS GLUCOCORTICOID PULSE THERAPY IN PATIENTS WITH GRAVES' ORBITOPATHY

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High dose, intravenous (iv) glucocorticoid (GC) (ivGC) pulse therapy, is used in several autoimmune diseases and also in Graves' Orbitopathy (GO) and has been associated with acute liver damage (ALD), resulting in a fatal outcome in a few cases. No certain risk factors for ALD have been established, because of which here we performed a large retrospective cohort study.

We assessed the relationship between ALD and several potential risk factors in 1,076 consecutive patients (287 males, 789 females; age 48·8±11·7 yr, range 17-79 yr) with GO given ivGC from January 1st 1998 to December 31st 2012, in our GO Clinic

The risk factors evaluated were: gender; age; cumulative MPA dose; MPA dose per infusion; obesity; BMI; clinically overt previous liver diseases; previous exposure to HBV or HCV; nonorganspecific autoantibodies known to be associated with autoimmune hepatitis; administration of oral prednisone after and during ivGC; liver steatosis; diabetes; hypertension; hyperlipemia; total cholesterol values; and, triglycerides values. ALD was defined as an increase of ALT ≥300 U/l.

Fourteen cases of ALD were recorded, resulting in a 1•3% morbidity. Thirteen patients recovered and one died, resulting in a 0•09% mortality. There was a significant, positive correlation of ALD with age and methylprednisolone acetate (MPA) cumulative dose, and ALD was more common (RR=3•9; P=0•02) in patients aged ≥53 yr (10/420=2•38%) than in those aged <53 yr (4/656=0•6%). In patients aged ≥53 yr there was a significant positive correlation of ALD with MPA cumulative dose, and also with MPA dose per infusion. Thus, the frequency of ALD in this age group was greater (RR 5•35; P=0•01) in patients with an MPA dose per infusion ≥0•67 gr (7/128, 5•46% vs 3/292, 1•02%). Regardless of age, no cases of ALD were observed for MPA doses per infusion <0•57 gr.

Age and MPA dose are significant risk factors for ALD, with the following practical implications: i) the total MPA cumulative dose should not exceed 8·5 gr (the average dose in patients without ALD); ii) in patients aged ≥53 yr age selection and observation should be quite strict; and iii) MPA dose should not exceed 0•57 gr per infusion, a measure to be applied regardless of age.

CO47 - THE PATHOGENESIS OF CONGENITAL HYPOTHYROIDISM : HIGHLY FREQUENT MULTIGENIC INVOLVEMENT REVEALED BY NEXT GENERATION SEQUENCING (NGS)

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Congenital hypothyroidism (CH) is the most common (3-6 cases / 10,000 live births) endocrine disorder that affects infants from birth, results from a partial or complete loss of thyroid function and is a leading cause of mental retardation in children. CH occurs when the thyroid gland fails to develop or function properly. In 80-85% of cases, the thyroid gland is absent, abnormally located, or hypoplastic. In the remaining cases, a normal-sized gland is present, but production of thyroid hormones is decreased or absent. The pathogenesis of CH is still undetermined in most of affected patients. Several candidate genes have been associated with both dysgenetic or functional thyroid defects but their involvement has so far been evaluated in cohorts of patients selected according to the clinical phenotype (eg, dyshormonogenic vs dysgenetic forms, or isolated vs syndromic CH). By Following this approach, variants in these candidate genes are present in a share of less than 10% of patients evaluated. In this study, we report a new approach based on systematic analysis of data generated by the processing of genomic DNA according to protocol NGS (Next Generation Sequencing) of 177 patients with CH, and confirmed through automated Sanger sequencing, of a panel of 11 candidate genes known (NKX2.1, PAX8, FOXE1, GLIS3, JAG1, TSHR, SLC26A4, TG, TPO, DUOX2, DUOXA2). In contrast with current knowledge, only 68/177 patients (38.4%) had no rare (defined as Minor Allele Frequency, MAF<0.01) or novel non-synonymous variants in these candidate genes, whereas carriers of at least 1 rare/novel variant were 63/177 (35.6%), and 2 or more novel/rare variants on the same gene or different genes were detected in 46/177 (26%) of the patients. The genes most frequently involved by rare variants / new non-synonymous are TG (n = 34) and DUOX2 (n = 31). Interestingly, FOXE1 variants were identified in three CH cases with gland, apparently normal, in situ (GIS) and no other midline defect, while DUOX2 or TG or SLC26A4 variants were found in dysgenetic forms. These data indicate that, in contrast with current understanding, variations in the currently known candidate genes can account for the CH pathogenesis in a large number of affected patients. Furthermore, the systematic approach NGS reveals a highly frequent multigenic origin of CH, comprising unexpected variations in genes that would have been excluded a priori from the screening, on the basis of the phenotypic expression.

Partially supported by the research fund: RF-2010-2309484

CO48 - GRAVES' DISEASE (GD) PHENOTYPE: IS SOMETHING CHANGING? EVALUATION OF A LARGE COHORT OF NEWLY DIAGNOSED CONSECUTIVE GRAVES' PATIENTS SEEN AT A SINGLE CENTER.

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In most endocrinology textbooks GD phenotype is characterized by the so-called "Merseburg triad": hyperthyroidism in 100% of cases, goiter in about 80-90% and Graves' Orbitopathy (GO) in 50%. GO is the most common extrathyroidal manifestation of GD, but we recently reported that the prevalence of GO in Graves' patients at diagnosis is lower (about 25%) than previously reported (Tanda ML et al. JCEM 2013). Aim of this study was to evaluate the prevalence of goiter in a large cohort of 265 consecutive patients with newly diagnosed Graves' disease (203 women and 62 men, mean age 48.2 years) seen at our Institution over a 5-year period (2010-2014). The presence or absence of goiter was assumed by ultrasonography, the upper normal estimated volume being 14 ml in women and 18 ml in men. Goiter was defined as small if within 1.5-times larger than normal, moderate if >1.5 and <2.5-times, large if >2.5-times. All patients were hyperthyroid; 132 patients (50%) had no goiter, 71 (27%) had a small goiter, 42 (16%) had moderate goiter and only 20 (7%) had a large goiter. Thyroid volume was significantly associated with serum FT4 and FT3 levels (FT4 $p < 0.0001$; FT3 $p = 0.01$). TSH-receptor antibody (TRAb) tests were positive in all patients at diagnosis and serum TRAb levels were higher in patients with moderate and large goiter than in patients with small or no goiter ($p = 0.003$). At diagnosis, only 60 patients (22.7%) had GO and this was mild in most cases. The prevalence of GO was significantly ($p = 0.04$) higher in patients with a moderate or large goiter than in those with small or no goiter. In conclusion, it seems that the phenotype of newly diagnosed Graves' disease has changed, because the majority of patients has no goiter, no (or mild) GO and milder degree of hyperthyroidism.

CO49 - NITRIC OXIDE SIGNALING AND EPIGENETIC MODIFICATION IN A MOUSE MODEL OF DIABETIC CARDIOMYOPATHY

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Diabetic cardiomyopathy, as major complication of diabetes mellitus, is a multifactorial disorder caused by combination of hyperglicemia, inflammation, calcium handling as well as oxidative stress in which reduction of nitric oxide (NO) bio-availability impairs both endothelial and cardiac function. NO is a potent epigenetic regulator of the activity of histone deacetylases (HDACs) and acetylases (HATs). Here, we investigated the involvement of NO signaling in controlling chromatin remodeling enzymes, including HDACs and HATs, in a mouse model of diabetic cardiomyopathy. Exposure to high glucose (HG, 30mM) for 72 hours of the mouse cardiomyocyte cell line HL1 induced the following changes: *i.* increase in class I and III HDACs, specifically HDAC1, 2 and Sirtuin1 but not class II HDACs (HDAC5); *ii*. increase in histone acetylase p300 protein levels and HAT enzymatic activity; *iii*. increase in tri-methylation of lysine (K) 27 of histone H3 (H3K27me3) paralleled by a reduction in acetylation of K9 of H3 (H3K9Ac). To explore the effect of the reduced availability of NO in diabetes, gene expression analysis was performed in HL1 exposed to HG in the presence or absence of NO donor (DETA/NO, 100microM). Protein-coding gene profiling by Cardiotoxicity qPCR array revealed a specific HG-induction of biomarkers of cardiac-damage involved in remodeling and fibrosis including Nexilin, Versican, Crem and Adra2. In parallel, non-protein coding gene profiling by Long non-coding qPCR array (LncRNAs) revealed a cluster of 18 lncRNAs differentially expressed in HL1 cells exposed to HG compared to control. Of note, HG-dependent induction of Nexilin, Versican, Crem and Adra2 as well as the LncRNAs FOXn2-as, MALAT1, SNHG3, SNHG6, Gtl2-as, H19 antisense, mHOTAIR, lincECN1 was counteracted by DETA/NO suggesting a direct link between NO pathway and transcriptional regulation of genes associated to cardiac injury.

In vivo, diabetic cardiomyopathy was induced in CD1 mice by streptozotocin (STZ) injection. Molecular analyses were performed at 1, 3 and 6 months (6M) after STZ treatment. Cardiac dysfunction was assessed by Azan-Mallory staining and induction of A- and B-type natriuretic peptide mRNA. In the heart of STZ-6M mice, induction of H3K27me3 and HATs activity as well as reduction of H3K9Ac was detected. In addition, HDAC2 and the signature of cardiotoxicity-associated genes, similar to HG-HL1, was induced at mRNA level. Confocal microscopy confirmed that nexilin was upregulated and delocalized suggesting destabilization of cardiac Z-disk.

In conclusion, this study reveals that NO contrasts the effect of HG possibly via HDACs and HATs activation and gene expression control in development of diabetic cardiomyopathy

CO50 - HIGH ADIPONECTIN LEVELS AND LOW GLOMERULAR FILTRATION RATE ARE INDEPENDENT PREDICTORS OF ALL-CAUSE MORTALITY IN PATIENTS WITH TYPE 2 DIABETES

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Both serum adiponectin and glomerular filtration rate (GFR) are known predictors of mortality risk in patients with type 2 diabetes (T2D). Because of the strong association between these two variables, it is not known whether their roles on mortality rate are interwoven or, in contrast, independent from each other. Answering this question was the aim of the present study.

The associations between serum adiponectin and eGFR (by Modification of Diet in Renal Disease equation) levels with all-cause mortality were investigated in 1,212 patients with T2D from two different Italian samples: 847 patients from the Gargano Mortality Study (GMS; follow-up=7.6±2.1 years; 145 events) and 365 patients from the Foggia Mortality Study (FMS; follow-up=7.3±1.4 years; 78 events).

At baseline, serum adiponectin was inversely associated with eGFR in GMS, in FMS as well as in the two studies combined (p values ranging 0.001 - <0.0001).

For each SD increase of adiponectin or eGFR, the HRs (95% CI) for all-cause mortality were 1.46 (1.29-1.66) or 0.53 (0.44-0.64) ($p<0.0001$ for both) in GMS, 1.50 (1.26-1.79) or 0.58 (0.46-0.74) ($p<0.0001$ for both) in FMS and 1.49 (1.34-1.64) or 0.54 (0.46-0.63) ($p<0.0001$ for both) in the two studies combined. In the combined sample, conditional analysis (i.e. a model comprising both adiponectin and eGFR) showed that the above reported associations with all-cause mortality remained significant ($p<0.0001$ for both adiponectin and eGFR). Results did not change when sex, smoking habits, BMI, diabetes duration, HbA1c, anti-diabetic, anti-hypertensive and anti-dyslipidemic treatments were added to the model ($p<0.0001$ for both).

This is the first study in a large sample of patients with T2D reporting that, despite adiponectin and GFR are strongly associated each other, they have an independent predicting role on all-cause mortality, thus suggesting that the biology linking high adiponectin levels and kidney dysfunction with mortality rate in diabetic patients is different. Further studies are needed to unravel such biological links.

CO51 - PROPOSED OF A NEW SIMPLIFIED VISCERAL ADIPOSITY INDEX (VAI-2).

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Our research group proposed in 2010 the Visceral Adiposity Index (VAI) as an indicator of adipose distribution and function. To date, according to the engine search Scopus this index has been cited in 81 papers, in the majority of which it is concluded that VAI could be proposed as a useful tool for early detection of a condition of cardiometabolic risk, before the latter develops into an overt metabolic syndrome. However, there are some studies in which is questioned the usefulness of the VAI, arguing that the formula is complex and that the index does not have a predictive power greater than the single parameters that compose it.

To explore the association of a simplified VAI (VAI-2) with diabetes, hypertension, cerebro- and cardiovascular events, we conducted a cross-sectional study on 1477 Primary Care Patients of the AlkaMeSy Study.

VAI-2 has been simplified by replacing the first part of the VAI (model of adipose distribution: MOAD) with WC/80 (in women) and WC/94 (in men); VAI-2 comprises only the variables WC, Triglycerides and HDL cholesterol.

At a logistic regression analysis VAI and VAI-2 (adjusted for age, smoking and LDL cholesterol at the time of the event), showed a significant independent association with diabetes [(OR 1.39, IC95%: 1.14-1.70, p=0.001) and (OR 1.36, IC95%: 1.14-1.63, p=0.001), respectively], hypertension [(OR 1.47, IC95%: 1.24-1.75, p<0.001) and (OR 1.56, IC95%: 1.32-1.84, p<0.001), respectively], angina or myocardial infarction [(OR 1.92, IC95%: 1.46-2.53, p<0.001) and (OR 1.78, IC95%: 1.39-2.29, p<0.001), respectively], TIA or ischemic stroke [(OR 1.75, IC95%: 1.37-2.24, p<0.001) and (OR 1.57, IC95%: 1.25-1.96, p<0.001), respectively]. Through a Roc analysis we also identified the respective cutoff of VAI and VAI-2 that are associated with the presence of diabetes (1.66 and 1.68), hypertension (1.48 and 1.49), angina or myocardial infarction (1.86 and 2.08), TIA or ischemic stroke (1.57 and 1.75). No significant differences in c-statistic between VAI and VAI-2 was found for all endpoints studied.

Our study shows that VAI-2 has the same predictive value of VAI, therefore given the simplicity of the formula, can be considered a viable alternative in the evaluation of the visceral adipose function and of the its associated cardiometabolic risk.

CO52 - STAT3 INHIBITION INDUCES PCSK9 IN HEPATOCYTES, POTENTIALLY LINKING HYPERTRIGLYCERIDEMIA AND INSULIN RESISTANCE

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PCSK9 (Proprotein Convertase Subtilisin Kexin Type 9) regulates the circulating low-density lipoprotein cholesterol (LDL) levels by inducing LDL receptor (LDLR) degradation. However, PCSK9 levels are positively correlated with insulin resistance, liver steatosis and plasma very low density lipoprotein-triglycerides (VLDL-TG) concentrations. This evidence suggests that PCSK9 could also be implicated in lipid homeostasis. Obesity is characterized by low-grade chronic inflammation, elevated circulating cytokines, and hepatic overexpression of suppressor of cytokine signaling (SOCS) proteins, altogether major determinants of hypertriglyceridemia associated to insulin resistance. In the present study, we have investigated the role of SOCS3 in the transcriptional regulation of PCSK9 in HepG2 cell line. As expected, forced overexpression of SOCS3 determined a complete abrogation of STAT3 phosphorylation, associated to: 1) induction of fatty-acid synthase mRNA levels (3.59±0.40 fold); 2) activation of SREBP transcriptional activity (1.58±0.15 fold); 3) increase apoB production (3.47±0.09 fold). SOCS3 overexpression also determined a significant increase of PCSK9 mRNA (7.82±1.73 fold) and protein, determined by Western blot analysis and ELISA assay (2.18±1.13 fold) without significant changes of HMG-CoA reductase and LDLR levels as well as of cholesterol biosynthesis. Pharmacological inhibition of STAT3 or JAK proteins also induced PCSK9 levels by 2.06±0.75 and 3.30±0.3 fold, respectively. Interestingly, insulin significantly induced STAT3 phosphorylation, fatty-acid synthase and PCSK9 mRNA levels to a similar extent in both control and SOCS3-overexpressing cells, although the overall mRNA levels of PCSK9 and fatty-acid synthase were significantly higher in HepG2 SOCS3 cells. In conclusion, we provide evidence that biological and/or pharmacological inhibition of the JAK/STAT pathway increased PCSK9 levels, both in the absence and in the presence of insulin stimulation, a possible determinant of PCSK9 transcription in insulin resistant patients.

CO53 - A NOVEL FUNCTIONAL CROSS-TALK BETWEEN THE IGF-I RECEPTOR AND DISCOIDIN DOMAIN RECEPTOR 1 IN BREAST CANCER CELLS

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Background: The insulin-like growth factor-I receptor (IGF-IR) plays a key role in regulating mammalian development and growth by binding IGF-I and IGF-II with high affinity. IGF-IR and its ligands are frequently deregulated in cancer and may contribute to cancer progression in dependence to microenvironmental cues. However, several phase II/III trials of anti-IGF-IR therapies have shown limited efficacy. The mechanisms of resistance to IGF-IR therapies and the clinically applicable strategies for overcoming drug resistance are still undefined. Discoidin domain receptor 1 (DDR1) is a collagen receptor tyrosine kinase, which is activated by collagen binding. It is also frequently overexpressed in cancer and implicated in cancer progression and metastasis. Previously, in transfected fibroblasts, we found that IGF-IR functionally interacts with DDR1. **Aims:** We investigated whether DDR1-IGF-IR crosstalk may play a pro-oncogenic role in breast cancer cells and whether it could represent a valuable therapeutic target. **Materials and methods:** We employed human breast cancer cells lines, characterized by different IGF-IR and DDR1 expression profiles. We evaluated the functional impact of DDR1 overexpression and silencing on IGF-IR signaling and trafficking, and IGF-I dependent biological effects. **Results:** In human breast cancer cells we found that DDR1 constitutively associated with the IGF-IR. This interaction was enhanced by IGF-I stimulation, which promoted rapid DDR1 tyrosine-phosphorylation and co-internalization with the IGF-IR. Cell fractionation studies confirmed that IGF-IR and DDR1 constitutively associate in cell membrane and cytosolic fractions, and that IGF-I stimulation increases this association. Significantly, DDR1 was critical for IGF-IR endocytosis and trafficking into early endosomes, IGF-IR protein expression and IGF-I intracellular signaling and biological effects. In particular, cell proliferation, migration and colony formation were inhibited by DDR1 silencing and enhanced by DDR1 overexpression. Moreover, we found that a recently described specific DDR1 tyrosine kinase inhibitor (DDR1-IN-1) partially inhibited both basal and IGF-I stimulated proliferation in MCF-7 cells. **Conclusions:** These results suggest that DDR1 enhances IGF-IR - dependent protumoral effects in breast cancer cells. Therefore, the DDR1 – IGF-IR cross-talk could be considered a new valuable target for cancers resistant anti IGF-IR therapies.

CO54 - INTERACTION BETWEEN COMMON GENETIC VARIABILITY OF OSTEOPROTEGERIN (OPG), BETA CELL FUNCTION AND SUBCLINICAL ATHEROSCLEROSIS IN PATIENTS WITH NEWLY DIAGNOSED TYPE 2 DIABETES.

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Background: Osteoprotegerin (OPG), a member of the tumor necrosis factor receptor superfamily, is a modulator of the RANKL/RANK system in bone metabolism and it has been implicated in vascular calcification of patients with type 2 diabetes (T2DM). *Aim*: To explore the potential connections between OPG, insulin secretion/sensitivity and subclinical atherosclerosis in patients with newly diagnosed T2DM (VNDS; NCT01526720). *Methods:* In 793 GAD-negative patients with newly diagnosed T2DM (mean±SEM age: 58.8±0.41 years; BMI: 30.0±0.22 kg/m^2; FPG: 7.17±0.08 mmol/L; HbA_{1c}: 6.86±0.05%) we: 1. genotyped 12 tag SNPs reportedly covering 95% of OPG common variability: 2. assessed metabolic phenotypes: insulin sensitivity (SI) by the euglycemic insulin clamp, derivative (DC) and proportional control (PC) of beta cell function by state-of-art modelling of glucose/C-peptide curves during OGTT; 3. assessed subclinical atherosclerosis phenotypes by US-doppler scan of carotid and peripheral arteries of lower limb (N=554) and by standard electrocardiogram (ECG; N=554). All analyses were adjusted for age, sex, smoking. *Results*: Both minor alleles of two *OPG* tag-SNPs rs4355801 (A) and rs2073618 (G) showed a defective PC of beta cell function ($p<0.05$ or less), under a dominant model. We then computed an *OPG* score: the higher was the rs4355801-rs2073618 genotype score, the lower was PC ($p=0.003$). The minor allele of rs4355801, but not rs2073618, was associated with less severe atherosclerosis in peripheral arteries ($p=0.03$). *Conclusions*: Our data highlight that in Italian patients with newly diagnosed T2D genetic variability of OPG may be implicated in both beta cell (dys)function and atherosclerosis.

CO55 - SCLEROSTIN SERUM LEVELS IN HYPOGONADAL MEN AND EVIDENCE OF ANDROGEN EFFECT ON HUMAN OSTEOCYTES

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Osteocytes are the most abundant cells in bone, accounting nearly the 95% of all cells in the adult skeleton. The osteocyte network plays a central role in directing bone response either to mechanical loading or to unloading, leading correspondingly to bone formation or resorption. Sclerostin (SOST) is a glycoprotein integral to osteocyte function as a signal to damp the action of osteoblast bone deposition through the negative modulation of Wnt-signaling pathway. Any agent targeting the osteocyte functions and SOST release will have a major impact on the bone-remodeling process, such as osteoporosis. Steroid sex-hormones are major determinants of bone mass and hypogonadal men are at increased risk of osteoporosis, but the possible effect of androgens on osteocyte function is unknown.

The aim of this study was to investigate the possible action of androgens on human osteocytes in vitro and in vivo.

The expression of the androgen receptor (AR) on both decalcified human trabecular bone samples and human primary osteocyte cultures by double-immunofluorescence showed colocalization of AR on SOST-positive osteocytes. This expression was also confirmed by western blot analysis. To evaluate the functional state of AR in osteocytes, we assessed the eventual nucleus translocation of AR signal after DHT stimulation. As expected, we observed that in osteocytes stimulated with DHT, AR staining was detected as diffuse nuclear spots and negligible signal in the cytoplasm, wheras the staining for AR was mainly cytoplasmatic and peri-nuclear in the control sample. Incubation of cultured osteocytes with DHT led to a statistically significant decrease of SOST protein levels in the cell pellet (P=0,01 and P<0,001). These data were also confirmed by RT-PCR experiments, which showed a decreased expression of SOST after stimulation with DHT (P=0,005). Serum SOST levels were determined in 25 young hypogonadal men (HP) and in 58 age-matched eugonadal controls (CTRL). Serum SOST was significantly higher in HP with respect to CTRL (138,02 ± 36,2 pg/mL vs 100,3 ± 33,0 pg/mL; P=0,05), Within HP subjects, serum T levels were negatively correlated with SOST levels (R2=0,312; P= 0,02).

Taken together, these data suggest for the first time a direct role of androgens on SOST release by osteocytes in a AR-dependent manner and increased SOST levels in hypogonadal men. Studies are ongoing to identify the signalling pathways and cell process associated to androgen stimulation in this cell population.

CO56 - LOW EXTRACELLULAR SODIUM PROMOTES ADIPOGENIC COMMITMENT OF HUMAN MESENCHYMAL STROMAL CELLS: A NOVEL MECHANISM FOR CHRONIC HYPONATREMIA-INDUCED BONE LOSS

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Hyponatremia represents an independent risk factor for osteoporosis and fractures, affecting both bone mineral density and quality. The mechanisms through which this occurs are not yet completely understood, but a direct stimulation of osteoclastogenesis and bone resorptive activity in the presence of reduced extracellular sodium concentrations ($[Na^+]$) has been shown. However, to date, the effects of reduced $[Na^+]$ on osteoblasts activity and bone formation have not been investigated. The aim of this study was to investigate the effects of a chronic reduction of extracellular $[Na^+]$, independently of osmotic stress, on human mesenchymal stromal cells (hMSC) obtained from bone marrow, which represent the common progenitor for osteoblasts and adipocytes. We first investigated the effect of low extracellular $[Na^+]$ on hMSC homeostasis and we found a significant inhibition of hMSC adhesion and viability, but not an alteration of the surface antigen expression profile and of the immune-modulatory properties. We subsequently demonstrated that low extracellular $[Na^+]$ were able to modulate the production by osteoblasts of factors (MCP-1 and CXCL-12) that stimulate osteoclast recruitment and bone resorption. Next, we tested whether chronic hyponatremia was able to alter the cellular commitment of bone marrow hMSC. We found that the exposure to reduced $[Na^+]$ did not affect hMSC ability to commit toward the osteogenic and the adipogenic lineages, as demonstrated by the unaltered gene expression of specific markers at the end of each differentiation. However, the dose-dependent increase in the number of adipocytes as a function of reduced extracellular $[Na^+]$, observed by Oil-Red-O staining, suggested a preferential commitment toward the adipogenic phenotype at the expense of osteogenesis. This observation was further supported by the amplification of the inhibitory effect on the expression of osteoblastic markers exerted by adipocyte-conditioned media in the presence of low $[Na^+]$. Finally, the analysis of cytoskeleton remodelling by immunofluorescent microscopy showed that low $[Na^+]$ were associated with an evident disruption of tubulin organization in hMSC-derived osteoblasts, thus indicating a negative effect on bone quality. These results add new evidence suggesting that hyponatremia should be carefully taken into account by clinicians because of its negative effects on bone, in addition to the known neurological effects, and indicate for the first time that impaired osteogenesis may be involved.

CO57 - ROLE OF MEN1 GENE LARGE DELETIONS IN MULTIPLE ENDOCRINE NEOPLASIA TYPE 1 SYNDROME

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MEN1 is an autosomal dominant disorder characterized by tumours in multiple endocrine glands, most commonly parathyroid, enteropancreatic and anterior pituitary glands. To date, germline mutations in the *MEN1* gene have been identified in about 70–80% and 30% in familial and sporadic MEN1 cases, respectively. The *MEN1* gene exons spans 9 kb of the genome encoding the menin protein with tumor suppressor function. More of 2/3 of *MEN1* mutations, spread across the coding region, lead to a truncated protein, confirming a loss-of-function mechanism. A lack of a genotype-phenotype correlation has been observed. Approximately 20–30% of MEN1 patients do not have *MEN1* mutations detected with conventional mutation screening methods. Up to date 12 large germline *MEN1* deletions have been reported in unrelated MEN1 probands, accounting for around 3% of MEN1 cases analyzed.

The aim of this work was to evaluate if the *MEN1* mutation detection rate can be increased by the occurrence of large monoallelic *MEN1* gene deletions. We used Multiplex Multiplex Ligation-dependent Probe Amplification (MLPA) assay to identify gene copy number variations in a group of 25 *MEN1* index cases (7 familial and 18 sporadic) negative for *MEN1* mutations on direct sequencing. Our initial cohort consisted of 54 MEN1 probands, 33 of whom with a family history of the disease. At *MEN1* sequencing analysis we found 29 germline mutations (54%), 26 of whom occurring in familial cases (79%). 72% of all detected mutations were frameshift, nonsense or affecting splice site junctions. MLPA experiments were performed on DNA obtained by patients' blood samples, using the SALSA MLPA probemix kit P244-B1 (MRCHolland), according to the manufacturer's instructions.

We found four *MEN1* large deletions; a deletion spanning the whole gene in 2 kindreds, one encompassing the 5'UTR region, exons 1 and 2 in one sporadic case, and a deletion of exons 9-10 in a familial case.

We established that in our study alteration of the *MEN1* gene can explain around 90% of MEN1 familial cases of Italian origin, since *MEN1* large deletions account for 10% of all germline *MEN1* mutations in MEN1 kindreds. We didn't observe any association between the type of mutations and the clinical characteristics of the disease. We recommend to include MLPA assay in the routine analysis for *MEN1* mutations, especially in families without detected germline mutations, as this type of large *MEN1* deletions were detected in 40% of *MEN1* mutation negative cases.

CO58 - INCIDENCE OF CARDIOVASCULAR EVENTS IN PRIMARY HYPERPARATHYROIDISM AT FOLLOW-UP: THE ROLE OF PARATHYROIDECTOMY.

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Increased cardiovascular mortality in patients with primary hyperparathyroidism (PHPT) is well established from existing evidence, though overall improvement in cardiovascular events has not been observed after parathyroidectomy.

The present study evaluated the mortality and the incidence of cardiovascular diseases (CVD) during a long term follow-up [68 (37-122) months] in a large series of PHPT patients (n=652, males 168, aged 59.9±0.5), comparing surgically treated with successful parathyroidectomy (PTX) *vs* clinically monitored (CM) PHPT patients. CVD were classified as major (coronaropathy, stroke, transient ischemic attack) and minor events, due to atherosclerosis and cardiac remodeling (arrhythmopathy, cardiac valvulopathy and cerebral vasculopathy). Of the 652 PHPT patients diagnosed from 1996 to 2014, data of follow-up were available from 386 patients (103/283; M/F). Clinical features of the PHPT patients with a significant follow up were similar to those of the whole PHPT series. Data were collected by a structured phone interview (287 PTX and 99 CM patients). Median follow-up did not differ between PTX and CM. At the time of diagnosis, PTX patients were younger than CM (57.5 ±0.7 vs 67.4 ±1.0, PTX vs CM; $P<0.01$) and presented a more severe biochemical PHPT, with higher calcium levels.

The overall incidence of major CV events in PHPT were higher (5%) than Italian healthy population (2.5%, data from Studio Cuore), confirming the higher cardiovascular risk of PHPT. Mortality rate at follow-up was low and similar between PTX and CM patients (1.7% vs 3%). The incidence of major cardiovascular events was similar in the two groups (5% vs 6%, PTX vs CM). Interestingly, we observed an increased risk of developing minor cardiovascular events in clinically monitored patients compared to PTX patients, with an adjusted hazard ratios (HR) of 3.6 (1.17-11.57, P=0.03) at 54 months. In conclusion, 1) Parathyroidectomy seemed to reduce the risk of occurrence of minor CV events but not the incidence of major events 2) Large, multicentre, long-term intervention studies would be required to prove definitive reduction in the cardiovascular risk following parathyroidectomy in PHPT.

CO59 - THE EMBRYONIC STEM CELL MICRORNA MIR372 IS ABERRANTLY EXPRESSED IN PARATHYROID TUMORS.

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We investigated the involvement in parathyroid tumorigenesis of the miR-371-373 cluster, that has been previously detected in two-thirds of parathyroid carcinomas (PCas) concomitantly with the aberrant expression of the microRNA belonging to the close cluster C19MC. miR-372 is known to be highly expressed in human embryonic stem cells and downregulated upon differentiation. Moreover, miR-372 promotes human somatic cell reprogramming. Here we reported that miR-372 was expressed at high levels (more than 4 folds) in 48 out of 79 parathyroid tumors compared with the expression levels in normal parathyroid glands (n=5). miR-372 was expressed in more than 75% of both atypical parathyroid adenomas (PAds; n=18; 5.9±0.8 folds, P=0.003) and PCas (n=15; 5.1±1.1 folds, P=0.01) at levels significantly higher than those in typical PAds (n=46; 2.1±0.7 folds). miR-372 overexpression in HEK293 and MCF7 cells significantly downregulated mRNA and protein levels of the cyclin inhibitor kinase CDKN1A/p21 and of the large tumor suppressor kinase 2 (LATS2). *CDKN1A* and *LATS2* mRNA levels were significantly downregulated by the miR-372 overexpression also in typical PAds-derived cells. miR-372 overexpression attenuated the Go/G1 checkpoint in the cell cycle, reducing the proportion of cells in the Go/G1 phase from 70 to 59%. Moreover, miR-372 has been related with the "stemness" Wnt/ß-catenin signaling pathways. We found that miR-372 levels were positively correlated with the nuclear b-catenin accumulation, investigated by western blot, and *AXIN2* mRNA levels in 16 typical PAds. Nonetheless, in typical PAds-derived cells (n=6) miR-372 was not affected by lithium chloride-induced nuclear ß-catenin accumulation.

In conclusion, 1) miR372 was aberrantly expressed in a subset of parathyroid tumors; 2) atypical PAds and PCas expressed miR-372 more frequently and at higher levels than typical PAds; 3) *CDKN1A/p21* and *LATS2* were targets of the miR-372 inhibitory effect in PAds-derived cells; 4) miR-372 aberrant expression might impair Wnt/ß-catenin pathway in parathyroid tumor cells.

CO60 - EFFECT OF GNAS TRANSCRIPT MANIPULATION ON HUMAN MESENCHIMAL STEM CELLS DIFFERENTIATION TOWARDS OSTEOCYTE CELL LINEAGE: INSIGHT INTO THE PATHOPHYSIOLOGY OF ECTOPIC OSSIFICATION IN GNAS-RELATED DISORDERS

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Epi/genetic defects at the imprinted GNAS locus, that encodes the α subunit of the stimulatory G protein (Gsα), have been associated with a heterogeneous group of rare diseases, termed as Pseudohypoparathyroidism.

Most GNAS-based disorders have the common feature of episodic de novo formation of heterotopic ossifications (HO) in subcutaneous tissues. The mosaic tissue distribution of HO suggests that pathogenesis involves an abnormal differentiation of precursor cells, and that GNAS defects provide a sensitized background, but the molecular mechanism is still under investigation.

To date, investigations have been mainly conducted in mouse models, thus we have developed an in vitro human model from surgically removed samples of subcutaneous fat (adipose-derived mesenchymal stem cells, ADMSCs) to study the determinants underlying HO formation in GNAS-related diseases. The multilineage differentiation ability of ADMSCs was confirmed via biochemical assays, specific stainings and expression analysis of specific markers. We also determined the preservation of GNAS imprinting profile during cell culture and handling. Subsequently, cells were treated with siRNAs against Gsα-specific or non-specific GNAS transcripts, the efficiency of transfection (ranging from about 75% at day 4, up to about 50% at day 21) was evaluated, as well as the effect on cell differentiation toward osteoblasts. No significant differences due to different siRNA target sequences were observed.

The increase of alkaline phosphatase activity reflected cell maturation and non-induced silenced cells displayed an intermediate phenotype between cells grown in control and inducing medium (Abs 405nm at day 14: silenced=0.507±0.16, control=0.076±0.01, induced=0.861±0.13, $p<0.05$). Moreover, Gsα-deficient cells, although not able to completely differentiate and depose mineralized matrix, began to lay down few, interspersed and very small calcium deposits.

Future experiments will expand our knowledge in molecular mechanisms underlying HO formation in GNAS-related disorders, in order to test pharmacological treatments against HO formation and progression.

CO61 - THE DPP-4 ENZYME CORRELATES WITH PLASMA TRIGLYCERIDES IN NON-OBESE SUBJECTS AND MEDIATES PALMITATE-INDUCED APOPTOSIS IN HUMAN CARDIAC PROGENITOR CELLS

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Hypertriglyceridemia is an independent risk factor for cardiovascular disease, and chronic exposure to elevated saturated fatty acids (SFA) leads to cardiomyocyte dysfunction and death, both in vivo and in vitro. Circulating levels of dipeptidyl peptidase-4 (DPP-4) have been shown to correlate with cardiovascular disease in humans. Thus, we investigated the potential link between plasma DPP-4 levels, triglyceridemia and the activation of apoptotic pathways in human cardiac progenitor cells (CPC). Plasma DPP-4 levels, evaluated with an ELISA assay in 20 non-diabetic subjects (M/F 9/11, age 47±11 y, BMI 33±9, triglycerides 135±99 mg/dL), were found to positively correlate with BMI ($r = 0.485$, $p<0.05$). In addition, DPP-4 levels were significantly and positively associated with plasma triglycerides in non-obese ($r = 0.847$, $p<0.05$) but not in obese ($r = 0.02$) individuals. Then, primary cultures of CPC were obtained from right auricle biopsies of patients undergoing elective heart surgery. When CPC were exposed to the SFA palmitate (0.25 mmol/L, up to 16 h), DPP-4 levels and activity in the culture medium were found to be increased 2- to 4-fold ($p<0.05$), and this was associated with increased phosphorylation of the stress kinase JNK and the JNK substrate c-Jun ($p<0.05$). In addition, exogenous DPP-4 (up to 500 ng/ml) activated the JNK pathway, inducing a 2.5 fold increase in c-Jun phosphorylation ($p<0.05$). Finally, palmitate augmented CPC apoptosis, evaluated by measuring cytoplasmic oligosomes and Caspase-3 activation, ($p<0,05$), but DPP-4 silencing with a specific siRNA prevented CPC apoptosis, as well as c-Jun phosphorylation ($p<0.05$). In conclusion, the DPP-4 enzyme correlates with plasma triglycerides in vivo in non-obese subjects, is released from CPCs in response to palmitate in vitro, and contributes to SFA-triggered stress signals leading to cardiac cell dysfunction and death.

CO62 - EVIDENCE OF BETA CELL DEDIFFERENTIATION IN HUMAN TYPE 2 DIABETES

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Diabetes is associated with a deficit of insulin-producing beta cells. Animal studies show that beta cells become dedifferentiated in diabetes, reverting to a progenitor-like stage, and partly converting to other endocrine cell types. To determine whether similar processes occur in human type 2 diabetes, we surveyed pancreatic islets from 15 diabetic and 15 non-diabetic organ donors. We scored dedifferentiation, by immunohistochemistry, using markers of endocrine lineage (Synaptophysin and Chromogranin A), beta cell-specific transcription factors (NKX6.1, FOXO1, MafA), and a newly identified endocrine progenitor cell marker, aldehyde dehydrogenase 1A3 (ALDH1A3). Dedifferentiated cells, defined as Synaptophysin-positive/hormone-negative cells, accounted for 31.9% of b cells in type 2 diabetics *vs*. 8.7% in controls ($p<0.001$). The expression and localization of transcription factors required for maintenance of beta cells in rodents (FOXO1, NKX6.1, and MAFA) were altered in 84% of insulin positive cells in diabetics ($p<1x10^{-5}$) and associated with the expression of the progenitor cell marker ALDH1A3. Interestingly, insulin secretion in response to glucose was inversely correlated with the dedifferentiation score ($r=0.55$, $p<0.05$). Moreover, beta cell-specific transcription factors were ectopically found in glucagon- and somatostatin-producing cells of diabetic subjects. The data support the view that pancreatic beta cells become dedifferentiated and convert to alpha- and delta-“like” cells in human type 2 diabetes.

We envision dedifferentiation as a mechanism to protect beta cells from apoptosis by stealth, preserving them for re-differentiation under more favorable metabolic conditions. The findings should prompt a reassessment of goals in the prevention and treatment of b cell dysfunction.

CO63 - SCLEROSTIN AND INSULIN RESISTANCE IN PREDIABETES. EVIDENCE OF A CROSS-TALK BETWEEN BONE AND GLUCOSE METABOLISM

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Objective: A gene mutation of Wnt/β-catenin signaling cascade is present in rare patients with the insulin resistance syndrome. Sclerostin is a circulating peptide inhibiting Wnt/β-catenin signaling. Our aims were to evaluate serum sclerostin in prediabetic subjects and to analyze its relationship with insulin-resistance and β-cell function.

Research Design and Methods: We performed a cross-sectional study including 43 healthy normal glucose tolerant (NGT) individuals and 79 individuals with impaired glucose regulation (IGR) undergoing OGTT. A subgroup (n=18 with NGT and n=30 with IGR) underwent a euglycemic hyperinsulinemic clamp with tritiated glucose.

Results: Sclerostin levels were higher in IGR as compared to NGT (50.8±2.4 vs. 38.7±2.3 pmol/l; p=0.01), positively correlated with HOMA-IR (r = 0.62; p<0.001) and negatively with insulin-mediated total body glucose disposal (r =- 0.40; p<0.001). Fasting endogenous glucose production (EGP), hepatic and adipose tissue insulin resistance indexes were positively correlated with sclerostin levels (r = 0.48 and r = 0.62; r = 0.61; p<0.001). Fasting and OGTT insulin clearance were inversely correlated with sclerostin serum levels (r = -0.52 and r= -0.44; respectively; both p<0.001). Sclerostin levels were not correlated with β-cell function parameters. In multiple linear regression analysis, sclerostin levels improved the prediction of HOMA-IR and insulin-mediated total body glucose disposal by 29 and 13 % (p<0.05).

Conclusions: Sclerostin levels are increased in prediabetic individuals and correlated with insulin-resistance in skeletal muscle, liver and adipose tissue. These data suggest that sclerostin might play a role in determining insulin resistance possibly acting on insulin clearance and degradation.

CO64 - EX VIVO ISLET SIZE AND TRANS-DIFFERENTIATION CORRELATE WITH IN VIVO BETA-CELL GLUCOSE SENSITIVITY IN HUMANS

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Neogenesis from duct cells, increased islet size and trans-differentiation of α cells to β cells likely represent compensatory mechanisms to cope with the increased insulin demand in insulin resistance states. To correlate in-vivo insulin secretion (IS) and sensitivity with ex-vivo islet characteristics, 18 non-diabetic patients (10 F/8 M, 51±15 yrs., BMI 27.9±5.3 kg/m2) scheduled for pancreatoduodenectomy underwent a 2-h hyperglycemic clamp and a hyperinsulinemic euglycemic clamp. β cell glucose sensitivity (GS) during the hyperglycemic clamp was calculated as the ratio of insulin secretion and glucose increments. Pancreas samples were collected during surgery for IHC for glucagon, insulin and somatostatin+ cells to assess islet morphology. Neogenesis was evaluated by the quantification of scattered islets and ductal cells by CK19 immunostaining and trans-differentiation of α to β cell was quantified by counting the percentage of insulin and glucagon double positive cells. When subjects were classified as insulin sensitive or resistant (using the median glucose uptake during the euglycemic clamp [4.9 mg•kg-1•min-2] as a cut-off), insulin resistant subjects displayed a significantly higher basal IS (127±23.7 vs. 91.7±6.78 pmol•min-1•m-2, p=0.04), while both the incremental 1st phase IS (226±82.7 vs. 510±99.8 pmol•min-1•m-2, p=0.04) and β cell glucose sensitivity (47.7±10.5 vs. 91.5±17.4 pmol•min-1•m-2•mM-1, p=0.03) were significantly lower. Analysis of the entire group revealed an inverse correlation between islet size and β cell glucose sensitivity (r= -0.49; p<0.05) and glucose uptake, and between β cell glucose sensitivity and percentage of double+ cells (r=-0.72; p=0.03). No correlation was found between neogenesis markers and β cell glucose sensitivity. Our data suggest that insulin resistance determines alterations in islet morphology and β cell function, with a strict correlation between function and morphology. Impaired β cell glucose sensitivity might represent a major stimulus for the induction of trans-differentiation of α to β cells and increased islet size.

CO65 - GLUCAGON-LIKE PEPTIDE-1 (GLP-1)-RECEPTOR INDEPENDENT EFFECTS OF DIPEPTYDYL PEPTIDASE-4 (DPP-4) INHIBITION IN ANGIOGENIC CELLS AND MACROPHAGE POLARIZATION

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Background: DPP-4 inhibitors (DPP-4i) represent a valid strategy to enhance GLP-1 mediated β-cell insulin secretion in diabetes. DPP-4, which is expressed as soluble form and as membrane-bound protease (CD26), selectively cleaves a variety of substrates. DPP-4i may mediate vascular protection and anti-inflammatory effects independently of GLP-1 action. Circulating angiogenic cells (CACs) and pro-inflammatory macrophages (M1), represent key cell populations involved in the atherosclerotic process as they participate in the reparative and pro-inflammatory responses, respectively. The effects of DPP-4 inhibition in these two cell populations are currently unknown.**Aim:** to evaluate GLP-1 receptor-independent effects of DPP-4 inhibition in primary human CACs and M1. **Methods:** Circulating mononuclear cells were isolated from healthy donors' buffy coats and cultured in media enriched with specific growth factors, EGM-2 bullet kit (Lonza) and IFNγ+LPS, to obtain CACs and M1, respectively. CACs and M1 were incubated for 24h with DPP-4i (KR 62436, Sigma Aldrich), at concentrations selected on the basis of concentration-response curves of DPP-4 inhibition, and with Exendin 9-39, to achieve GLP-1 receptor blockade. Apoptosis and function of CACs were assessed by caspase 3/7 activation and fibronectin adhesion assays, respectively. Pro-inflammatory mediator (IL1β, IL8, IL6, COX2, SOD2 SOCS1, MCP-1 and TNFα) gene expression was measured by qPCR and protein production confirmed by ELISA assay. **Results:** DPP-4i dose-dependently reduced DPP-4 activity. At 2μM of DPP-4i, there was a significant gradient of inhibition in soluble (-70%) vs membrane-bound (-50%) DPP-4 ($p<0.05$). In CACs, DPP-4i decreased staurosporine-induced apoptosis by 25% and improved their adhesive capacity by 50% (both $p<0.05$). In M1, pro-inflammatory gene (IL1β, IL6, COX2, SOCS1, and TNFα $p<0.05$ or lower) and protein (TNFα and IL6; $p<0.001$) expression in M1 were reduced by 40%-70%. The molecular pathways mediating these effects in CACs and M1 are currently under investigation. **Conclusions:** These results suggest that DPP-4 inhibition *per se* improves CAC resistance to apoptosis and adhesive capacity and curbs the M1 inflammatory response, thereby potentially relenting, or even stopping, atherogenesis, independently of glucose control and of GLP-1 receptor activation.

CO66 - NONALCOHOLIC FATTY LIVER DISEASE IS INDEPENDENTLY ASSOCIATED WITH EARLY LEFT VENTRICULAR DIASTOLIC DYSFUNCTION IN PATIENTS WITH TYPE 2 DIABETES

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Backgorund & Aims: accumulating evidence suggests that non-alcoholic fatty liver disease (NAFLD) is associated with left ventricular diastolic dysfunction (LVDD) in nondiabetic individuals. To date, there are very limited data on this topic in people with type 2 diabetes and it remains still uncertain whether NAFLD is independently associated with LVDD in this patient population.

Methods: we performed a liver ultrasonography and trans-thoracic echocardiography (with speckle-tracking strain analysis) in 222 consecutive outpatients with type 2 diabetes without a history of ischemic heart disease, chronic heart failure, valvular heart disease, and known hepatic diseases.

Results: patients with NAFLD (*n*=158; 71.2%) were more likely to be male, overweight/obese, and had longer duration of diabetes, higher hemoglobin A1c and lower estimated glomerular filtration rate (eGFR) than those without NAFLD. They also had significantly (*P*<0.05 or less) higher values of E/e' ratio (9.6±2 *vs*. 6.9±2), Tau (51.3±12 *vs*. 37.5±12 ms), LV end-diastolic pressure (17.7±1.8 *vs*. 15.9±1.5 mmHg), indexed left atrial volume (31.7±9 *vs*. 28.8±8 ml/m^2) and lower ejection fraction (62.7±6 *vs*. 65.4±7%). Age, hypertension status, smoking history, medication use, LV volumes, indexed LV mass and E/A ratio were not significantly different between the two groups. NAFLD was associated with a three-fold increased rate of mild and/or moderate LVDD (present in 59% of the whole sample), even after adjusting for age, sex, body mass index, hypertension (*i.e*., blood pressure ≥140/90 mmHg and/or treatment), duration of diabetes, hemoglobin A1c, eGFR, LV ejection fraction, and indexed LV mass (adjusted-odds ratio 3.08, 95% CI 1.5-6.4, *P*=0.003).

Conclusion: our results indicate that NAFLD is strongly associated with early LVDD in type 2 diabetic patients with preserved systolic function, independently of multiple clinical risk factors and echocardiographic parameters.

CO67 - REDUCTION OF P27 EXPRESSION CORRELATES WITH SOMATIC MEN1 GENE MUTATIONS IN SPORADIC PARATHYROID ADENOMAS

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MEN1 is the main gene responsible for tumorigenesis of syndromic and sporadic primary hyperparathyroidism (PHPT). Up to date somatic mutations of the *MEN1* gene (20-35%) represent the main molecular defects of sporadic PHPT. Germline mutations of the Cyclin-Dependent Kinase Inhibitor 1B (*CDKN1B*) gene encoding p27 have recently been associated with multiple endocrine tumors in rats and humans. Menin, as a component of a Histone Methyltransferase Complex, directly regulates the expression of the *CDKN1B* gene specifically binding to transcriptional regulatory elements of its promoter. P27 is mainly localized in the nucleus in quiescent cells, where negatively regulates the progression of G1 to S phase of the cell cycle, by inhibiting cyclin A, E/CDK2 complexes, whereas in proliferating cells a fraction translocate to the cytoplasm where it can be degradated by proteolysis. Loss or reduced nuclear expression of p27 have been frequently detected in various human malignancies, while *CDKN1B* somatic mutations are rarely involved in tumorigenesis.

We evaluated the expression of p27 protein in a group of 50 sporadic parathyroid adenomas *CDKN1B* gene mutation-negative and its statistic correlation with *MEN1* gene mutations.

Immunohistochemistry was performed using the Ventana Benchmark immunostaining system. A monoclonal anti-p27 antibody (Ventana) was used to detect p27 expression. Nuclear and cytoplasmic p27 expression was scored as "negative" in case of no staining in any cell, or ≤20% of positive cells associated with weak/equivocal intensity. In addition, *MEN1* gene alterations were studied by automatic sequencing the tumor DNA and performing Loss of Heterozygosity (LOH) analysis at 11q13 (MEN1) locus. Forty-six percent (23/50) of the sporadic adenomas had negative p27 nuclear staining. Positive cytoplasmic staining was detected in 9 adenomas (18%). Somatic *MEN1* mutations (associated with LOH in all, but one sample) were identified in 15 of 50 (30%) samples. Eleven of 15 *MEN1* mutation-positive adenomas (73%) had a negative nuclear p27 staining. Moreover, we observed that no *MEN1* mutation was detected in samples with positive p27 cytoplasmic staining.

The occurrence of *MEN1* gene mutations in our cohort is in agreement with the percentage previously reported in the literature in sporadic parathyroid adenomas. Herein we found that *MEN1* mutations significantly segregated in tumors negative for p27 nuclear and cytoplasmic staining, suggesting that menin inactivation may be directly related to the downregulation of p27 expression through the inhibition of *CDKN1B* gene transcription rates.

CO68 - INDEPENDENT ASSOCIATION OF LOW VITAMIN D WITH POOR FUNCTIONAL INDEPENDENCE AND PHYSICAL ACTIVITY IN PEOPLE WITH CHRONIC SPINAL CORD INJURY: A CROSS-SECTIONAL AND PROSPECTIVE ANALYSIS

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Although significant associations between inadequate 25(OH)D status and reduced muscle mass, strength and physical performance have been reported in able-bodied elderly populations, no data exists on the association between 25(OH)D and physical function in people with spinal cord injury (SCI), who exhibit a very high prevalence of vitamin D deficiency. In the present study we explored the cross-sectional and 1-year prospective association of 25(OH)D with both functional independence (FI) in activity of daily living and leisure time physical activity (LTPA) in people with chronic SCI. We enrolled 100 consecutive patients (72 males and 28 females), aged 51.7±18.8 years, admitted to a rehabilitation program at the San Raffaele Institute of Sulmona. Vitamin D deficiency (25(OH)D <20 ng/mL) was found in 78 patients: they exhibited a significantly higher BMI, lower FI degree and were engaged in a significantly poorer weekly LTPA. At the cross-sectional linear multiple regression analysis of log-transformed values, after full adjustment for possible confounders, other than the tetraplegic status, also lower 25(OH)D levels showed significant independent associations with poorer FI, as evaluated by the Spinal Cord Independence Measure (SCIM) scale [beta (95%CI): 0.27 (0.11, 0.44), p=0.001], and with minutes/week of LTPA [0.71 (0.12, 1.29), p=0.01]. In the prospective part of the study, 46 patients were re-evaluated after 1 year. Lower baseline 25(OH)D levels were independently associated with a decrease in FI [0.27 (0.05, 0.49), p=0.02] and LTPA [0.03 (0.02, 0.04), p<0.0001], after adjustment for sex, age, smoking, BMI, comorbidities, pain, injury duration, level and completeness of the lesion. These associations persisted [0.24 (0.01, 0.47), p=0.04 and 0.02 (0.01, 0.04), p=0.0003, respectively] after further adjustment for dichotomized post-follow-up levels of 25(OH)D (<20 or ≥20 ng/mL). In conclusion, in people with chronic SCI, a low 25(OH)D level may represent an independent indicator of poor FI and LTPA, as well as an independent predictor of their decline.

CO69 - SERUM CHITOTRIOSIDASE IN POSTMENOPAUSAL WOMEN WITH SEVERE OSTEOPOROSIS

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Background: Recently, it has been shown that the expression of human chitotriosidases (Chit) increases significantly during the osteoclast differentiation and their activity. Chit seems to be involved in osteoclast function. We aim to evaluate Chit activity in postmenopausal women affected by severe osteoporosis and investigate its relation with a bone resorption marker such as β-CrossLaps (CTX).

Methods: In this cross-sectional study, 91 postmenopausal women affected by osteoporosis and 61 with either osteopenia or normal bone mineral density (BMD) were screened. All subjects were assessed by dual energy X-ray absorptiometry (DXA) and X-ray vertebral morphometry. Osteoporotic subjects were considered eligible if they were affected by at least one vertebral osteoporotic fracture (Group A= 57 subjects). Osteopenic or healthy subjects were free from osteoporotic fractures (Group B= 51 subjects). Enzymatic Chit and serum CTX were measured in the whole population.

Results: Group A showed higher serum levels of beta-CTX compared to group B (0.40±0.26pg/mL vs 0.29±0.2, p=0.022). Chit was significantly higher in group A (472 nmol/mL/h ± 313 vs 1042±613, p<0.001) than in group B even after adjustment for age (p<0.001). Spearman correlation test revealed a negative correlation between chit and BMD at each sites (lumbar spine: r -0.38, p=0.001, femoral neck: r -0.35, p=0.001, total femour: r -0.39, p<0.001). Furthermore a positive correlation between chit and PTH was observed (r 0.26, p= 0.013). No significant correlation was found between chit and beta-CTX (r 0.12, p=0.229). After a multivariate analysis a positive correlation between severe osteoporosis and chit (p<0.001), beta-CTX (p=0.013) and age (p<0.001) was observed.

Conclusion: This is the first clinical study showing a correlation between chit and severe post-menopausal osteoporosis. Further large and prospective studies are needed to evaluate if chit may be a promising clinical biomarker and/or therapeutic monitor in subjects with osteoporosis.

CO70 - ASSOCIATION BETWEEN 25(OH)-VITAMIN D AND TESTOSTERONE LEVELS: EVIDENCE FROM MEN WITH CHRONIC SPINAL CORD INJURY

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As an independent linear association between 25-hydroxyvitamin D (25(OH)D) and testosterone levels is controversial, this study aimed to explore this topic in men with chronic spinal cord injury (SCI), who exhibit a high prevalence of both androgen and vitamin D deficiency. Deficiency of 25(OH)D (<20 ng/mL) was found in 36 out of 49 patients (73.5%). They exhibited significantly lower total testosterone and free testosterone levels, higher parathyroid hormone (PTH) and HOMA-IR, a poorer functional independence degree, and were engaged in poorer weekly leisure time physical activity (LTPA). The levels of 25(OH)D were strongly correlated with free testosterone ($r = 0.60$; $p < 0.0001$). Other significant correlates were total testosterone ($r = 0.42$; $p = 0.002$), PTH ($r = -0.49$, $p = 0.0003$), functional independence degree ($r = 0.36$; $p = 0.01$) and weekly LTPA ($r = 0.41$, $p = 0.003$). The prevalence of 25(OH)D deficiency significantly increased throughout the highest to the lowest tertile of both total and free testosterone levels. At the linear regression models, lower 25(OH)D levels were associated with both lower free testosterone [beta (95%CI): 0.28 (0.02, 0.53), $p = 0.0007$] and total testosterone [0.28 (0.02, 0.53), $p = 0.03$], after adjustment for age, smoking, alcohol consumption, comorbidities and HOMA-IR. However, after full adjustment, also including functional independence degree, BMI and LTPA, only the association of lower 25(OH)D with lower free testosterone was still significant [0.42 (0.17, 0.67), $p = 0.001$]. In conclusion, in men with SCI, 25(OH)D correlates with total and free testosterone and exhibits an independent linear association with free testosterone. Regardless of this independent link, hypovitaminosis D and androgen deficiency share life style-related risk factors to take into account in the rehabilitative approach to patients with SCI.

CO71 - ESTROGEN MEDIATES METABOLIC SYNDROME-INDUCED ERECTILE DYSFUNCTION: A STUDY IN THE RABBIT

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Objectives: ERα is critical in mediating the harmful effects of hyperestrogenism in fetal or neonatal life on the developing penis. In contrast, little is known on the impact of an excess of estrogens on penile function in adulthood. The aim of this study was to investigate the effect of estrogens on metabolic syndrome (MetS)-associated erectile dysfunction (ED), in an animal model of MetS.

Methods: To understand the role of sex steroid milieu, we treated subgroups of MetS rabbits with either testosterone (T) or tamoxifen, a classical ERs antagonist. We evaluated acetylcholine (Ach)- penile responsiveness as well as the expression of genes related to penile smooth muscle relaxation and contractility.

Results: MetS was associated to elevated estradiol (E2) and low T levels. E2, but not T, was independently and negatively associated with genes able to affect penile erection. Smooth muscle-related markers decreased as a function of E2 and were positively associated with all the variables investigated. Increasing concentrations of circulating E2 were negatively associated with Ach-induced relaxation. In HFD rabbits, in- vivo T dosing significantly improved MetS, and normalized circulating E2. Conversely, in-vivo tamoxifen dosing reduced visceral adiposity and partially restored T level. Ach-induced relaxation was severely impaired by HFD and significantly restored, up to the control level, by both tamoxifen and T. In rabbit smooth muscle cells culture 17 β estradiol significantly reduced the expression of αSMA, SM22 and PDE5.

Conclusions: Tamoxifen reverted completely these effects. In conclusion, HFD-induced ED is more associated with a high estradiol, than to a low T, milieu.

CO72 - INCREASED PLATELET HYPER-REACTIVITY IS A CARDIOVASCULAR RISK FACTOR IN KLINEFELTER SYNDROME

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Objectives. To determine whether Klinefelter syndrome subjects (KS) are at higher risk of platelet hyper-reactivity.Methods. A case-control study was conducted. KS and controls were tested for light transmission aggregometry. Platelet aggregation (max-A%) was defined as maximal light transmittance reached within 6 min after the addition of 0.2 or 0.4 mM arachidonic acid (AA). A ≥50% irreversible light transmittance (LT-50%) following platelet stimulation was employed to define an adequate platelet aggregation. AC-50% was defined as the minimal agonist concentration needed to achieve a > 50% stable aggregation. We compared the mean max-A% and the prevalence of LT-50% achievement in KS and in controls.Results. Forty KS and 46 healthy matched controls were enrolled. After applying exclusion criteria, 23 KS under adequate hormonal replacement therapy were found eligible. AA (0.2 mM) induced a >50% aggregation in 69.6% of KS and in 15.2% of controls ($p< 0.001$). AA (0.4mM) determined a > 50% aggregation in all cases and controls. The maximal aggregation achieved after 0.4 mM AA was higher in KS than in controls ($p< 0.001$). Accordingly, the AC-50% was 0.26 mM AA for KS and 0.36 mM for controls ($p< 0.001$). 8-iso-PGF2α and 11-dehydro-TXB2 are metabolite produced by activated plateleted and released in humane urine. They were higher in KS than in controls ($p< 0.001$ and $p=0.001$ respectively). Moreover, an inverse correlation was found between AC-50% and 8-iso-PGF2α ($p< 0.001$) as well as 11-dehydro-TXB2 ($p<0.001$). In a multivariate linear regression model, KS predicted a lower AC-50% ($p< 0.001$) and higher levels of 8-iso-PGF2α ($p< 0.001$) as well as of 11-dehydro-TXB2 ($p <0.001$). No significant correlations were found between AA (0.2 and 0.4mM) induced > 50% aggregation, metabolic syndrome, testosterone and estradiol levels in KS.Conclusions. We showed that KS subjects, under adequate testosterone replacement therapy, may present augmented platelet hyper-reactivity, in an independent manner by metabolic syndrome and steroid hormone levels, suggesting a genetic link between this condition and 47 XXY karyotype. Platelet hyper-reactivity is an additional cardiovascular risk factor in KS condition, that we suggest to consider for an adequate prevention treatment to reduce cardiovascular risk and mortality.

CO73 - ROLE OF MICROENVIRONMENT ON NEUROBLASTOMA SK-N-AS SDHB SILENCED CELL METABOLISM AND FUNCTION

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Solid tumors are very complex tissues comprising not only cancer cells, but also non-malignant stromal cells such as endothelial cells, fibroblasts, immune cells and extracellular matrix, forming the so called tumor microenvironment. In the last few years, it has become more and more evident the pivotal role of the tumor microenvironment in modulating cancer progression and metastasis. Tumor microenvironment has thus become a potential therapeutic target.

To obtain an experimental model resembling the in vivo conditions of the succinate dehydrogenase B subunit (SDHB)-mutated paragangliomas (PGL), we evaluated the effects of SDHB silencing on metabolism and proliferation in the human neuroblastoma cell line (SK-N-AS), cultured alone or in association with human fibroblasts.

Silencing, verified by Western Blot and densitometry analysis, caused a 70% decrease in protein expression, an almost complete loss of the complex specific enzymatic activity and a significant increase in HIF1 and HIF2 expression, thus resembling the in vivo tumor cell phenotype.

When compared to wild type SK-N-AS cells, SDHB silenced cells showed an altered metabolism characterized by an unexpected significant decrease in glucose uptake and by an increase of lactate uptake. Moreover, silenced cells showed a significant increase in cell proliferation and metalloproteinase activity. When co-cultured with human fibroblasts, control cells showed a significant decrease in glucose uptake and a significant increase in cell proliferation versus their mono-cultured counterparts. These effects were even more strikingly evident in co-cultured silenced cells which showed a 70% decrease in glucose uptake and a 92% increase in cell proliferation vs their mono-cultured counterparts.

Our data demonstrate, for the first time, that SDHB impairment causes a metabolic and functional derangement of neural crest-derived tumor cells and that microenvironment, here represented by fibroblasts, strongly affects their tumor metabolism and growth capacity.

CO74 - CUSHING'S SYNDROME DIAGNOSIS: THE RENAISSANCE OF URINARY FREE CORTISOL?

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Introduction and aim: International guidelines have recommended as initially screening for Cushing's Syndrome (CS) one test among: urinary free cortisol (UFF, performed by liquid chromatography–tandem mass spectrometry, LC-MS/MS), late night salivary cortisol (LNSC) or 1-mg dexamethasone suppression test (DST). We retrospectively analysed the diagnostic performance of UFF, LNSC or DST in a large cohort of consecutive patients.

Materials and Methods: we collected 511 subjects, referred to our Endocrinology Unit between 2012-2014 with medical conditions suggestive of hypercortisolism (299 patients) or with an incidental finding of adrenal node (212 patients with adrenal incidentaloma, AI). After complete diagnostic work-up (with dynamic testing, imaging and histological confirmation when available), we considered CS 46 subjects (26 pituitary, 13 adrenal and 7 ectopic); 255 non-CS and 191 AI (excluding from the initial group 2 adrenal CS, 16 primary hyperaldosteronism and 3 pheo). UFF was measured with a LC-MS/MS method (threshold >170 nmol/L); LNSC (cut-off >5.24 ng/mL) and serum cortisol after DST (considering cut-off either 50 or 138 nmol/L) were measured with a radio-immunometric kit.

Results: We collected 1551 tests: 396 DST, 472 LNSC and 683 UFF (238 patients performed all 3 tests; 289 DST+LNSC, 296 DST+UFF and 313 LNSC+UFF). Comparing non-CS+AI versus CS, DST revealed a sensitivity (SE) 100% and specificity (SP) 77% at 50 nmol/L cut-off and SE 87% and SP 95% at 138 nmol/L cut-off (AUC 0.977); mean LNSC SE 86% and SP 92% (AUC: 0.966); mean UFF SE 98% and SP 91.5% (AUC 0.978). Comparing AI vs CS, DST showed SE 100% and SP 62% at 50 nmol/L and SE 87% and SP 93% at 138 nmol/L (AUC 0.965); mean LNSC SE 86% and SP 92% (AUC: 0.971) and mean UFF SE 98% and SP 91% (AUC 0.977). Considering only non-CS versus CS, DST showed SE 100% and SP 91% at 50 nmol/L and SE 87% and SP 97% at 138 nmol/L (AUC 0.990); LNSC revealed SE 86% and SP 92% (AUC: 0.963); UFF revealed SE 98% and SP 92% (AUC 0.980).

Conclusions: In this study, UFF manifests high clinical accuracy to detect CS, probably because we use LC-MS/MS method and we calculate a local threshold. In addition, LNSC demonstrates high diagnostic accuracy to detect CS between AI and non-CS. Considering DST the critical issue is the serum cortisol cut-off: we suggest using the higher one (138 nmol/L) when considering patients with AI.

CO75 - ARMADILLO REPEAT CONTAINING 5 (ARMC5) GENE MUTATIONS IN PATIENTS WITH BILATERAL MACRONODULAR ADRENAL HYPERPLASIA (BMAH): A MULTICENTER EXPERIENCE

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Inactivating germline mutations of ARMC5 have been recently identified as a genetic cause of BMAH. We aimed to identify ARMC5 mutations in a cohort of patients with BMAH presenting clinical or subclinical hypercortisolism.

We evaluated the presence of ARMC5 mutation in 42 cases (39 apparently sporadic and three familial cases from two kindred) by direct sequencing. In addition, ARMC5 gene was analyzed in tumoral DNA of eleven nodules from nine patients and in three additional cases from archived paraffin-embedded tissue. Different computational tools were used to predict the possible effects of the identified amino acid substitutions on protein function

We identified nine nucleotide variants. Two were non-sense mutations, one of which is novel and segregates in a family with the disease. Three were novel frame-shift mutations predicted as pathogenic as lead to a downstream stop codon with an early termination of translation. Four were missense substitutions, three of them predicted to be damaging as well. In addition, two heterozygous nucleotide changes, already reported as rare SNPs, have been identified. Familial screening was performed in seven first-degree relatives of four index cases, in three of which the same ARMC5 mutations as the proband has been detected. Analysis of tumoral DNA evidenced LOH in the carrier of a non-sense mutation supporting the concept that ARMC5 acts as tumor suppressor gene. No additional LOH or mutations have been detected in further available cases.

Based on computational analysis we hence identified an ARMC5 pathogenic mutation in 11/42 patients (26%), one of which in a BMAH family. This report further supports previous studies demonstrating that ARMC5 alterations are frequent in BMAH and that its identification may have implications for the clinical care of BMAH families

CO76 - USEFULNESS OF SIMULTANEOUS MEASUREMENT OF PLASMA DEXAMETHASONE, CORTISOL AND CORTISONE BY LC-MS/MS AFTER LOW-DOSE DEXAMETHASONE SUPPRESSION TEST: PRELIMINARY RESULTS

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Background: The low-dose dexamethasone (DEX) suppression test (DST) is a sensitive *screening* test for endogenous Cushing's Syndrome (CS) while its specificity varies considerably. Reasons for false-positive results include poorly absorption, excessively metabolism and impaired clearance of DEX. Thus, the attained DEX levels following peroral administration varies substantially among individuals giving the rationale for suggesting DEX measurements as part of the DST.

Aim: To evaluate if the simultaneous measurement of plasma dexamethasone, cortisol and cortisone after DST may improve its diagnostic performance, we developed a highly sensitive and specific method using LC-MS/MS.

Methods: Ten patients (5 with active CS and 5 healthy controls) were analyzed. All the patients received 1 mg DEX at 23.00 h. Plasma samples were collected posttest 08.00 h and cleaned up by solid-phase extraction before analysis. Liquid chromatographic separation was carried out under reversed-phase conditions prior detections by tandem mass spectrometry. The analytes were determined in the presence of deuterated internal standard cortisol-d2 and dexamethasone-d4. Analytical instrumentation used: Agilent 1100 Series HPLC coupled with triple quadrupole 6410. Primary mass transitions monitored for cortisol, cortisone and dexamethasone were m/z 363/121, 361/163 and 393/373 respectively.

Results: The LOD (Limit Off Detection) for the dexamethasone, cortisol and cortisone was 0,2 ng/ml (0,51 nmol/L), 0,1 ng/ml (0,27 nmol/L) and 0,25 ng/ml (0,69 nmol/L) respectively. The recovery rate of steroids in the analytical method used was 95-100%. The linearity test is >0,95. All the patients except one attained plasma DEX levels >5 nmol/L after 1 mg DST (range 4,76−27,09 nmol/L; median 6,64 nmol/L) with no association with posttest cortisol levels.

Conclusions: Our method provides high sensitivity and specificity. DEX measurement may identify subjects with inadequately low plasma DEX levels (<5 nmol/L) and may therefore be useful in retesting subjects with possibly false positive DST.

CO77 - COMBINED EFFECTS OF SIROLIMUS AND MITOTANE IN THE INHIBITION OF GROWTH IN HUMAN ADRENOCORTICAL CARCINOMA CELLS

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Adrenocortical cancer (ACC) is a rare cancer with poor prognosis and scant treatment options. Mitotane alone, or in combination with cytotoxic chemotherapy, represents the referral current treatment for patients with unresectable ACC. Recent studies have shown that mTOR inhibitors suppress growth of ACC cells. This study aimed at evaluating the effects of mitotane in combination with mTOR inhibitors.

In H295 and SW13 cells we tested the effects of a 6 day treatment with increasing doses of mitotane in the presence or absence of selected doses of sirolimus on cell proliferation as measured by the total DNA content. The tested doses of mitotane ranged between $10^{-7}$and 10^{-5}M in both H295 and SW13 cells, sirolimus was tested at concentrations of $5x10^{-9}$ and 10^{-6}M in H295 and $5x10^{-11}$M and 10^{-10} M in SW13.

In H295, mitotane significantly inhibited cell proliferation at all concentrations tested, with an IC_{50} of $4.5x10^{-6}$M and a maximal inhibition of 87% as compared with vehicle-treated controls ($p<0.001$). In SW13, mitotane significantly inhibited cell proliferation at concentrations higher than $2.5x10^{-6}$M, with an IC_{50} of $1.6x10^{-5}$M and a maximal inhibition of 81% as compared with vehicle-treated controls ($p<0.001$). In both H295 and SW13 sirolimus significantly inhibited cell proliferation at both concentrations tested and when combined with mitotane, it showed statistically significant additive effects. This additivity was observed only with low mitotane doses (between $10^{-7}$and $5x10^{-6}$M). Using mitotane doses higher than $5x10^{-6}$M the cell proliferation inhibition was already nearly maximal and no significant additive effects could be observed.

The current study demonstrates that sirolimus has additive antiproliferative effects when combined with low mitotane doses. These doses correspond to concentrations lower than the therapeutic range of mitotane. If this effect can also be achieved *in vivo*, our data suggest that the addition of sirolimus to mitotane might be useful in ACC patients when the therapeutic range of mitotane range is not reached.

CO78 - STEROID PROFILING BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS) IN PATIENTS WITH NON-SECRETING ADRENOCORTICAL ADENOMAS AND SUBCLINICAL HYPERCORTISOLISM

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Steroid profiling by liquid chromatography tandem mass spectrometry (LC-MS/MS) offers an accurate and simultaneous detection of a panel of steroids with several advantages over traditional assays. However, only a few studies have investigated the steroid profiling in sporadic adrenocortical adenomas. Our aim was therefore to investigate steroid secretion by LC-MS/MS in patients with non-secreting adrenocortical adenomas (NSA) and subclinical hypercortisolism (SH), by analysing cortisol (F), 21-deoxycortisol (21-DF), 11-deoxycortisol (S), 17-hydroxyprogesterone (17-OHP), androstenedione (A), dehydroepiandrosterone (DHEA), testosterone (T), progesterone (P), 21-deoxycorticosterone (DOC), and corticosterone (B). Steroids were evaluated in basal condition and 60´ after stimulation with 250 µg of 1,24 ACTH. Patients (n=94) were analysed at baseline and compared to 190 sex- and age-matched controls. DHEA levels were progressively decreased in NSA and SH, respect to controls. A and T levels were also reduced in females. A significant positive correlation between basal DHEA and ACTH was found (correlation coefficient 0.403, $P<0.001$), supporting the hypothesis that decreased stimulation of contralateral cortex by reduced ACTH levels is the likely cause of DHEA reduction. Considering the known anti-glucocorticoid effects of DHEA in adipocytes, we tested the potential effects of reduced DHEA on metabolic alterations. Increased BMI (correlation coefficient 2.01, $P<0.001$) and cortisol after dexamethasone test (correlation coefficient 0.14, $P<0.001$), and reduced DHEA (correlation coefficient -5.70, $P=0.034$), were all factors independently associated with the increase of waist circumference. Stimulation study was performed on 56 patients and revealed that subjects with SH had higher levels of F, 21-DF, DOC, B, and lower levels of DHEA and A respect to NSA. Steroid profiling with LC-MS/MS highlighted important clues into the secreting pattern of adrenocortical adenomas. Further studies are needed to address the clinical implication of the increased levels of gluco- and mineralocorticoid precursors in the pathogenesis of cardiovascular and metabolic correlates in SH.

CO79 - ANTI-MÜLLERIAN HORMONE AND INSULIN-LIKE 3 LEVELS IN HEALTHY NORMAL-WEIGHT OVULATORY AND ANOVULATORY EUMENORRHEIC LATE ADOLESCENT FEMALES

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Objective: Are anti-müllerian hormone (AMH) and insulin-like 3 (INSL3) levels in healthy normal-weight ovulatory and anovulatory eumenorrheic late adolescent females different? Are anovulatory cycles in eumenorrheic late adolescent females the expression or not of a precocious ovarian dysfunction leading to a hyperandrogenic condition later on?

Study Design: This study analyzed AMH and INSL3 levels in forty healthy eumenorheic late adolescent females, (aged 16-19 ys), selected from a cross-sectional epidemiological study performed on the prevalence of hypernadrogenic states. The subjects were divided into ovulatory (n:28) and anovulatory (n:12) groups in accordance to a cluster analysis based on progesterone (P) distribution measured in the latter part of the cycle. Both groups were compared for anthropometric, biochemical and hormonal parameters.

Results: INSL3 and AMH were detectable in all samples. Testosterone (*P*=0.01), the free-androgen index (FAI) (*P*=0.051), gonadotropins (LH: *P*=0.02; FSH: *P*=0.004) and AMH (*P*=0.02) levels were significantly higher in the anovulatory group with respect to their ovulatory counterpart. A trend toward significantly higher INSL3 concentrations (*P*=0.08) was also shown in the anovulatory group. A positive correlation between INSL3 levels and androgens such as androstenedione (*r*=0.38; *P*=0.02), testosterone (*r*=0.44; *P*=0.004) and FAI (*r*=0.42; *P*=0.006) and a negative borderline significant correlation (*r*=-0.30; *P*=0.055) between AMH and P were shown in all subjects.

Conclusion: Healthy eumenorrheic late adolescent females with sporadic anovulation display higher AMH and INSL-3 blood concentrations in association with higher androgen levels compared with age- and BMI-matched subjects with ovulatory cycle, suggesting evidence of an earlier ovarian dysfunction.

CO80 - COMBINED EFFECTS OF TWO GENE POLYMORPHISMS (IRS-1 G972R AND PC-1 K121Q) IN THE POLYCYSTIC OVARY SINDROME (PCOS)

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A number of polymorphisms of genes involved in insulin signaling are associated with PCOS, but they were studied separately in the literature. Thus, we ignore the impact of their coexistence.

To start addressing this issue at least on serum metabolic and hormonal parameters, we genotyped 100 consecutive PCOS women (by Rotterdam criteria) and 45 age-matched healthy women as controls for two of the said polymorphisms: G972R of IRS-1 and K121Q of PC-1. Statistics was by the χ^2 test or the two-tailed Student's *t* test.

Comparing the PCOS group with the control group, the rate of homozygosity or heterozigosity was significantly greater (50 *vs*. 24.5%, P=0.004) for IRS-1 polymorphism, but insignificantly greater (20 *vs.* 13.3%, P=0.33) for PC-1 polymorphism. Homozygosity for either polymorphism existed only in the PCOS group (2/100 or 2/100 *vs*. 0/45 or 0/45), but did not coexist in the same 2 PCOS women. Thus, data of homozygotes and heterozygotes were pooled. In the PCOS group, compared with controls, the genotypes IRS-1 hetero/PC-1 wild type (WT) (36 *vs.* 17.8%, P=0.03) and IRS-1 hetero/PC-1 hetero (14 *vs*. 6.7%, P=0.20) were overrepresented at the expense of IRS-1 WT/PC-1 WT (44 *vs*. 68.8%, P=0.005), while IRS-1 WT/PC-1 hetero was similarly represented (6 *vs*. 6.7%). In the PCOS group, IRS-1 hetero/PC-1 WT had the highest HOMA-IR (2.9±2.0), fasting insulin (FI=14.8±10 µU/ml), insulin area under the curve at OGTT (mean=8628), triglycerides (98.8±68.3 mg/dl), total and calculated free testosterone (TT=69.4±29.9 ng/dl; cFT=1.4±0.8 pg/ml). The first 4 values decreased in the IRS-1 hetero/PC-1 hetero PCOS group (2.2±2.2; 11.7±10.4 µU/ml; 7827; 72.2±18.5 mg/dl), while TT and cFT were similar (68.1±23.7 and 1.3±0.8); this genotype also had the highest HDL levels (65.7±16.5 mg/dl), which were among the lowest in the IRS-1 hetero/PC-1 WT PCOS group (56.1±16.8). Thus, such IRS-1 hetero/PC-1 hetero was the PCOS subgroup with these 7 values closest to those of the 45 controls, in whom values were 1.5±1.2, 6.6±5.8 µU/ml, 6745, 66.1±26.4 mg/dl, 48.3±20.6 ng/dl, 0.8±0.4 ng/dl and 67.6±12.8 mg/dl.

Our data demonstrate that: 1) G972R of IRS-1, not K121Q of PC-1, is significantly overrepresented in our PCOS population; 2) the R allele of IRS-1 confers a pejorative effect on metabolic and, to a lower extent, hormone indices of the PCOS phenotype; 3) such effect is ameliorated by the Q allele of PC-1. Our data underscore that genetic variations in insulin signaling contribute to the extent and the variability of metabolic and hormonal derangement.

CO81 - HEART RATE REPRESENTS AN EARLY MARKER OF CARDIOVASCULAR RISK IN YOUNG PATIENTS WITH PCOS: ROLE OF INSULIN OR WEIGHT?

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Objective: PCOS is one of the most common endocrine diseases affecting women up to 10%, during their reproductive years. This syndrome has been recognized to be associated with an increased cardiovascular risk mostly due to its metabolic co-morbidities. Moreover, changes in heart rate, mediated by the balance between sympathetic and vagal activity which could be impaired in insulin resistant states, contributing to the global cardiovascular risk. **Aim of the study:** we tested the hypothesis that a correlation among insulinemic status, weight, blood pressure (BP) and heart rate (HR) exists in PCOS. **Materials and methods:** LH,FSH,PRL, androgens, estrogens, PRA and aldosterone, OGTT (75g) for glycemia and insulin, HOMA-ir, ISI and lipids were analyzed in 54 young PCOS-women. BMI (Kg/m2), waist and hip circumference, acne, alopecia and Ferriman-Gallwey score were considered. HR, systolic(S) and diastolic(D) BP(24h-ABPM) were recorded. Patients were then subdivided in hyperinsulinemic (**hINS**: insulin-peak>70mUI/L,HOMA-ir>2), in normoinsulinemic (**nINS**: insulin-peak<60 mUI/L, HOMA-ir< 2), and further in **obese** (BMI > 30) and **lean** (BMI<24,9). Statistical analysis were performed using independent Student's t-test and Pearson correlation coefficient method. **Results:** HR, as well as BP, was significant increased ($p<0.05$) in hINS subjects (HR:73.3±7.0; BPS:116.1±7.3; BPD:66.2±4.3) compared to nINS (HR:67.1±2.8; BPS:109.2±2.7; BPD:58.5±3.5), during the 24-h recording. The nocturnal decrease of HR and BP was smaller in hINS patients (HR:17.8±5.4; BPS:7.9±4.3; BPD:13.2±5.5%) compared to nINS (HR:25.0±4.3; BPS:15.0±4.3;BPD; 20.9±6.3%). No significant difference was found when considering the weight of patients only **Conclusions:** HR is significantly increased in hINS patients thus suggesting that hyperinsulinemia could influenze the sympathetic/vagal activity partially independent from obesity. An increased HR could be considered therefore as a simple cardiovascular risk marker in young hyperinsulinemic PCOS women.

CO82 - TIM 16 INHIBITION ENHANCES SENSITIVITY TO DOXORUBICIN IN HUMAN BREAST CANCER CELLS

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TIM 16 protein is a component of the translocase complex TIM 23 located in the mitochondrial inner membrane, encoded by the Magmas gene, overexpressed in human pituitary adenomas. Magmas silencing in ACTH secreting rat pituitary adenoma cells leads to a greater sensitivity to pro-apoptotic stimuli. We recently demonstrated that Magmas overexpression protects rat GH secreting pituitary adenoma cell lines towards apoptosis. Moreover, we found that compound 5, a TIM16 inhibitor, is not cytotoxic but enhances the proapoptotic effects of staurosporine by reducing mitochondrial membrane potential (MMP) activation in a medullary thyroid carcinoma cell line, suggesting that compound 5 may be useful for cancer treatment in association with chemotherapeutic drugs.

Since breast cancer (BC) displays high chemoresistance the aim of our study was to investigate Magmas expression in human breast cancer cell lines and to verify if compound 5 could increase the effects of a chemotherapeutic agent, such as Doxorubicin.

We found that Magmas protein in the MCF7 breast cancer cell line is highly expressed as compared to MCF12A normal breast tissue cell line.

Treatment with compound 5 did not influence cell viability, while Doxorubicin decreased this parameter by 20-30% in both cell lines. Only in MCF7cells, co-treatment with compound 5 enhanced by 15-20% the antiproliferative effects of Doxorubicin, as assessed both by cell viability assay and BrDu incorporation. Moreover, in MCF7 cells BAX levels are enhanced after treatment with compound 5 and Doxorubicin, while the same treatment did not affect BAX levels in MCF12A cells. MitoTox Glo assay showed that the enhancing effects of compound 5 on Doxorubicin action are due to mitochondrial toxicity. In addition, treatment with compound 5 strongly decreased MMP in MCF7 cells, while it had weaker effects on MCF12A cells.

In summary, these data suggest a role for the use of compound 5 as a sensitizing agent for chemoresistant breast cancer treatment.

CO83 - SERUM FREE TESTOSTERONE INDEPENDENTLY PREDICTS METABOLIC SYNDROME IN WOMEN WITH POLYCYSTIC OVARY SYNDROME

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Metabolic syndrome is a frequent finding in women with polycystic ovary syndrome (PCOS), consistent with the high prevalence of insulin resistance in these subjects. However, the role of androgen excess in this alteration remains unclear. To investigate this issue, 188 PCOS women, recruited in the Verona 3P Study, underwent accurate clinical, endocrine and metabolic assessment. Serum total and free testosterone were measured by liquid cromatography-mass spectrometry and equilibrium dialysis, whereas insulin sensitivity (M value) was assessed by the glucose clamp technique. Metabolic syndrome was diagnosed by the 2009 IDF criteria. In these women (mean age±SD 24.0±5.5 yr, BMI 29.0±7.7 Kg/m2) insulin resistance (M value <75th centile in controls) occurred in 69.9%, and hyperandrogenemia, evaluated by serum free testosterone levels, in 70.0% of subjects. Fifty-eight (30.9%) of these women met the criteria for diagnosis of metabolic syndrome. Among the components contributing to this diagnosis increased waist circumference (71%) and reduced HDL-cholesterol (54%) were the most common abnormalities, whereas other alterations were less frequent. In this cohort, logistic regression analysis was carried out including either metabolic syndrome or each component of the syndrome as dependent variable, and age, insulin sensitivity and either total testosterone (model 1) or free testosterone (model 2), as independent variables. Both in models 1 and 2 insulin sensitivity was an independent predictor of metabolic syndrome, as well as of each component of the syndrome, whereas age was an additional independent predictor of fasting glucose and triglyceride alterations. However, in model 2 also free testosterone was an independent predictor of metabolic syndrome, as well as of alterations in blood pressure, HDL-cholesterol and waist circumference.

In conclusion, our results confirm the main role of insulin resistance in the abnormalities characteristic of the metabolic syndrome in PCOS women. However, hyperandrogenemia may independently contribute to these alterations.

CO84 - VISCERAL ADIPOSITY REPRESENTS A RELIABLE PREDICTIVE PARAMETER OF EFFICACY OF METFORMIN IN THE TREATMENT OF POLYCICTIC OVARY SYNDROME

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Visceral adiposity and insulin resistance (IR) are very common features of polycystic ovary syndrome (PCOS). Metformin (MET) is a well-validated insulin-sensitizing drug commonly used in the treatment of PCOS. The current study aimed at investigating whether body mass index (BMI), marker of obesity, waist circumference (WC), marker of visceral adiposity degree, visceral adiposity index (VAI), marker of visceral adiposity function, and HOMA-IR, marker of IR, may be potential predictive parameters of responsiveness to treatment with MET in PCOS. Fifty-nine consecutive patients (27±6 years) started MET at the dose of 1500 mg/day. Clinical [Menstrual cycles (MC), Ferriman-Gallwey score (FGS), BMI, WC], hormonal [LH/FSH and androstenedione (AND)] and metabolic parameters (total-(T) and HDL-cholesterol (C), triglycerides (TG), fasting (F) and 120-min (after glucose load) glucose (G) and insulin (I), VAI and HOMA-IR) were evaluated at baseline and after 6-months therapy. Compared to baseline, MET treatment induced a significant improvement in MC (p=0.000), FGS (p= 0.000), BMI (p=0.000), WC (p=0.000), AND (p=0.000), TC (p=0.000), HDL-C (p=0.000), TG (p=0.000), FI (p=0.004), 120-min I (p=0.000), VAI (p=0.000) and HOMA-IR (p=0.004). Patients were stratified according to BMI, WC, VAI and HOMA-IR. BMI≥25 Kg/m2 was found in 90%, WC >80 cm in 86%, VAI<1 in 84% and HOMA-IR≥2.5 in 61%. No significant difference was found in percent change (Δ) of clinical, metabolic and hormonal parameters after MET treatment in patients stratified by BMI and WC and HOMA-IR. Conversely, ΔTC (p=0.01), ΔHDL-C (p=0.004), ΔTG (p=0.004) and ΔLH/FSH (p=0.01) were significantly higher in patients with VAI<1 as compared to those with VAI>1. ΔFGS significantly correlated with ΔBMI (r=0.36, p=0.005), ΔWC (r=0.57, p=0.000) and ΔVAI (r=0.27, p=0.03). At regression analysis, ΔWC was the best predictor of ΔFGS (t=3.05, p=0.004). In conclusion, the results of the current study demonstrated that visceral adiposity function represent an interesting parameter for targeting MET therapy in patients with PCOS, since VAI, marker of visceral adiposity function and indirect marker of cardiometabolic risk, is a good predictor of effectiveness in metabolic and hormonal parameters and visceral adiposity degree is the best predictor of the improvement in hirsutism, one of the main clinical feature of PCOS.

PD01 - GALECTIN-3 AND HBME-1 IMPROVE THE ACCURACY OF CORE BIOPSY IN INDETERMINATE THYROID NODULES.

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Background. Core needle biopsy (CNB) has been recently described as an accurate complementary test in thyroid inconclusive cytology (FNAC). Here we retrospectively investigated the role of Galectin-3, Cytokeratin-19 and HBME-1 panel to increase the accuracy of CNB in diagnosing thyroid nodules with prior indeterminate FNAC.

Methods. The study included 74 nodules of which 15 were cancers at CNB, 19 had uncertain microhistology, and 40 nodules had benign CNB report. The above immunohistochemical (IHC) panel was analyzed in all cases.

Results. At final histology 19 malignant and 55 benign lesions were found. All 15 cancers and 40 benign nodules diagnosed at CNB examination were confirmed. Out of the uncertain CNB, 4/19 (21%) were malignant and the remaining 15/19 (79%) benign. When considering the IHC panel of these three markers, the higher sensitivity and NPV were found for HBME-1, and the most accurate combination these markers was Galectin-3 plus HBME-1. When we combined CNB and IHC, both sensitivity and specificity of CNB increased from 79 to 100% and from 73 to 96%, respectively. The combination of CNB plus Galectin-3 and HBME-1 could correct indentified 19/19 cancers and 53/55 benign lesions.

Conclusions. CNB can diagnose the majority of thyroid nodules with previous indeterminate FNAC, and the accuracy of CNB is increased by adding Galectin-3 and HBME-1. We encourage to adopt CNB as a second-line approach to indeterminate thyroid nodules, and apply IHC in those lesions with uncertain CNB. This approach should significantly improve the pre-surgical diagnosis of patients.

PD02 - MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF EARLY THYROID MORPHOGENESIS IN TRANSGENIC ZEBRAFISH EMBRYOS

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Introduction Thyroid dysgenesis is the major cause for congenital hypothyroidism which affects about 1/3000 newborns. However, very little is known about the pathogenetic mechanisms leading to thyroid dysgenesis, partly because of a poor understanding on how intrinsic factors and extrinsic signaling cues are integrated to regulate thyroid morphogenesis. Zebrafish embryos offer several salient properties to study basic mechanisms of organ development. In this study, we performed a detailed morphological and molecular characterization of early thyroid morphogenesis in zebrafish. Materials and Methods Thyroid development was studied from anlage formation (22 hpf) to folliculogenesis (55 hpf) using transgenic zebrafish expressing distinct fluorescent proteins in foregut endoderm, thyroid precursor cells, thyroid follicular cells and cardiovascular cell lineages. In addition, transgenic biosensor lines were used to develop a dynamic 3D-atlas of important signalling pathway activities (e.g., BMP, FGF, Wnt, Notch) in the thyroid region. Fluorescent reporter expression was monitored in live embryos using epifluorescence and confocal microscopy. Fluorescent in situ hybridization and immunofluorescence staining was performed with fixed embryos and stained embryos were analyzed by confocal imaging. Results First thyroid precursor cells can be identified at 23 hpf in two bilateral regions juxtaposed to the midline and dorsal to the cardiac cone. By 26 hpf, a continuous midline thyroid anlage has formed adjacent to the apical pole of the cardiac tube. Thyroid precursor cells are in direct cell-cell contact with endothelial cells of the aortic arch artery and distal ventricular myocardium. The initially monolayered cluster of thyroid precursor cells transforms into a thyroid bud which detaches from the ventral floor of the pharynx by 48 hpf. From 30 to 55 hpf, the number of thyroid cells remains fairly constant and no cell proliferation is detectable in the thyroid primordium. Among the signaling pathways activities investigated, the thyroid primordium was characterized by very high BMP activity as evident from high expression of phosphorylated smad5 as well as from strong dmKO2 expression in BMP activity reporter fish. Interestingly, markers of BMP signaling decreased rapidly after thyroid detachment from the pharyngeal epithelium. Conclusions Zebrafish is a powerful model system for studies on basic mechanisms involved in thyroid morphogenesis. Here we harnessed the optical transparency of zebrafish embryos and the availability of transgenic reporter lines to perform confocal analyses of the thyroid region aiming at the development of a dynamic 3D atlas of early thyroid morphogenesis. These datasets will provide a critical resource to understand tissue-tissue interactions and will aid in the identification of thyroid phenotypes in different zebrafish models.

PD03 - THE INCREASED PAPILLARY THYROID CANCER INCIDENCE IN MT. ETNA VOLCANIC AREA IS ASSOCIATED WITH NON-ANTHROPOGENIC HUMAN BIOCONTAMINATION WITH TRACE ELEMENTS

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Background and Aim. High thyroid cancer (TC) incidence in Mt. Etna volcanic area suggests a possible relationship with environmental carcinogens. Chemical(s) possibly involved and vehicles of diffusion are not known. We measured 26 trace elements in drinking water and lichens (markers of atmosphere pollution), considered as potential vehicles of diffusion, and in urines of residents (to document biocontamination). We also evaluated the effect of chemicals found increased in the volcanic area on rat thyroids.

Methods. Element concentration was measured in tap water (278 samples), lichens (102 samples) and urines of residents (278) of volcanic and control areas by a quadrupole system (DRC-ICP-MS). Seven heavy metals (B, Cd, Hg, Mo, Pd, V and W) were added to drinking water of adult hypothyroid (low iodine diet + methimazole) S-D female rats at 2X of the concentration observed in the volcanic aquifer and thyroid histology was compared to that of control rats after 12 months.

Results. 1) Age-standardized thyroid cancer incidence is nearly doubled in the volcanic area (only papillary histotype, PTC); 2) Geometric means (GM) of As, B, Cd, Mo, Sb, Se and V were significantly ($p<0.001$) increased in both water and lichens and Hg, Mn, Pd, U and W only in water in the volcanic area. 3) B, Cd, Hg, Mn, Mo, Pd, Se, U, V and W were significantly increased in urines collected in the volcanic area and correlated with values in water ($r=0.69$; $p=0.001$) and lichens ($r=0.58$; $p=0.007$). 4) Thyroid micronodules were significantly increased in rats treated with seven elements increased in the volcanic area ($p=0.009$).

Conclusions. In the volcanic area the increased incidence of PTC is associated with atmosphere and water pollution with heavy metals and consequent human biocontamination. The effect of these elements on rat thyroid micronodularity supports the possibility of a cause-effect relationship.

PD04 - ANATABINE DECREASES THE EXPRESSION OF SODIUM/IODIDE SYMPORTER, THYROGLOBULIN, AND THYROIDPEROXIDASE IN FRTL-5 THYROID CELLS

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Tobacco use has been associated with amelioration of some autoimmune diseases, such as ulcerative colitis and Hashimoto thyroiditis. Using data from the third national health and nutrition examination survey, it was first reported in 2004 that smokers had a lower prevalence of thyroid peroxidase (TPO) and/or thyroglobulin (Tg) antibodies than nonsmokers, a finding later confirmed and expanded in several studies. It is possible that this beneficial effect relates to the alkaloid content of tobacco, given the known anti-inflammatory properties of nicotine. Anatabine, a minor alkaloids, has been recently shown to reduce disease incidence and severity in a mouse model of autoimmune thyroiditis (Caturegli et al., Endocrinology, 2012, 153: 4580) and Tg autoantibodies in patients affected by Hashimoto thyroiditis (Schmeltz et al., J Clin Endocrinol Metab, 2014, 99: E137). Based on these findings, we designed the present study to assess the effect of anatabine on the expression of thyroid specific genes. The thyroid FRTL-5 cell line was treated with or without anatabine and then evaluated for the expression of NIS, Tg, TPO, and thyrotropin receptor by qRT-PCR and immunoblotting. Anatabine significantly reduced the expression of NIS, Tg and TPO RNA, but not that of the thyrotropin receptor. This reduction was dose- and time-dependent, reaching a maximal effect at a dose of 1 mM and at 24 hours of treatment. The transcriptional effect of anatabine was confirmed at the protein level.

Our results suggest that the beneficial effect of anatabine on autoimmune thyroiditis relates not only to its immunological properties but also to the ability to decrease the "visibility" of classic thyroid autoantigens. Further studies are needed to unravel the mechanisms of action of anatabine and its potential use as a drug for the treatment or prevention of autoimmune thyroiditis.

PD05 - CHRONIC LYMPHOCITIC THYROIDITIS (CLT) IN AN AREA CLOSE TO A PETROCHEMICAL COMPLEX: CYTOLOGICAL ASSESSMENT AND CORRELATION WITH HISTOLOGY AND ULTRASONOGRAPHY.

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Introduction. The incidence of Hashimoto's thyroiditis (HT)/CLT has increased over time and environmental pollution could be one reason. HT/CLT is detected more frequently in histologically proven thyroid cancer (TC) compared to benign thyroid lesions (BTL). We evaluated cases of CLT in patients from South-Eastern Sicily referred for US-FNAC, comparing those living close to a large petrochemical complex (zone A) to those living in a control area (city of Siracusa - zone B), located around 15 Km south of the industrial district.

Patients and Methods. We retrospectively evaluated CLT in thyroid FNAC of 391 patients of zone A and compared with those of 622 patients of the control zone B. Then we examined the association between CLT and the histological nature of the nodule (benign [BTL] or malignant [TC]) in thyroidectomized (Tx) patients: 53 [37 females (F), 16 males (M), zone A) vs. 80 (62 F, 18 M, zone B).

Results. Comparisons are given as zone A vs. zone B. CLT was found in about 32% of slides in zone A vs. 23% in zone B (P=0.002). In Tx patients, overall rate of CLT was 33.8% (43.4% vs. 27.5%, P= 0.058). Overall rate of TC was 45.1% (43.4% vs. 46.2%). Within TC, the rate of CLT was 38.3% (52.2%, vs. 29.7%, P= 0.08), in agreement with rates in the preoperative cytological category of high risk for TC (SIAPEC 2014 classes THY3B+4+5 pooled, 47.2% vs. 28.8%, P= 0.07). The rate of CLT in BTL was 30.1%, but unlike TC, there was no inter-zone difference (20.8% vs. 25.6%). By echogenicity, the highest rate of CLT was in the hypoechoic TC nodules of zone A (females, 87.5%), and the lowest in isoechoic either TC or BTL of zone B (males, 12%). Hypoechoic CLT-positive TC nodules in zone A had the smallest maximum diameter (10.1±3.0 mm), while isoechoic CLT-negative BTL had the largest (29.4±15.4 mm). Worthy of note, the distribution of the hypo- and isoechoic nodules differed in zone A vs. B (P= 0.044), as they were similarly represented in zone A (47% vs. 53% isoechoic) while the hypoechoic were underrepresented in zone B (30% vs. 70% isoechoic).

Conclusions. It is the greater rate of CLT, not TC, that contributes to the 2-fold greater extent of association of CLT with TC in the polluted zone compared to the control area. The petrochemical complex-related pollution is an environmental factor involved in the development of CLT and, likely, in the CLT association with thyroid neoplasms; it also influences echogenicity of nodules and its extent of association with CLT and it prompts TC to arise at a relatively low level of growth of the nodule.

PD06 - BIOLOGICAL ACTIVITY OF NOVEL THYROID HORMONE ANALOGUES: ROLE OF NA+ TAUROCHOLATE COTRANSPORTING POLYPEPTIDE IN LIVER SELECTIVITY

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Background. The interest in the potential effect of thyromimetics in lowering serum cholesterol is growing. Thyroid hormone actions on lipid metabolism are exerted in the liver and mediated by the T3 receptor TRβ1. The creation of molecules transported into hepatocytes by liver-specific transporters can increase the liver selectivity of thyromimetics. Sodium taurocholate co-transporting polypeptide (NTCP), a solute carrier protein primarily expressed on the basolateral membrane of hepatocytes, is particularly interesting. **Objectives**. The role of NTCP in the liver preferential uptake of a series of new thyromimetics was analyzed. **Methods**. The compounds to test (KB141, KB5588, KB6628, KB6823, KB3488, KB3493, KB3495, KB4933, KB4956, KB5035, KB5160, KB5359, KB5525, KB5526, KB5866, KB6594, KB8038) were synthesized at Karo Bio AB. To explore the effect of NTCP on the nuclear availability of each compound, COS1 cells were co-transfected with TRβ1, NTCP, a construct coding for a TRE-dependent luciferase reporter and a control renilla reporter. Two days after transfection, cells were incubated for 24 h with 0.1-1000 nM of each compound. Incubation with the same concentrations of T3 was added as a control. The luciferase/renilla ratio was the measure of the compound transcriptional activity. **Results**. Like T3, KB141, KB5588, KB3488 and KB6823 demonstrated no differences in transcriptional activity in the absence or presence of NTCP. KB6628, KB5035, KB5866, KB5160 and KB4956 showed a 1.5-fold higher activity in cells transfected with NTCP compared to cells transfected with empty pcDNA3 vector. KB3493, KB3495, KB5359, KB5525, KB5526, KB4933, KB6594 and KB8038 showed an even greater difference as they had no activity in the absence of NTCP and a 4-fold higher activity in the presence of NTCP. **Conclusions**. NTCP is an attractive transporter to target thyromimetics to the liver.

PD07 - DIAGNOSTIC AND THERAPEUTIC ROLE OF THYROID REMNANT ABLATION WITH LOW ACTIVITY OF 131I IN PATIENTS WITH LOW AND INTERMEDIATE RISK PAPILLARY THYROID CARCINOMA (PTC).

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The real need to perform thyroid residual ablation with ^{131}I (RRA) is nowdays under debate, particularly in patients with low and intermediate risk PTC.

Objective: In this study we evaluated the diagnostic and therapeutic role of this procedure.

Patients: To this purpose we analyzed the data of 548 consecutive patients (pts) with PTC treated with total thyroidectomy, arrived at the Department of Endocrinology of Pisa in 2006 to perform RRA. All patients were treated in hypothyroidism with a standard activity of 30 mCi of ^{131}I (1110 MBq) followed by a Whole Body Scan (pWBS). Neck ultrasound and serum thyroglobulin (Tg) and thyroid hormones measurements were performed in all pts. On the basis of TNM we classified patients in two groups: low risk (LR; n=348) and intermediate risk (IR; n=200).

Results: In addition to the thyroid remnant, the pWBS showed the presence of further areas of ^{131}I uptake in 16/548 (2.9%): 7 LR (2.2%) and 9 IR (4.3%) (p=0.09). The mean value of serum Tg was 86.329±112.398 ng/ml in LR and 242.411±260.779 ng/ml in IR (p=0.1). In 11/16 pts (4 LH, 7 IR) pWBS showed the presence of latero-cervical lymph node metastases, 9 out of 11 were also detected by neck ultrasound followed by fine needle aspiration citology (FNAC). The pWBS showed mediastinal uptake in 1/16 (1 IR), lung metastases in 3/16 (2 LR, 1 IR) and bone metastases in 1/16 (1 LR). Only 7/548 (1.3 %) (5 LR and 2 IR) metastases were detected by pWBS only. At the end of follow-up (median 7.8 years), 8/16 pts were free of disease (5 LR, 3 IR) while the other 8 had persistent disease: 5 “biochemical” disease (1 lung and 4 lymph nodes) and 3 “structural” disease (1 bone, 1 mediastinum and 1 lymph node). Remission was achieved in 4 cases after one single ^{131}I activity, in 1 case after surgical treatment and in the last 3 cases after several ^{131}I courses.

Conclusions: The pWBS after RRA played an important diagnostic role in only 1.3 % of PTC pts with no difference between LR and IR groups. Serum Tg was unable to predict pWBS positive cases. Three out of 3 cases with lung and 8/11 lymphnodes metastases revealed by pWBS have been cured by 131-I. Unfortunately, we do not know what could happen to these subjects, especially those with lung metastases, if the pWBS was not performed

PD08 - METFORMIN REVERTS THE SECRETION OF CXCL8 INDUCED BY TNF-ALPHA IN PRIMARY CULTURES OF HUMAN THYROID CELLS: AN ADDITIONAL INDIRECT ANTI-TUMOR EFFECT OF THE DRUG.

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Metformin displays both direct and indirect anti-tumor effects. CXCL8 is a crucial downstream mediator of Nuclear-Factor-κB signaling related to the growth and progression of thyroid cancers. Targeting CXCL8 results in prolonged survival and reduced metastatic spread in in-vivo animal models of thyroid tumors. Objective: This study aimed to evaluate whether metformin inhibits the secretion of CXCL8 induced by Tumor-Necrosis-Factor-α (TNF-α) in primary cultures of normal and tumor human thyroid cells as well as in thyroid cancer cell lines. Methods: Normal human thyrocytes, papillary thyroid cancer cells, and thyroid cancer cell lines (TPC-1 and BCPAP) were stimulated with TNF-α (10 ng/mL) alone or in combination with metformin (0.01, 0.1, 1, 2.5, 5, and 10mM). CXCL8 levels were measured in the cell supernatants after 24 hours. Results: Metformin significantly and dose-dependently inhibited the TNF-α-induced CXCL8 secretion in both normal thyrocytes (ANOVA: $F = 42.04$; $P < .0001$) and papillary thyroid cancer cells (ANOVA: $F = 21.691$; $P < .0001$) but not in TPC-1 and BCPAP cell lines. Conclusion: Metformin inhibits the TNF-α-induced CXCL8 secretion in primary cultures of normal thyroid cells and differentiated thyroid cancer cells at least of the most frequent poorly aggressive phenotype. The recruitment of neutrophils within the thyroid gland is a crucial metastasis-promoting factor, and it depends on the amount of CXCL8 produced by both tumor cells and by the more abundant normal thyroid cells exposed to TNF-α. Thus, the here-reported inhibiting effect of metformin on TNF-α-induced CXCL8 secretion could be considered as a further indirect anticancer property of the drug.

PD09 - AHR GENE EXPRESSION AND FUNCTION IN PAPILLARY THYROID CARCINOMA

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Background: recent studies demonstrated that aryl-hydrocarbon receptor (AhR) plays a role in promotion and progression of epithelial tumors. We recently demonstrated that AhR protein is overexpressed in papillary thyroid carcinoma (PTC) tissues from acromegalic patients and that this increase is higher in BRAFV600E-mutated PTCs (Mian et al., 2014). To date, AhR role and expression in thyroid differentiated carcinoma has not been clarified yet. **Aim of the study:** the aim of the study was to ascertain AhR role and expression in PTC. **Methods:** AhR gene expression was analyzed in 50 consecutive PTC and in their healthy thyroid tissue, using Real-time, western blot (WB) and immunohistochemistry. Moreover, analyses were performed for BRAF and RAS mutations. 3 different drugs (RAF265, SB590885 and ZSTK474), acting on MAPK and PI3K/Akt pathways, were added, alone or in combination, to 3 thyroid carcinoma cell lines (BCPAP, TT and 8505C). Then, Real-time and WB were performed to evaluate their effect on AhR. BRAV600E mutation effects on AhR activity was also investigated after gene transfection, using a Dual-Luciferase Reporter Asssay System. **Results:** 66% (33/50) of the patients were females, 34% (17/50) were males, with a mean age at diagnosis of 46 years old. At the time of diagnosis, 28/50 were at stage I, 2/50 at stage II, 14/50 at stage III and 6/50 at stage IV. AhR expression was significally higher in PTC than in thyroid normal tissue ($p<0,0001$), especially in patients carrying BRAFV600E mutation ($p<0,0001$). Moreover, BCPAP cell-lines (thyroid papillary carcinoma BRAFV600E-mutated) treated with BRAF-inhibitors (RAF265 and SB590885), showed a significant decrease in AhR protein expression, and this was also confirmed through gene-transfection assays. **Conclusions:** AhR increased expression in BRAFV600E-mutated PTC and his reduction after *in vitro* administration of BRAF inhibitors, both suggest a correlation between AhR and BRAFV600E and so a possible link between thyroid carcinogenesis and environmental xenobiotics.

PD10 - THE CHARACTERIZATION OF NEW THYROTROPIN RECEPTOR MUTATIONS REVEALS THE STRUCTURAL AND FUNCTIONAL ROLE OF TWO HIGHLY CONSERVED RESIDUES.

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Congenital Hypothyroidism (CH) is one of the most frequent congenital endocrine diseases, with a frequency of 1:4000 newborn. Different genes are involved in its pathogenesis, and thyrotropin receptor (TSHR) alterations play a dominant role. In our study a cohort of 85 hungarian patients with permanent CH has been selected and subjected to genetic screening by TSHR direct sequencing. Among others, two new missense variants in highly conserved residues with peculiar characteristics, N432D and P449L, have been identified and characterized. The N432 residue is one of the most conserved in the receptor's first transmembrane helix. Our results showed that N432D variant lead to the production of an abnormal protein not able to reach the cellular membrane, as demonstrated by flow cytometry, microscopy and absence of cleavage in α and β subunits. The characterization of post-translational modifications revealed maturation up to high mannose carbohydrates addition but lack of complex carbohydrates, a situation indicative of ER early retention and absence of golgi translocation. Moreover, despite TSHR degradation usually occours through proteasome, this mutant is also eliminated through lysosomes. P449 residue is localized in the first intracellular loop of the receptor that is relevant for interaction with protein G. Our data demonstrated that P449L variant follows a maturation process similar to wild type receptor, with an even slightly higher membrane expression. Nevertheless, both receptor-associated signalling pathways are altered, with stronger deleterious effects on Gq11/PLC compared to Gs/cAMP. In conclusion, N432 and P449 residues are fundamental for the correct maturation and the signal transduction processes, respectively. N432D variant significantly alters protein maturation and degradation while P449L variant severely impairs receptor functionality.

PD11 - LONG –TERM OUTCOME OF PAPILLARY THYROID CANCER PATIENTS WITH BIOCHEMICAL INCOMPLETE RESPONSE

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Background: Papillary thyroid cancer (PTC) patients are classified as in remission or with persistent disease according to thyroglobulin (Tg) testing and imaging. The finding of abnormal serum Tg values without structural evidence of disease (SD) on imaging studies can rely on either normal or malignant thyroid remnants, and is called biochemical incomplete response (BIR). The aim of this study is to evaluate the outcome of patients with BIR 6-12 months after initial treatment (IT). **Methods:** A consecutive cohort of 504 PTC patients was retrospectively collected according to the following inclusion criteria: total thyroidectomy + radioiodine remnant ablation; TSH-stimulated Tg testing (TSH/Tg) about one year after IT and a second time during follow-up (F/U); yearly basal Tg and neck ultrasound; negative Tg antibodies. BIR was defined as basal Tg ≥1 ng/mL and/or TSH/Tg >2 ng/mL. **Results:** At the time of first F/U assessment, 329 (66%) patients had no evidence of disease (NED), 88 (17%) patients had SD, and 87 (17%) patients had BIR. The latter cases served as the study cohort. They were initially classified as low (59%) or intermediate (41%) risk of recurrence according to American Thyroid Association guidelines, and they had a median F/U of 11 (3-25) years. Spontaneous Tg decline below the abnormal threshold was observed in 76% of the patients based on a second TSH/Tg testing, and in 97% according to basal Tg measurement. Only 2 (2.3%) patients were found with SD during F/U. **Discussion:** The majority of patients with BIR had a spontaneous decline of Tg values. Detection of SD was rare. Given the excellent prognosis, in these patients, over treatment would be more harmful than beneficial.

PD12 - INTERMEDIATE RISK DIFFERENTIATED THYROID CANCER (DTC) PATIENTS SHOW THE SAME RATE OF ABLATION OF LOW RISK DTC PATIENTS WHEN TREATED WITH 30 MCI OF 131I AFTER STIMULATION WITH RECOMBINANT HUMAN TSH (RHTSH)

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Background: Radioiodine remnant ablation (RRA) is, along with total thyroidectomy, the initial treatment of patients with differentiated thyroid carcinoma (DTC). Nowadays, there's a general agreement to reduce the treatments with ^{131}I, and if needed, to use the lowest effective activity.

Methods: We evaluated data of 505 patients with DTC (272 with low and 233 with intermediate risk) at the time of remnant ablation (30 mCi of ^{131}I after rhTSH) with anti-thyroglobulin antibodies (TgAb) <20 U/ml. We excluded from our study "very low" and "high" risk DTC patients. Patients were reassessed after 12 months of follow-up and those with undetectable thyroglobulin (Tg) (baseline if ultrasensitive or <1 ng / ml when stimulated with rhTSH) and negative neck US were considered ablated and their response to initial therapy was defined as "excellent" according to the new guidelines.

Results: as expected, in the intermediate-risk group there were more males ($p = 0.037$), Tg values at the time of ablation were higher ($p = 0.02$) and lymph node metastases were more frequent ($p = 0.008$) with respect to the low-risk group. Other epidemiological and clinicopathological parameters were similar in the two groups. Although a slightly difference in the rate of ablation was observed, 199/267 (74.5%) low-risk pts and 154/225 (68.5%) intermediate risk pts showed an excellent response with no apparent statistical difference ($p = 0.136$).

Conclusions: 1) In this group of patients with DTC treated with total thyroidectomy, RRA with 30 mCi of ^{131}I after rhTSH has been equally effective in terms of remission of disease in both low and intermediate-risk pts; b) Intermediate-risk pts at the time of RRA have higher Tg values and more lymph node metastases at neck US, but this does not seem to affect the excellent response to initial therapy

PD13 - HYPONATREMIA IMPROVEMENT IS ASSOCIATED WITH A REDUCED RISK OF MORTALITY: EVIDENCE FROM A META-ANALYSIS

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Background: Hyponatremia is the most common electrolyte disorder encountered in clinical practice and it is associated with increased morbidity and mortality. However, there is no clear demonstration that the improvement of serum sodium concentration ($[Na^+]$) counteracts the increased risk of mortality associated with hyponatremia. The aim of our study was to perform a meta-analysis that included the published studies that addressed the effect of hyponatremia improvement on mortality.

Methods: A Medline, Embase and Cochrane search was performed to retrieve all English-language studies of human subjects published up to June 30th 2014, using the following words: "hyponatremia", "hyponatraemia", "mortality", "morbidity" and "sodium". Fifteen studies satisfied inclusion criteria encompassing a total of 13,816 patients. The identification of relevant abstracts, the selection of studies and the subsequent data extraction were performed independently by two of the authors, and conflicts resolved by a third investigator.

Results: When all studies were considered, any improvement of hyponatremia was significantly associated with a reduction of overall mortality (OR=0.57[0.40-0.81]). The results were even more impressive by performing a sensitivity analysis, which considered only those studies (n=8) reporting a threshold for serum $[Na^+]$ improvement to $\geq$130 mmol/L (OR=0.51[0.31-0.86]). The favorable effect of hyponatremia improvement on mortality rate was confirmed in those studies (n=4) reporting data at 12 months of follow-up (OR=0.55[0.36;0.84]), and a trend toward a reduction of mortality rate was observed at 36 months (n=3, OR=0.67[0.45;1.02]). Meta-regression analyses showed that the reduced mortality associated with hyponatremia improvement was more evident in older subjects and in those with lower serum $[Na^+]$ at enrollment.

Conclusions: This meta-analysis documents for the first time that the improvement of serum $[Na^+]$ in hyponatremic patients is associated with a reduction of overall mortality.

PD14 - THE ARYL HYDROCARBON RECEPTOR (AHR) REPRESSOR EXPRESSION CORRELATES WITH THE IN-VITRO AGGRESSIVENESS OF A CELLULAR MODEL OF GH-SECRETING PITUITARY ADENOMA

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Aryl Hydrocarbon Receptor (AHR) Repressor is a bHLH/Per-ARNT-Sim transcription factor that modulates the dioxin receptor AHR. AHRR inhibits AHR functions by competing for dimerization with the AHR nuclear translocator ARNT, thus preventing its binding to promotorial elements of genes mostly involved in cellular proliferation and differentiation. AHRR downregulation has been reported in human malignancies including colon, breast and lung cancer. In-vivo and in-vitro studies on several cancer cell lines demonstrated that a reduced AHRR expression led to an increased malignant phenotype associated with resistance to apoptosis, increased cellular motility and angiogenic potential. In pituitary tumors a decreased expression of AHR has been associated with a more invasive phenotype. In addition, mutations in the AHR interacting protein AIP, have been found in families with pituitary adenomas predisposition and young acromegalic patients. Aim of this study was to correlate AHRR expression levels with the development of cellular malignant phenotypes in the somato-lactotroph cell line GH3.AHRR was stably silenced in GH3 by shRNA approach. Two different stable clones were selected through mRNA and protein expression evaluation by qPCR and western blot respectively. MTT viability assay, the ^{3}H-thymidine incorporation assay, clonogenic assay, cytofluorimetric analysis and caspase 3-7 assay were performed to evaluate the effects of AHRR knocking down. All experiments were performed also in presence of 50 nM α- and β naphtoflavone (α/β NF), two well-known AHR modulators.

MTT assay demonstrated a decreased viability of nearly 40% compared to non-silenced controls regardless of α- NF or β-NF treatment ($p<0.01$). A similar less aggressive behavior was observed in clonogenic assay, in which knocked down cells showed a reduced capability to form colonies compared to non-silenced controls (35% ± 20%, $p<0.01$). AHRR-silenced clones showed an increased DNA synthesis after 48h (42%± 3%, $p<0.01$), while AHRR expression changes apparently do not alter cell cycle progression as observed in a cytofluorimetric assay. Moreover, after 8h Camptothecin treatment silenced cells showed to be more prone than the non-silenced to undergo apoptosis.

The data presented herein demonstrated that, unlike other malignanciens, in a cellular model of GH-secreting pituitary adenoma, AHRR could have oncogenic potential. Although the promising results obtained in this study, further investigations are needed to clarify the magnitude of AHRR expression possibly in somatotropinomas derived primary cells, and its potential use as therapeutic target

for GH-secreting pituitary adenomas.

PD15 - OCCURRENCE OF HYPOGONADOTROPIC HYPOGONADISM IN PATIENTS WITH PREVIOUS AUTOIMMUNE GROWTH HORMONE DEFICIENCY

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Some GH-deficiencies, previously considered as idiopathic, have been seen to be caused by autoimmune hypophysitis with pituitary antibodies directed to GH-secreting cells. Twenty-two adults with GH deficiency (GHD) diagnosed since childhood, whose pituitary function and antipituitary antibody (APA) pattern have been investigated before starting replacement therapy with recombinant GH (which was continued until reaching their adult age) were retested one year after the stopping of rGH treatment to investigate the possible variation of their pituitary function and autoantibody pattern.Pituitary function and presence of antipituitary antibodies (APA) by immunofluorescence had been investigated in all patients at diagnosis and subsequently one year after the stopping of rGH treatment. Sera of patients positive for APA were retested by double imunofluorescence to identify the pituitary cells targeted by these antibodies. A magnetic nuclear resonance (MR) of hypothalamic-pituitary region had been performed at diagnosis and repeated after stopping of therapy .At diagnosis, 16 out of 22 patients had shown presence of APA at high titres, identified by double immunofluorescence as targeting only GH-secreting cells (group 1), while the remaining 6 had been found APA negative (group 2). When retested after stopping of therapy, 12 out of 16 patients in group 1 persisted APA positive, while the remaining 4 became negative with recovery of pituitary function. None of 6 patients in group 2 resulted APA positive despite 5 of them showed still GHD. Among the 12 patients persisting APA positive, 5 showed APA still targeting only GH-secreting cells associated with persisting isolated GHD. Instead, 3 showed APA targeting both GH and gonadotropin-secreting cells, associated with combined GH deficiency and delayed puberty or hypogonadotropic hypogonadism (HH), 4 showed APA targeting only gonadotropin-secreting cells associated with isolated pubertal delay or HH. Our results indicate that an autoimmune aggression to pituitary cells in childhood, despite involving at start only GH-secreting cells causing isolated GHD, may subsequently involve other pituitary-secreting cells, particularly gonadotropin- secreting ones, causing delayed puberty or more severe HH.

PD16 - ENDOCRINOLOGICAL STUDY OF PATIENTS AFFECTED BY MIOTONIC DISTROPHY TYPE 1

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Myotonic dystrophy type 1 (MD1) is an autosomal dominant inherited progressive disorder. It is caused by trinucleotide repeat expansions (CTG) in the *DMPK* gene, located on chromosome 19q13.3. Adult onset dystrophy begins with muscle weakness and myotonia that affect not only skeletal muscle, but also smooth muscles. Other elements are insulin-resistance, heart troubles with conduction defects, respiratory dysfunction, daytime sleepiness, mild cognitive impairment, liver abnormalities and endocrine disorders.

Aim of our study was to go deeper into current endocrinological and andrological knowledge as concerning MD1.

We measured the concentration of a large number of hormones in venous blood comparing 22 patients with genetically confirmed MD1 (Group 1; mean age 40,32 ± 11,86 SD) with 200 healthy subjects (Group 2; mean age 38,45 ± 13,69 SD); we also perform testicular and thyroid ultrasound in MD1 group.

Mann-Whitney U test showed a significant difference between MD1 men and control subject in terms of thyroid and gonadic function. 25% of the MD1 men had CT levels over the normal range. Both MD1 men and women had ACTH (20% of men and 16,6% of women) and cortisol (20% of men and 8,33% of women) levels meaningfully higher than the control group. 20% of MD1 men had also high values of IGF1 and 10% of PTH. The last one was above the upper normal limit also in 16,6% of MD1 women. In all MD1 ≤40 years old men FSH values were over the upper normal limit and INHB values were below the lower normal limit. In this subgroup LH was high in 80% and PRL in 40%. Testosterone was reduced in 60% of MD1 men, free testosterone in 100%, E2 in 20%, DHEAS in 60% and Δ^4 increased in 20%. In ≥41 years old MD1 men, E2 was reduced in 40%, FSH increased in 40% and LH in 20 %, INHB was reduced in 100%, PRL increased in 20%, testosterone reduced in 40%, free testosterone and DHEA-S were reduced in 80%. None of MD1 ≤45 years old women was in menopause: E2 values were below the lower normal limit in 50% of these patients, FSH was reduced in 33,3%, LH was reduced in 33,3% and increased in 16,6%, INHB and testosterone were reduced in 33,3%, DHEA-S was reduced in 16,6%. In the ≥46 years old MD1 subgroup, 3 women were in menopause. Among fertile women, 100% had INHB levels below the normal range and 33,3% low E2 levels. Within the whole subgroup, PRL and Δ^4 were increased in 16,6%, DHEA-S was reduced in 66,6%.

There is a high frequency of endocrine abnormalities in MD1 patients. These findings lead to emphasize the importance of endocrine monitoring and screening in this population, in order to start the fitting therapy.

PD17 - EXPOSURE TO BENZENE MODIFIES SSTR2-ZAC1 SIGNALLING PATHWAY IN GH3 PITUITARY ADENOMA CELLS

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Higher prevalence of acromegaly in greatly polluted areas suggests the involvement of environmental factors in the pathogenesis of pituitary tumors. We recently reported that the pollutants polyclorobiphenyl, bisphenol-A , 1,1-dicloro-2,2-bis(clorophenyl)ethane, di-2-etylesylphtalate, benzene (BZ) and methylparaben do not modify the viability and the proliferation of rat pituitary adenoma cells GH3, but they modify the gene expression of the growth hormone (GH), the somatostatin receptor type 2 (SSTR2), the zinc-finger protein ZAC1 and the aryl hydrocarbon receptor (AHR) interacting protein AIP. These alterations could explain the more aggressive behavior of GH-secreting adenomas in highly polluted areas and sustain their resistance to SSA. In the present study we evaluated the effects of BZ, one of the most common environmental pollutants. GH3 cells were treated with 10 ng/l BZ up to 72 hours. At different times, we evaluated the expression of GH, AIP, AhR, SSTR2 and ZAC1. After 72 hours, BZ caused a significant increase of GH and SSTR2 and a reduction of ZAC1. In addition, after 24 hour exposure, an increase of AHR and a reduction of AIP were observed. In a second series of experiments, GH3 viability was evaluated after treatment with 10^{-9}-10^{-6} M octreotide in the absence and in the presence of 10 ng/l BZ. Octreotide reduced GH3 cell viability, but this effect was lost in the presence of BZ. Our data, even still preliminary, suggest that BZ can modify the biological behavior of GH-secreting pituitary cells, changing their sensitivity to octreotide.

(Grant PRIN, AA 2010-2011 cod-DI1112000360001).

PD18 - DISEASE CONTROL CRITERIA IN ACROMEGALY PATIENTS DURING SOMATOSTATIN ANALOGUES (SSA): GH PROFILE OR RANDOM GH?

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Introduction: acromegaly is due to increased GH secretion usually sustained by a GH-secreting pituitary adenoma. It is well known that SSA can control GH hypersecretion in 60% of patients and tumor volume in 30%. Control of hormonal disease activity is associated with normal mortality rate and therefore to verify the optimal biochemical control is of critical importance to adapt the dose and the choice of alternative treatment. GH levels less than 2.5 ng/l and normal for age IGF-I levels have been suggested to be safe biochemical parameters for the control of acromegaly. In 2010 a Consensus recommended new biochemical criteria: random GH<1 ng/ml and normal for age IGF-I levels. Methods: we compared the reliability of mean GH profile (GHP)<2.5 and random GH (GHR)<1 ng/ml as good marker of disease activity in acromegaly; we also evaluated the association between GH levels (mean and random), IGF-I and IGFBP-3 levels. To this goal in an observational and retrospective study, we enrolled 34 SSA responder acromegaly patients (25 F,33-86 years). The clinical response had been defined by normal IGF-I levels and no clinical activity. In all subjects the dose of SSA had been stable in last 2-5 years. In all subjects in phase 1 (before 2010) mean GHP, IGF-I and IGFBP3 and in phase 2 (after 2010) GHR, IGF-I and IGF-BP3 had been evaluated. Statistical analysis was performed using Wilcoxon test for the comparison of GH profile and GH random, while the correlation between GH and IGF-I and BP3 levels was analyzed by Spearman test. Results: both mean profile (2.2 ng/ml) and random GH (1.17 ng/ml) correlates with IGF-I levels (186.8 ng/ml and 175.0 ng/ml; p<0.01). In all subjects in both phases of the study IGF-I (phase 1: 186.8±10.0; phase 2: 175.0±37.3 ng/ml) and IGFBP-3 (phase 1: 2.7±0.1; phase 2: 2.5±0.1 μg/dl) levels were normal for age. GHR (2.2±0,48 ng/ml) levels were similar (p=0.1) to GHP (1.17±0.57 ng/ml). Concordance between GHP<2.5 ng/ml and normal IGF-I (85.3% of patients) was significantly different (p<0.01) to that of GHR<1 ng/ml and normal IGF-I (29.4%). Conclusions: our study shows that in SSA responder acromegaly patients, GHP<2.5 ng/ml better than GHR<1.0 ng/ml correlate with normal IGF-I levels, thus indicating that evaluation by GHP would more reliably reflect an appropriate disease control.

PD19 - EFFECTS OF URSODEOXYCHOLIC ACID THERAPY ON CHOLELITHIASIS PREVENTION IN ACROMEGALIC PATIENTS TREATED WITH SOMATOSTATIN ANALOGS

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Cholelithiasis, the most serious adverse effect of somatostatin analogues (SSA) therapy, has been reported in 5-60% of acromegalic patients after 2 years of therapy. SSA-induced gallstones (GS) are effectively dissolved by ursodeoxycholic acid (UDCA) in a high percentage of patients, when administered with the evidence of GS. However, to date, no study has evaluated the effects of UDCA started together with SSA, in order to prevent GS. The current study aimed at investigating the effects of UDCA in preventing GS development in acromegalic patients treated with SSA. Sixty-four patients (35 F, 29M, mean age 48.5 years) from two major referral Italian centers entered the study. BMI, lipid fractions, GH, IGF-I and gallbladder ultrasound were evaluated in 24 patients receiving UDCA at the SSA starting (Group A) and in 40 age-matched patients treated with SSA only (Group B). In the whole patient cohort, GS occurred after a median SSA duration of 24 months. GS prevalence was significantly lower in Group A (4.1%) than in Group B (35%, p=0.01) whereas biliary sludge prevalence was similar in both groups (4.1% in Group A, 5% in Group B, p=0.6). Cholecystectomy was required in none of Group A and in 7.5% of Group B (1 receiving high-dose SSA, p=0.4), whereas UDCA was required in 17.5% of Group B and in 4.1% of Group A. GS were asymptomatic in 86.6% of cases. SSA duration (p=0.9) and prevalence of dyslipidemia (p=0.6) were similar in both groups, whereas obesity prevalence was higher in Group B (45%) as compared to Group A (20%, p=0.01). However, GS prevalence was similar among patients of different BMI quartiles. At GS diagnosis, biochemical control was achieved in 73.3% of cases (26.7% on high-dose SSA). In conclusion, UDCA administration at SSA starting seems able to reduce GS development in acromegaly, suggesting its use as potential preventive therapy in routine clinical practice.

PD20 - EFFICACY AND SAFETY OF LOW DOSE TOLVAPTAN IN THE TREATMENT OF HYPONATREMIA DUE TO SIADH AFTER SEVERE TRAUMATIC BRAIN INJURY

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Hyponatremia is the most common electrolyte disorder encountered in clinical practice and represents a clinical and social burden. The vasopressin receptor antagonist, Tolvaptan, is a new and interesting drug for the treatment of the syndrome of inappropriate ADH secretion (SIADH). We describe 4 cases of hyponatremia secondary to SIADH in patients with severe traumatic brain injury (TBI) and post-traumatic epilepsy in 3 cases. Three patients (2 M, 1 F) developed hyponatremia in the post-acute phase of TBI, during hospitalization in the Intensive Rehabilitation Unit (IRU), admitted in state of extremely severe disability. Hyponatremia was associated with seizure and continuous drowsiness in 2 cases, and with drowsiness and poor motor initiative in 1 case, causing reduced response to the rehabilitation. Hyponatremia was initially treated with sodium supplementation (i.v. or enteral) and/or water restriction, without complete benefit. After endocrinologist consultation, hypoadrenalism and hypothyroidism were excluded and SIADH was diagnosed. Therapy with oral Tolvaptan was started (5 mg/die for 5-30 days, then 7.5 mg/die) with normalization of serum sodium, disappearance of seizures and amelioration of neurological picture. Two patients continued Tolvaptan (7.5 mg/d or 7.5 mg every 2 d) for 6-8 months: cognitive and motor functioning scales slightly improved, but they still presented severe disability at discharge of the IRU. One patient continued low dose of Tolvaptan for 3 months and after drug withdrawal serum sodium remained stable. This patient had a good response to the rehabilitation, presenting moderate disability at discharge from IRU. No side effects of the drug were observed. The fourth male patient presented hyponatremia associated with central adrenal insufficiency during the post acute phase of severe TBI. Thereafter, adrenal function returned to normal without seizures for 2 yr. Three years later, seizures reappeared associated with hyponatremia due to SIADH. Adrenal function persisted normal. Tolvaptan was started (15 mg/d for 5 d, 7.5 mg/d for 10 d, then 7.5 mg for 4 d/wk) and no more seizures appeared. The patient is undergoing therapy with low dose Tolvaptan since 12 months, without side effects. We demonstrated that an adequate treatment of hyponatremia is associated with neurological improvement and lack of seizure in TBI patients. Low dose Tolvaptan is safe and effective in the treatment of hyponatremia due to SIADH after severe TBI.

PD21 - COMPARATIVE GH/IGF-I SYSTEM ASSESSMENT IN SYSTEMIC LUPUS ERYTHEMATOSUS AND SYSTEMIC SCLEROSIS

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Different rheumatic and musculoskeletal disorders, including osteoarthritis (OA), rheumatoid arthritis (RA), diffuse idiopathic skeletal hyperostosis, as well as fibromyalgia are characterized by elevated circulating GH levels. Furthermore, reduced somatostatin levels seem to be strongly associated with joint inflammation (as seen in RA), as well as in elderly patients with the inflammatory complications of knee OA. Previous studies have also suggested that alterations in this hypothalamic/pituitary axis may contribute to systemic lupus erythematosus (SLE) progression. Similarly, IGF-I is reported involved in the development of systemic sclerosis (SS). Against this background, we have measured inflammatory markers (VES, CRP), renal function, 25(OH) vitamin D, IGF-I, IGFBP3 and ALS levels in age comparable female patients affected by SS (N=11, medium age 59,45 years ± 13,35 SD) or SLE (N=7, medium age 49,42 years ± 16,24 SD). Exclusion criteria were uncontrolled diabetes mellitus, severe cardiomiopathy, patient younger than 30 or older than 75 years. The two groups were also compared for their chronic treatments and antropomethric parameters. No statistically significant differences in VES, CRP, 25(OH) vitamin D levels and renal function were found between the two groups. Conversely, SLE patients showed an higher activity of GH/IGF-I system compared to SS group. Indeed, differently from the other elements of the ternary complex, ALS values resulted significantly higher in SLE patients (p=0,0327). The detection in the 2 study groups of comparable IGF-I and IGFBP3 values could be explained by the fact that patients with SLE were treated with corticosteroids. These medications are known to mask GH action on IGF-I and, by consequence on IGFBP3 levels, while the corticosteroid effect on ALS seems not significant. In conclusion, GH/IGF-I could play a role in different rheumatic diseases. Our preliminary data show a possible hyperactivation of GH/IGF-I system in SLE. These results, if confirmed, could have future implications on medical treatment of this disease, reason why the recruitment of a larger caseload is ongoing.

PD22 - FLAVOR IMPAIRMENT: A NEGLECTED SENSORINEURAL DISABILITY IN PATIENTS WITH KALLMANN SYNDROME

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Background. Kallmann Syndrome (KS) associates congenital hypogonadism to olfactory impairment. Our aim was to evaluate flavor function and flavor-related handicap in KS patients.

Patients and Methods. 30 patients with KS, 12 with normosmic hypogonadism (nIHH), 24 with acquired anosmia (AA) and 58 healthy controls entered the study. All participants filled questionnaires for dietary habits, olfaction-related quality-of-life, and self-determined smell, flavor and taste abilities prior to undergo standardized olfactometry and gustometry. Each subject underwent flavor test, developed with orally-administered aqueous aromatic solutions, consisting in identifying 21 different compounds by choosing each out of five alternative items. Flavor score (FS) was calculated as the sum of correct answers (range 0-21).

Results. Flavor perception by self-assessment was similar between KS, nIHH and controls, and was largely reduced in only AA. FS was similar between KS (5.4±1.4) and AA (6.4±1.9), and lower than nIHH (16.2±2.4,$p<0.001$) and controls (16.8±1.7,$p<0.0001$). FS showed strong reproducibility and correlated with olfactory scores in the overall population. KS and AA patients identified aromatics eliciting trigeminal stimulation better than pure odorants. Olfaction-related quality-of-life was more impaired in AA than in KS.

Conclusions. This is the first report showing flavor impairment in KS. This contrasts with what generally evidenced in routinely clinics, since KS patients, contrarily to AA, do not complain flavor inability, perhaps owing to the congenital nature of dysfunction. Flavor injury should be accounted as a specific KS impairment, because of important detrimental effects on physical and mental health and on quality-of-life. KS patients might be apprised of flavor inability also to prevent unexpected and life-threatening accidents.

PD23 - THE ROLE OF PROCONVERTASE 1/3 IN THREE CASES OF TRANSFORMATION OF SILENT CORTICOTROPH-CELL ADENOMA INTO ACTH-SECRETING ADENOMA ASSOCIATED WITH CUSHING'S DISEASE.

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Introduction. Corticotroph-cell adenomas may sometimes change their pattern of hormonal secretion, but the mechanisms responsible for these transformations have not been completely elucidated yet.

Methods/design. We retrospectively reviewed the records of 1259 consecutive endoscopic endonasal surgical procedures for pituitary adenomas from 1998 to 2013. Of these, 132 were ACTH-secreting adenomas associated with Cushing's disease (CD) and 44 were silent corticotroph-cell adenomas (SCA). During the follow-up, 7 patients (4 men and 3 women) showed a transformation of their clinical expression from SCA to CD or, more rarely, vice versa. Of these, to date only 3 patients with corticotroph-cell adenomas changing their pattern of hormonal secretion during the follow-up were re-operated. Then, we examined the expression of proconvertase 1/3 (PC1/3), which plays an important role in the POMC processing within the pituitary, in tissue specimens obtained from these 3 patients with SCA that had developed clinical and laboratory features of Cushing's disease at the time of recurrence, using both immunohistochemistry and quantitative real time-polymerase chain reaction.

Results. The data indicated that the immunohistochemical PC1/3 expression was negative or weak and focally positive in tissue specimens obtained in the 3 patients at the time of first operation (SCA), whereas we observed a strong expression in the majority of the neoplastic cells in the same 3 patients at the time of recurrence, when they had become CD. The PC1/3 expression, as evaluated using immunohistochemistry, showed a significant correlation with the PC1/3 levels obtained by qRT-PCR in assessing the increase of PC1/3 expression from SCA to CD. Twelve cases of SCA without changing their phenotype during the follow-up were used as controls: both the immunohistochemical PC1/3 expression and level of PC1/3 obtained by qRT-PCR were absent or weak in scattered neoplastic cells.

Conclusion. The study provides insight into the role of prohormone convertase 1/3 (PC1/3) in the transformation of phenotype from SCA to CD. Though rare, the possible change in the pattern of hormonal secretion by pituitary tumours is a very intriguing issue indeed, especially in the case of corticotroph-cell adenomas.

PD24 - PREVALENCE OF FAMILIAL ISOLATED PITUITARY ADENOMAS (FIPA): A SINGLE CENTER EXPERIENCE

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Less than 5% of pituitary adenomas (PA) are estimated to develop in a genetic setting, mostly in a Multiple Endocrine Neoplasia type 1 (MEN1) or Familial Isolated Pituitary Adenomas (FIPA) context. About 15% of FIPA have been associated with germline mutations in the *AIP* gene, mainly young onset GH/PRLsecreting PA. However, the prevalence of FIPA is not well defined. **Material and methods**: A series of 501 PA patients followed at the Neuromed Institute was systematically asked for documented cases of PA in their kindred. Patients were characterized for tumour phenotype, age at diagnosis and tumour volume. Screening for primary hyperparathyroidism was performed in most patients, including all familial cases. **Results**: Overall 46/493 (9.3%) patients had some familial history of PA (no familial information in 8 cases). This prevalence was reduced to 31/493 (6.3%) when only the probands were considered. There were one genetically proven MEN1 kindred with prolactinomas (n=3), one MEN1-like kindred (with acromegaly and NFPA) and 29 FIPA kindreds. Therefore, FIPA patients and probands respectively represented 41/493 (8.3%) and 29/493 (5.9%) of the whole series. FIPA kindreds included 12 homogeneous kindreds (2 with acromegaly, 10 with prolactinomas), 10 heterogenous kindreds with different phenotypes, the others are awaiting final classification. Overall, patients with a familial history of PA were affected by prolactinomas – 26/262 (10%) -, acromegaly – 11/59 (18%), NFPA -8/150 (5%) or Cushing's disease -1/21 (4%), with a non-significant female predominance (33/328 F vs 12/163 M, Pns). The median age at diagnosis in familial cases (29 yrs, range 9-77) was significantly lower than in sporadic cases (38 yrs, range 8-82)(P=0.0044 by Wilcoxon). Germline AIP mutations were found in the 2 families with homogeneous somatotropinomas, whereas AIP polymorphisms were observed in 2 heterogeneous kindreds and no AIP changes were found in 9 additional kindreds. All AIP^{mut} patients had young onset macro-somatotropinomas. **Conclusion**. This case-finding study suggests that familial forms of PA, especially FIPA, are more frequent that usually reported, and a familial history should be searched for in all PA patients. Although the relatively low MEN1 prevalence in this series may be explained by recruitement biais since the primary pituitary presentation of MEN1 occurs in a minority of cases, FIPA appear to be much prevalent than MEN1 in the PA population. This should be usefully kept in mind for the management of PA patients and their relatives.

PD25 - TADALAFIL MODULATES AROMATASE ACTIVITY AND ANDROGEN RECEPTOR EXPRESSION IN HUMAN OSTEOBLAST IN VITRO

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Introduction: Tadalafil (TAD) administration in men with erectile dysfunction is associated with increased testosterone / estradiol (T/E_2) ratio due E_2 reduction. Since this molecule is used in chronic in urologic patients, it might imply potential effects in other tissues.

Aim: To characterize the effects of the exposure of human osteoblasts to the selective Phosphodiesterase type5 (PDE5) inhibitor TAD to evaluate changes in osteoblasts homeostasis.

Methods: Human osteoblast-like cells SAOS-2 (OBS) were cultured *in vitro* and tested for viability during growth. Cells were then treated with/without different concentrations of TAD for several time points.

Main outcome measure: PDE5, Aromatase (ARO), androgen (AR) and estrogen (ER) receptor, P1NP osteoblastic markers and Wnt/b-catenin protein pathway expression were determined in OBS *in vitro* by RT-PCR, western Blot, ELISA assay.

Results: OBS expressed significant levels of PDE5 transcript further confirmed by the presence of protein. Exposure of cells to increasing concentrations of TAD (10^{-8} - 10^{-6} M) significantly decreased both PDE5 mRNA and protein expression and also inhibited ARO mRNA and protein expression. Accordingly to the inhibition of ARO, it was observed an increase in T levels in the supernatants. Moreover, TAD significantly decreased total ER and AR receptors ratio mRNA, increased AR nuclear translocation, suggesting a preferential androgenic vs. estrogenic pathway activation. OBS markers were modulated upon TAD exposure. TAD also increased cell proliferation indicating, along with differentiation markers, a modulation of osteoblast homeostasis.

Conclusions: We firstly demonstrated that TAD inhibits ARO activity determining an increase in testosterone levels. Interestingly, TAD increased AR protein and its nuclear translocation, strongly suggesting a new control pathway for target tissues. In conclusion, these findings might be important in the holistic clinical evaluation of patients who might need long-term PDE5i treatment.

PD26 - CHRONIC PDE5 INHIBITORS AND ENDOTHELIAL FUNCTION IN MEN WITH T2DM: RESULTS FROM A METANALYSIS STUDY

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OBJECTIVE: Diabetes mellitus (DM) is associated with endothelial dysfunction, because of reduced nitric oxide-dependent vasodilation and increased production of pro-inflammatory factors, leading to increased long-term cardiovascular risk. Since the effects of phosphodiesterase-5 inhibitors (PDE5i) on endothelial function have not been systematically investigated, we conducted a meta-analysis of the available randomised clinical trials (RCTs).

DESIGN: A thorough search of the literature was carried out. Relevant studies were considered according to RCT study design, enrolment of men with Type 2 DM, chronic administration of PDE5i and evaluation of endothelial function through both hemodynamic and endothelial inflammation-related parameters using the Cochrane criteria.

RESULTS: A total of 15 studies were eligible, but only six RCTs met the inclusion criteria and were analysed. They included 476 diabetic men, 239 randomized to the PDE5i Sildenafil and 237 to placebo. Four RCTs evaluated flow-mediated dilation (FMD) demonstrating a weighted mean increase of 2.19% (95%-confidence interval: 0.48-3.90). However, this result showed a high degree of variability (I2 98%). After sensitivity analysis and exclusion of two studies through funnel plot, a new analysis confirmed a significant, Sildenafil-related FMD improvement of about 5.88% (3.19-8.37). Sildenafil improved endothelin-1 and high-sensitivity C-reactive protein of about -0.94 pg/mL and -0.36 mg/L, respectively, but statistical significance was not reached (p=0.69 and p=0.22, respectively). Finally, chronic Sildenafil administration significantly reduced interleukin (IL)-6 serum levels (-0.82 pg/mL) (confidence interval: -1.58 to -0.07).

CONCLUSION: For the first time we demonstrate an overall beneficial effect of chronic Sildenafil administration on endothelial function in diabetic men. Chronic Sildenafil treatment improves FMD and IL-6, leading to a beneficial effect on hemodynamic and serum pro-inflammatory makers. Larger prospective, longitudinal studies aiming at confirming the effect of chronic PDE5i on endothelial function should focus on these parameters.

PD27 - INFERTILE MEN HAVE FREQUENTLY LEYDIG CELL DYSFUNCTION: STUDY ON HYPOGONADISM, VITAMIN D AND BONE MASS IN 5177 SUBJECTS

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Male factors are responsible for half of the cases of couple infertility. Whatever the cause, spermatogenic disruption is clinically and hormonally recognized by low sperm count and Sertoli cell markers. However, recent evidence showed that Leydig cell impairment is also frequent in subjects with primary testicular damage, as evidenced for example by reduced INSL3 and 25(OH)-vitamin D levels. The latter is caused by reduced expression of CYP2R1, a major enzyme involved in 25-hydroxylation of cholecalciferol, and lower 25(OH)-vitamin D levels are well known cause of low bone mass. Furthermore, testosterone (T) production by the Leydig cells might be also impaired in men with primary spermatogenic damage. To clarify these aspects that previous reports analyzed only separately and on limited number of subjects, in this study we evaluated the presence and type of hypogonadism, 25(OH)-vitamin D status and bone mass in a very large cohort of infertile males. Among subjects referred to our tertiary Universitary Centre for semen analysis during the period January 2011-June 2014 (11,516 semen analysis) we report here the data of men who completed the andrological program, including semen culture (n: 10,394), history and physical examination (n: 7,527), hormone analysis (FSH, LH, T, 25(OH)-vitamin D; n: 5,884), and ultrasound of the testes (n: 5,177). Men with total sperm count <10 million/ejaculate (n: 2,583) underwent also genetic analysis (karyotype, Y chromosome microdeletions, CFTR mutations; n: 2273) and DEXA (n: 855). Azoospermia was present in 9.3% of cases (n: 481/5,177), oligozoospermia (with or without reduced motility and/or normal sperm morphology) in 40.6% (n: 2302), asthenozoospermia in 12.2% (n: 632), and normozoospermia in 34.5% (n: 1787). Main causes or risk factors were varicocele (28%), genetics (15%), obstruction/sub-obstruction of seminal tract (12%), cryptorchidism (6%), infections/iatrogenic causes/ejaculation disorders/prior surgery (14%) and idiopathic forms (25%). Primary hypogonadism (T<10.4 nmol/L, LH>8 IU/L) was found in 25.7% of cases, secondary hypogonadism (T<10.4 nmol/L, LH<1.5 IU/L) in 1.3%, subclinical hypogonadism (T>10.4 nmol/L, LH>8 IU/L) in34.2%. Men with all forms of hypogonadism have frequently insufficient (48.5%) or deficient (25.4%) 25(OH)-vitamin D levels and higher risk of low bone mass, osteoporosis (16.8%) and osteopenia (31.5%). This study, performed in a very large cohort of subjects, showed that hypogonadism and low vitamin D levels are very frequent in infertile males. Both conditions, caused by Leydig cell dysfunction, are implicated in the frequent low bone mass seen in these patients. Metabolic and other clinical conditions associated with low T and low vitamin D levels need therefore to be accurately evaluated in these subjects, and treatment should consider also these aspects other than specific treatment only of infertility.

PD28 - AN INTEGRATED APPROACH WITH VARDENAFIL ORODISPERSIBLE AND COGNITIVE-BEHAVIORAL SEX THERAPY FOR THE TREATMENT OF ERECTILE DYSFUNCTION

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Introduction: Erectile Dysfunction (ED) is considered a multifactorial disease, where organic and psychological aspects are often interconnected.

Aim: To compare the efficacy of combined vardenafil orodispersible tablet (VARD) and Cognitive-Behavioral Sex Therapy (CBST) with VARD alone in improving sexual symptoms in both male and female partners.

Methods: 30 male patients with ED, and their partners, were randomly assigned to two different groups and treated for 10 weeks with VARD (Group A) or VARD+CBST (Group B). International Index of Erectile Dysfunction (IIEF-15), Female Sexual Function Index (FSFI) and Index of Sexual Satisfaction (ISS) were respectively administered to male, female and both partners at time(T) 0, 1 (+5 weeks of therapy) and 2 (+10 weeks of therapy).

Results: Groups A and B were similar in their socio-demographic and clinical characteristics. T0 test scores did not significantly differ between the groups. In both group A and B the IIEF-Erectile Function (EF) domain showed a significant improvement from T0 to T1 ($p=0.005$ and $p<0.0001$ vs. T0, respectively) without any further change at T2 only in group A ($p=0.68$). In group A, FSFI and both male and female ISS did not show any significant change at T1 and T2 vs. T0. In group B, a significant improvement at final time-point in FSFI and male and female ISS scores was reported ($p <0.05$, T2vs.T0 in all scores). After adjustment for patient's age and baseline EF score, the female change of intercourse satisfaction (IS), was significantly related to male EF at T2 ($\beta=0.889$, $p<0.050$). Furthermore, female sexual satisfaction at T2, as assessed by ISS score, was significantly related to male orgasmic function (OF, $\beta=0.911$, $p<0.050$), after adjustment for age and male OF at T0. These associations showed a trend significantly different in the two groups, with an improvement in Group B.

Conclusion: In our study, both VARD alone and VARD+CBST improve EF. However, only VARD+CBST ameliorates couple sexual satisfaction and female sexual function.

PD29 - RELATIONSHIP BETWEEN DIABETES MELLITUS TYPE 1 AND MALE REPRODUCTIVE FUNCTION

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Diabetes mellitus type 1 (DM1), an autoimmune disease, affects an increasing number of young men in reproductive age. It has been estimated that its prevalence increases at a rate of ~3% per annum. Diabetes may affect male reproductive function by acting on the hypothalamic-pituitary-testicular axis, causing sexual dysfunction and disrupting male accessory gland function. According a recent study shows that men with DM1 have a smaller number of live births than controls. Despite such evidence, little is known about sperm parameters (mainly limited to conventional sperm parameters) and other aspects of the male reproductive function in these patients. Therefore, this study was undertaken to evaluate both conventional and non-conventional sperm parameters, serum gonadal hormones and didymo-epididymal ultrasound features in patients with DM1. To accomplish this, 30 patients with DM1 (aged 18-35 years) and 20 age-matched fertile healthy men were enrolled in this prospective study. Patients with diabetic neuropathy, other endocrine disorders or conditions known to alter sperm parameters were excluded from the study. Conventional sperm parameters were evaluated according to the WHO 2010 criteria. As far non-conventional sperm parameters, mitochondrial function (mitochondrial membrane potential, MMP), apoptosis and chromatin/DNA integrity were evaluated by flow cytometry following specific staining. Serum total testosterone, 17β-estradiol, LH, FSH and prolactin were measured in all patients and controls. Finally, testicular and epididymal morphometry was evaluated by ultrasound scan before and after ejaculation. Patients with DM1 had a significantly lower percentage of spermatozoa with progressive motility than controls. This abnormality was significantly lower in DM1 patients with long (>10 years) than short (<5 years) disease duration. In addition, the percentage of spermatozoa with high MMP was significantly lower in DM1 patients than in controls. This non-conventional parameter was significantly worse in patients with long or intermediate (5-10 years) vs. short DM1 duration. Disease duration correlated inversely with the percentage of spermatozoa with high MMP. Patients with DM1 had a significantly higher cephalic and caudal epididymal diameters after ejaculation compared to controls, suggesting a lack of the physiological epididymal post-ejaculatory shrinkage. This aspect of the epididymal physiology was significantly more compromised in patients with long vs. short disease duration. All the other parameters did not show any statistically significant difference. Finally, HbA1C did not correlate with any of the parameters evaluated. In conclusion, patients with DM1 had lower sperm progressive motility, impaired mitochondrial function (which precedes the onset of motility disturbance) and epididymal post-ejaculatory dysfunction which cannot be ascribed to endocrinopathy and/or neuropathy.

PD30 - TESTOSTERONE TREATMENT IS NOT ASSOCIATED WITH INCREASED RISK OF ADVERSE CARDIOVASCULAR EVENTS: RESULTS FROM THE REGISTRY OF HYPOGONADISM IN MEN (RHYME)

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Introduction & Objectives: Long-term safety and benefits of testosterone (T) treatment are not well understood. To address limitations of prior short-term and retrospective studies, a prospective registry was established to assess long-term prostate and cardiovascular (CV) outcomes and events. This presentation addresses CV events in the registry. **Materials & methods:** The Registry of Hypogonadism in Men (RHYME) is a multi-national patient registry of treated and untreated men with hypogonadism implemented in 25 sites across 6 European countries (DE/ES/IT/NL/SE/UK). Patients were excluded for a history of prostate cancer (PCa) or prior T therapy. Follow-up assessments were performed at 3-6, 12, and 24 months. Baseline and follow-up data collection included clinical and biochemical assessments. Reports of new onset CV events included deep vein thrombosis, myocardial infarction, stroke, pulmonary embolism, stenting, coronary artery bypass graft, atrial fibrillation, percutaneous coronary intervention, transient ischemic attack, or death due to ischemic heart disease or heart failure. Univariate and multivariate Cox proportional hazards models were used for survival analysis of CV events. Incidence rates were calculated as the number of CV events divided by the total person-time (time to the event or final contact). **Results**: Of the 999 patients enrolled from 2009 to 2013, 750 (75%) initiated some form of T therapy. A total of 55 reported CV events occurred in 41 patients. The proportion of men reporting testosterone utilization was slightly higher (78.1%) among the 41 men reporting CV events compared to the 958 men who reported no CV events (75.0%). Older age, history of CVD and presence of other conventional CV risk factors characterized those men who experienced new onset CV events during the period of follow up. The adjusted hazards ratio for CV risk given a history of angina was 2.6 (95% CI: 1.1-6.1). The overall CV incidence rate was 1,522 per 100,000 person-years. CV incidence rates for those on T or not on T were not statistically different (1,480 vs. 1,694 per 100,000 person-years; p=0.70). By age, the CV incidence rates in those <60 were lower than those ≥60 years of age (735 vs. 2,179 per 100,000 person-years; p<0.01). **Conclusion:** Testosterone treatment status was not a predictor of new-onset CV events in the RHYME cohort. CV incidence rates are comparable to the annual discharge rate for CVD in Europe in 2010 from the WHO European Region's Health with a rate of 2500 per 100,000. Our results should be cautiously interpreted, since RHYME was not powered specifically for CV event rates. *Funding support from Bayer Pharma AG and Besins Healthcare.*

PD31 - RAPID EFFECTS OF TESTOSTERONE IN HUMAN SKELETAL MUSCLE CELLS

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~~Despite its anabolic effects on muscle, testosterone (T) seems to affect muscle metabolism regulation as well. An increased risk for hyperglycemia, hypertension, dyslipidemia and cardio-vascular diseases (Traish et al., 2009) is associated to low T concentrations. T replacement in hypogonadal men is beneficial for type 2 diabetes (T2D), insulin resistance (IR) and metabolic syndrome (Traish et al., 2009); in females with polycystic ovary syndrome (PCOS), an higher risk for T2D development is related to increase bioavailable T concentrations (Coviello et al., 2006; Legro et al., 1999). From animal models, T seems able to affect skeletal muscle metabolism through non-genomic mechanism (Allemand et al., 2009; Hamdi et al., 2010). The aim of this study is to evaluate T rapid effect in human skeletal muscle cells (Hfsmc) undifferentiated (u) and differentiated (d). In cells treated with 100 nM T we analyzed: i) after 24h, GLUT1, GLUT3 and GLUT4 mRNA expression, by Real-Time qPCR; ii) after 30', GLUT4 protein expression and translocation by immunocytochemistry; iii) after 30', 2h, 6h and 12h, AKT, ERK1/2, GSK3 and mTOR expression and phosporylation by WB. Cells treated with 100 nM insulin (I) were used for comparison. Our preliminary data show that in u- and d-Hfsmc T significantly increased GLUT4 mRNA expression similarly to I; in d-Hfsmc GLUT3 mRNA expression also significantly increased; no changes were observed onto GLUT1 mRNA expression. In d-Hfsmc 30' T treatment seems to induce GLUT4 redistribution from intracellular sites to the plasma membrane, similarly to I. In u-Hfsmc T activated all analyzed pathways starting from 30' up to 12h. We found that T in human skeletal muscle cells exerted similar effect as I. In conclusion, it seems that the acute action of T onto human skeletal muscle cell metabolism reproduce an I-like effect. This observation deserves further investigation using different T doses and different experimental times.

PD32 - CENTRAL MOTOR COMMAND OF BICEPS BRACHII MUSCLE IS DEPRESSED IN MALE HYPOGONADISM

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Evidence is accumulating that both central and peripheral nervous systems are important targets of testosterone (T). In humans there is a lack of information about the responses of the neuromuscular system to low levels of circulating T, either at spinal and/or cortical levels. The main hypothesis of the present study was to verify if, and in which direction, low levels of circulating T may affects central drive to skeletal muscle and/or muscle neuromechanical performance at peripheral level by comparing voluntary and electrically evoked muscle contractions. A group of 9 hypotestosteronemic males (HG) volunteers and a control group (CG) of 10 healthy age-matched males were recruited. All volunteers underwent a blood collection to evaluate T concentration by LC-MS/MS before performing the same neuromuscular protocol by using high density spatial surface electromyography on biceps brachii (BB) muscle. HG subjects enrolled in the study, accepted to suspend temporarily the treatment with testosterone to have an adequate wash-out period. During test trial, the following parameters were evaluated: 1) Reference maximal single twitches (rST); 2) Maximal voluntary isometric contractions (MVC); 3) Torque-Velocity curve assessment; 4) Pre-fatigue maximal twitch; 5) Isometric fatiguing contraction at 80%MVC until exhaustion; 6) Post-fatigue maximal twitch. Statistical differences were evidenced between CG and HG for serum T (5.7 ± 1.7 vs 0.6 ± 0.5 ng/dl $p< 0.001$). At any angular velocity, CG exhibited an higher BB median frequency (BB_{MDF}) value with respect to HG. On average, normalized BB_{MDF} was 95.9%±23.3 in CG vs 73.8%±9.3 in HG ($p<0.05$). BB conduction velocity (BB_{CV}) was higher in CG with respect to HG ($p<0.05$), but at the highest angular velocity. At fatigue test, initial BB_{MDF} was higher ($p<0.05$) in CG (91.78 Hz±22.03) vs HG (70.94 Hz±11.06) as well as was the normalized slope (-0.64±0.14 vs -0.5±11). Differently, only BB_{CV} intercepts were different between CG and HG (4.75 ms^{-1} ±0.7 vs 4.18 ms^{-1} ± 0.6; $p<0.05$). In the fresh state, a statistically significant difference between groups was found for time to peak (TTP) only, being HG slower than CG. In CG, half relaxation time (HRT) decreased after fatigue while increased in HG and the two groups were different at $p<0.05$. Cumulative individual averages for BB_{MDF} and BB_{CV} were computed for every subject and plotted against T concentrations as well as rST BB_{CV} values. A significant ($p<0.05$) correlation with T concentration was found for BB_{MDF} and BB_{CV} during voluntary contractions, while rST CV was not correlated with T levels. Low levels of circulating T elicits central and peripheral effects on the performance of the neuromuscular system. In particular, central neural drive to the muscle is depressed and the hypogonadal muscle is mechanically slower than the control one.

PD33 - EXPRESSION OF SHORTER ISOFORMS OF RETINOBLASTOMA INTERACTING ZINC-FINGER PROTEIN 1 (RIZ1) IN SEMINOMAS

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RIZ1, the full-length product of RIZ gene, has a PR/SET domain on N-terminal region, and a PR-Set7 binding domain on C-terminal tail. Both are necessary for the onco-suppressor activity. In many malignant tissues it has been found preferentially either RIZ gene splicing isoform RIZ2, lacking PR/SET domain, or frame-shift mutations and/or exon deletions, coding proteins lacking C-terminal region. RIZ1 expression has been described in the testis, but the relative levels of alternative isoforms, the presence of gene product variants and their role in gonadal tumors has not been investigated yet. Aim of this study was to investigate RIZ expression in normal and malignant testis. Methods: 9 germ cell tumor(5 seminomas, SEM, 4 non-seminomas, NSEM) and 4 control testis tissue samples (2 from normal areas surrounding tumors, and 2 from testis biopsies) were studied. RIZ expression was analyzed by Real time RT-PCR on RNA extracted from frozen samples, using RPS28 as housekeeping gene. In order to identify the presence of altered length variants we used specific primers to amplify exon1 coding N-terminal protein head, and exons 9 and 10 coding C-terminal tail. RIZ protein was detected by immunochemistry in fixed sections, using Ab9710 (ABCAM) and MoAB1802 as primary antibodies to recognize RIZ1 N-terminal 1-100 aa, and RIZ1/2 C-tail last 300 aa, respectively. Results and discussion: RIZ transcript was present in all samples analyzed. However, exon 9 transcript level was higher than that exon 10 ($p<0.01$) in SEM. Protein was immune-detected in all samples with N-terminal directed antibody, but resulted poorly expressed or absent in SEM, when C-tail directed antibody was used. Thus, our study reveals a greater expression of RIZ gene isoforms with shorter tail in SEM, respect to NSEM and control testis samples. Present data suggest that these shorter RIZ isoforms lacking C-terminal region (and PR-Set7 binding domain) may exert a potential oncogenic and de-differentiating role in male gonad.

PD34 - CXCR4 PHARMACOGICAL INHIBITION REDUCES BONE AND SOFT TISSUE METASTATIC BURDEN BY AFFECTING TUMOUR GROWTH AND TUMORIGENIC POTENTIAL IN PROSTATE CANCER PRECLINICAL MODELS.

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Background. CXCR4 has been implicated as regulators of bone resorption and bone metastatic development, suggesting that agents able to suppress this signaling pathway may be used as pharmacological treatments. In this study we studied if two CXCR4 receptor antagonists, Plerixafor and CTE9908, may affect bone metastatic disease induced by Pca in preclinical experimental models. Methods. To verify the hypothesis that CXCR4 inhibition affects PCa metastatic disease, selective CXCR4 compounds, Plerixafor and CTE9908, were tested in preclinical models known to generate bone lesions. Additionally the expression levels of CXCR4 and SDF-1α were analyzed in a number of human tissues derived from primary tumors, lymph-nodes and osseous metastases of PCa as well as in a wide panel of human Pca cell lines to non-tumorigenic and tumorigenic phenotype. Results. Bone-derived PCa cells express higher CXCR4 levels than other PCa cell lines. This differential expression was also observed in human PCa samples. In vitro evidence supports the hypothesis that factors produced by bone microenvironment differentially sustain CXCR4 and SDF1-a expression with respect to prostate microenvironment determining increased efficacy toward Plerixafor. The use of SDF1-a neutralizing antibodies greatly reduced the increase of CXCR4 expression in cells co-cultured with bone stromal cells (BMSc) and to a lesser extent in cells co-cultured with prostate stromal cells (HPSc) and partially reduced SDF1-a Plerixafor efficacy. SDF-1a induced tumor cell migration and invasion, as well as MMP-9, MMP-2 and uPA expression, which were reduced by Plerixafor. The incidence of X-ray detectable bone lesions was significantly reduced following Plerixafor and CTE9908 treatment Kaplan-Meier probability plots showed a significant improvement in the overall survival of mice treated with Plerixafor and CTE9908. The reduced intra-osseous growth of PC3 and PCb2 tumor cells, as a result of Plerixafor and CTE9908 treatment, correlated with decreased osteolysis and serum levels of both mTRAP and type I collagen fragments (CTX), which were significantly lower with respect to controls. Conclusions. Our report provides novel information on the potential activity of CXCR4 inhibitors on the formation and progression of Pca bone and soft tissue metastases and supports a biological rationale for the use of these inhibitors in men at high risk to develop

clinically evident bone lesions.

PD35 - SUPAR AS MARKER OF MALE ACCESSORY GLAND INFLAMMATION

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The association between MAGI (Male Accessory Gland Infection) and infertility is well known in clinical practice.

Standard semen analysis, leukocytospermia and microbiological tests are not often enough for a diagnosis. A large amount of biochemical parameters in seminal plasma have been suggested as inflammation markers, however there is not yet a sensitive and specific biomarker that accurately identifies MAGI. We investigated the presence of suPAR (soluble urokinase-type Plasminogen Activator Receptor), known marker of systemic inflammation, in semen in order to evaluate its possible involvement in urogenital tract inflammation.

On the basis of andrological evaluation, including spermiogram and ultrasound signs, we selected 76 patients with MAGI and 30 healthy men as control group. Patients were classified according to semen culture in group A (n=28) with bacterial MAGI and group B (n=48) with abacterial MAGI. suPAR concentrations were assayed on seminal plasma.

suPAR was detectable in all seminal plasma samples and a significant difference ($p < 0.0001$) was found between controls and patients; in fact in MAGI (groups A and B together) suPAR was higher than in normal subjects (86.6±30.7 vs 39.7±17.2 ng/mL), and its levels were inversely correlated to total motility ($r= -0.288$, $p=0.011$), progressive motility ($r= -0.285$, $p=0.012$) and vitality ($r= -0.306$, $p=0.007$). No correlations were reported between suPAR and sperm concentration and between suPAR and normal morphology . Nevertheless no suPAR difference was observed between MAGI groups A and B (88.9±33.2 vs 85.4±29.5 ng/mL, respectively). The area under the curve (AUC) of receiver operating characteristic (ROC) analysis for suPAR was 0.95 (95% CI: 0.91-0.99), pointing out an high accuracy of suPAR test for MAGI. A suPAR cut-off value of 55.3 ng/mL was associated with a negative predictive value (NPV) of 0.71 and a positive predicitive value (PPV) of 0.95; at the same cut-off, sensitivity was 0.89 (95% CI: 0.79-0.96), specificity was 0.85 (95% CI: 0.62-0.97) and accuracy was 0.88.

We report the first evidence of suPAR presence in seminal plasma, focusing on its interesting role as reliable and sensitive marker of inflammation in differential diagnosis of MAGI.

PD36 - ROLE OF PTEN DELETION AND BRAFV600E MUTATION IN THE GENERATION OF TESTICULAR GERM CELL TUMORS.

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~~Testicular germ cell tumors (TGCT) represent the most common solid malignancy affecting males between the ages of 15 and 35, while ovarian germ cell tumours (OGCT) are a type of ovarian neoplasm principally affecting young women. Germ cell tumors (GCTs) account for about 95 % of testicular cancer cases and for only 2-3% of ovarian cancer cases (Siegel et al., 2011). Most TGCT are potentially curable, however approximately 5% of patients with TGCT develop chemoresistance and die from the disease. PTEN deletion and mutational activation of BRAF are frequent genetic alterations found in human TGCTs, suggesting that they might be directly involved in germ cell tumorigenesis. Furthermore, BRAF mutation positively correlates with the acquisition of resistence to cisplatin, the most commonly chemotherapic agent employed for the treatment of human TGCTs. We obtained heterozygous floxed Ptenloxp/+ BRafCA Spo11Cre mice showing ovarian teratomas and testicular tumors with an incidence of about 30% at 20 days post partum (dpp) . Since Spo11Cre is active at around 13.5 days post coitum (dpc) in female germ cells and at around 7 dpp in male germ cells, these results suggest that ovarian teratomas origin from early meiotic germ cells in the fetal period whereas GCT formation in males can be a postnatal event. By histological inspection, we found that cancer cells in testes showed features reminiscent of seminoma such as a diffuse, confluent multinodular pattern. However, by immunohistochemical staining, we observed that the cells within the tumor showed heterogeneous positivity for the pluripotency markers Oct4, Sox2, Nanog, Kit and Prdm14, suggesting that they can represent a mixed form of seminoma and embryonal carcinoma cells.
Our results indicate that deregulated MAP and PI3 Kinase activation can lead to postnatal male germ cells transformation.

Siegel R, Ward E, Brawley Jemal A .CA Cancer J Clin. 2011;61:212.

PD37 - UNALTERED RATIO OF CIRCULATING LEVELS OF GROWTH HORMONE (GH) ISOFORMS AFTER GHRH PLUS ARGININE ADMINISTRATION IN ADULTS WITH PRADER-WILLI SYNDROME

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~~Human growth hormone (GH) is a heterogeneous protein hormone consisting of several isoforms, the most abundant being 22kiloDalton(kDa)- and 20kDa-GH. The availability of analytical methods to measure these GH isoforms might represent a valuable diagnostic tool to investigate GH hyposecretory states, including Prader-Willi syndrome (PWS), one of the most common cause of syndromic obesity. Aim of the present study was to measure circulating levels of 22kDa- and 20kDa-GH in PWS adults (n=14; M/F: 5/9; genotype DEL15/UPD15: 12/2; age: 19.0±3.7 years; BMI: 29.9±8.7 kg/m2) after combined GH releasing hormone (GHRH) plus arginine (ARG) administration. The results were analysed subdividing the study population in obese vs. not obese (6/8) and GH deficient vs. not GH deficient (GHD) (6/8) subjects, according to appropriate BMI-related diagnostic cut-off limits of GH peak response to the provocative test.

GHRH plus ARG significantly stimulated the secretions of 22kDa- and 20kDa-GH in not obese (at 30, 45, 60 and 90 min and at 45, 60, 90 and 120 min vs. 0 min, $p<0.05$, with GH peaks of 15.8±10.3 ng/ml and 2.7±1.2 ng/ml, respectively) and in not GHD PWS (at 30, 45 and 60 min and at 45, 60 and 90 min vs 0 min, $p< 0.05$, with GH peaks of 12.5±9.0 ng/ml and 2.0±1.8 ng/ml, respectively). No significant GHRH plus ARG-induced changes in 22kDa- and 20kDa-GH were observed in obese or GHD PWS patients, the only exception being the increase of 22kDa-GH ($p<0.05$) 60 min after the stimulus administration in GHD group (with GH peaks of 6.9±4.7 ng/ml and 0.8±0.6 ng/ml in obese subjects and 8.5±6.0 ng/ml and 1.2±1.0 ng/ml in GHD subjects for 22kDa- and 20kDa-GH, respectively). The GH responses for both isoforms were significantly higher in not obese than in obese PWS patients (at 45 and 60 min for 22kDa-GH and at 45, 60, 90 and 120 min for 20kDa-GH, $p<0.05$), while no differences were detected between GHD vs. not GHD groups. As previously reported in healthy subjects, the ratios of circulating levels of 22kDa- to 20kDa-GH remained constant after GHRH plus ARG both in obese/not-obese and GHD/not-GHD groups, thus suggesting the preservation of a normal balance in GH isoforms in PWS.

PD38 - ANTICIPATORY AND CONSUMMATORY EFFECTS OF (HEDONIC) CHOCOLATE INTAKE ARE ASSOCIATED WITH INCREASED CIRCULATING LEVELS OF THE OREXIGENIC PEPTIDE GHRELIN AND ENDOCANNABINOIDS IN OBESE ADULTS

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Hedonic hunger refers to consumption of food just for pleasure and not to maintain energy homeostasis. Recently, consumption of food for pleasure was reported to be associated with increased circulating levels of both the orexigenic peptide ghrelin and the endocannabinoid 2-AG in normal-weighted subjects. To date, the effects of hedonic hunger, and in particular of chocolate craving, on these mediators in obese subjects are still unknown. To explore the role of some gastrointestinal orexigenic and anorexigenic peptides and endocannabinoids (and the related congeners) in chocolate consumption, we measured changes in circulating levels of ghrelin, GLP-1, PYY, AEA, 2-AG, PEA) and OEA in 10 satiated severely obese subjects after consumption of chocolate (choco) and, on a separate day, of a non-palatable isocaloric food (npfood) with the same bromatologic composition. Evaluation of hunger and satiety was also performed by visual analogic scale. The anticipatory phase and the consumption of food for pleasure were associated with increased circulating levels of ghrelin (AUC_{choco}: 110488.2±9905.7 pg/ml×min vs AUC_{npfood}: 87651.1±8611.8 pg/ml×min, $p<0.05$), AEA ($T60_{choco}$: 5.1±1.5 pmol/ml vs $T60_{npfood}$: 3.7±1.0 pmol/ml, $p<0.05$), 2-AG ($T60_{choco}$: 5.7±4.3 pmol/ml vs $T60_{npfood}$: 2.8±1.0 pmol/ml, $p<0.05$) and OEA ($T60_{choco}$: 37.1±13.3 pmol/ml vs $T60_{npfood}$: 28.5±9.5 pmol/ml, $p<0.05$). By contrast, the levels of GLP-1, PYY and PEA did not differ before and after the exposure/ingestion of either chocolate or non-palatable foods. Hunger and satiety were higher ($T70_{choco}$: 37.0±21.2 mm vs $T70_{npfood}$: 24.5±26.5 mm, $p<0.05$) and lower ($T70_{choco}$: 46.5±26.5 mm vs $T70_{npfood}$: 64.5±26.7 mm, $p<0.05$), respectively, in the hedonic session than in the non-palatable one. In conclusion, when motivation to eat is generated by highly palatable food, a peripheral activation of specific endogenous rewarding chemical signals, including ghrelin, AEA and 2-AG, is observed in obese subjects. These preliminary findings seem to suggest the possible effectiveness of ghrelin and endocannabinoid antagonists in the treatment of obesity.

PD39 - EVALUATION OF KISSPEPTIN LEVELS AND THEIR RELATIONSHIP WITH TSH IN CHILDHOOD OBESITY.

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Kisspeptin is a neuropeptide secreted in the anteroventral periventricular and arcuate hypothalamic nuclei involved in starting of pubertal development and also in other functions typical of adult life. Kisspeptin levels are sex dependent, so they are higher in prepubertal girls and in adult women compared to age-matched male. Kisspeptin role in the reproduction function is still largely unknown. Some experimental data suggest an inter-relationship with Pituitary-Thyroid axis, but few data are available in humans. On the other hand Kisspeptin has been evaluated in human obesity but most data concern adult people. To highlight this topic in childhood obesity we investigated Kisspeptin and TSH levels in correlations with metabolic evaluation in a group of prepubertal obese children. Kisspeptin levels in a population of 27 prepubertal children (13 males) aged 5-12 years, classified as overweight (n=3) or obese (n=24) according to Cole's criteria. Eight normal weight children, aged 6-12 years, were enrolled as controls. Several metabolic parameters were evaluated: glucose and insulin levels after oral glucose load, total- LDL- and HDL-cholesterol, triglycerides, uric acid, total proteins, C Reactive Protein. Leptin was evaluated using ELISA method (DRG Instruments GmbH, Germany). In order to evaluate kisspeptin levels, morning blood samples were collected; after acidification, peptides were extracted in a C-18 SEP-Column (Phenomenex Inc, USA). The eluted samples were evaporated and stored at -80°C until assayed. Kisspeptin (pg/ml) was measured using the RIA kit KISS1(61-121)-Amide-Metastin(1-54)-NH2 (Phoenix Pharmaceuticals, Burlingame, USA). TSH was evaluated with chemio-luminescent assay. Mean thyroid hormone levels were normal in all children (mean ±SEM: 3.78±0.11 pg/ml for fT3 and 11.63±0.41 pg/ml for fT4). TSH levels (mean ±SEM: 3.00±0.36 pg/ml for TSH) significantly correlated with Standard Deviation of BMI (SDS BMI) (R^2= 0.4; p-value=0,04). Kisspeptin levels ranged from 8.8-34.6 pg/ml, without significant diffences between sexes. They did not correlate with SDS BMI, but showed a significant correlation with the TSH levels (R^2= 0.1; p-value=0,3). The role of thyroid hormone in the regulation of Kisspeptin system remains unknown, experimental data in animals suggest a stimulatory role of fT3 on Kiss2 gene via indirect mechanism. Our preliminary data suggest that TSH might correlate with Kisspeptin in childhood obesity, probably linking metabolic status to the maturation of reproductive axis, but further studies are needed to clarify these complex inter-relationships.

PD40 - LOW AND CHRONIC BISPHENOL-A (BPA) EXPOSURE AFFECTS 3T3L1 ADIPOGENESIS, LEADING TO ADIPOSE TISSUE METABOLIC DYSFUNCTIONS.

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It is now clear that overweight, obesity and insulin resistance can be due to environmental chemical pollutant exposure, molecules able to accumulate in adipose tissue and interfere with metabolic signaling and inflammatory pathways. We have focused our attention on Bisphenol-A (BPA), an environmental endocrine disruptor with estrogenic activity, worldwide detectable at nanomolar levels, lipophilic component of polycarbonate plastics and resins. We have investigated the effects of low and chronic BPA exposure, able to accumulates in adipose tissue. 3T3L1 fibroblasts, a well established model of murine preadipocytes able to differentiate in adipocytes, were cultured in presence of 1 nM BPA for 3 weeks before the induction of adipogenesis and during the differentiation. Oil Red O staining was used to quantify the total lipid accumulation in mature adipocytes. Total RNA was extracted from the cells and real time RT-PCR was performed to analyze the expression of master genes involved in adipogenesis. Proteins were analyzed by Western Blot analysis with specific antibodies, while adipocyte glucose utilization assay was performed by a ABX Pentra 400 clinical chemistry analyzer. An increased lipid accumulation at Oil Red O staining and an alteration in adipogenesis process were evidenced in 3T3L1 differentiated cells cultured in presence of BPA. In particular, adipocytes, chronically cultured in presence of BPA, displayed a significant increase in the nuclear receptor peroxisome proliferator-activated receptor-γ (PPARγ) and the adipocyte fatty acid binding protein (aP2) mRNA ($p<0.05$) and protein ($p<0.01$) expressions, compared to control cells. Finally, we have observed a decrease in MAPK and AKT protein activations upon BPA and a decrease in glucose utilization after insulin stimulation. In conclusion, the BPA bio-accumulation during adipocyte differentiation may cause an up-regulation of PPARγ and aP2, with alteration in pathways relevant for differentiation and insulin sensitivity, leading to hypertrophic adipocytes. These effects may contribute in adult obesity-low grade inflammation and metabolic syndrome development. Further experiments are required in human adipocytes to confirm the BPA metabolic interferences.

PD41 - EFFECT OF PHYSICAL ACTIVITY ON OSTEOBLASTS HOMEOSTASIS IN OBESE SUBJECTS: IN VITRO CHARACTERIZATION

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Recently, obesity has been associated with mineral metabolism alteration and low bone mineral density (BMD). In particular we have previously demonstrated that exposure of osteoblasts to the serum of obese subjects significantly alters cell homeostasis *in vitro*. Thus, aim of the present study was to investigate whether serum of obese patients subjected to physical activity for different times could affect osteoblast activity *in vitro*. Obese individuals were evaluated at time 0 and after 4, 6, 12 months of individualized prescribed physical activity. Blood was collected at each time points. Cells were incubated with the serum of four groups of obese subjects before and after physical activity: 1) obese (OT0); 2) obese after for 4 months of physical activity (OT4); obese after for 6 months of physical activity (OT6) 4) obese after for 12 months of physical activity (OT12). As expected, the results obtained in this study confirmed our previous results showing that serum of obese individuals, not physically active, induced an alteration of osteoblast differentiation and activity by a negative modulation of Wnt/β-catenin pathway. Furthermore, serum taken from obese individuals who underwent a specifically individualized physical activity training was used to treat osteoblasts. Interestingly, exposure of osteoblasts to the serum of patients subjected to physical activity, induced a β-catenin nuclear accumulation with a recovery of Wnt/ β-catenin signaling. At the same time the results obtained also demonstrated a significant decrease in sclerostin (70%) and increase in p1NP production proportionally to the time of physical activity. In conclusion, our results show for the first time that sera of obese patients subjected to physical activity induce a recovery of osteoblastic cells differentiation by a Wnt/ β-catenin-dependent activation, strongly suggesting that a correct life style, including both nutrition and physical activity approach, can significantly improve the metabolic alteration induced by obesity.

PD42 - THE DELETERIOUS EFFECT OF BMI ON INSULIN SENSITIVITY IN MEN AND WOMEN: EVIDENCE OF SEXUAL DIMORPHISM

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Insulin sensitivity and insulin secretion are both genetically and environmentally determined. Recently, some studies have shown gender specific differences in insulin action. Obesity is a major predisposing risk factor for the development of insulin resistance; some preliminary evidences have suggested that the deleterious effect of increasing BMI on insulin sensitivity is different across sex, being more evident in men than in women. Aim of the study was to investigate the role of BMI on insulin sensitivity in a large sample individuals with a wide range of BMI and no treatments known to interfere with glucose and lipid homeostasis. A total of 829 (244 men and 585 women) individuals with BMI ranging 18.2-77.3 Kg/m^2 were studied. None of the study individuals were on treatments known to interfere with glucose and lipid metabolism. The Insulin Sensitivity Index (ISI) was calculated by plasma glucose and insulin levels during an oral glucose tolerance test (OGTT), according to the formula 10,000/√[fasting plasma glucose (mg/dl) x fasting plasma insulin (mU/l)] x [mean OGTT glucose concentration (mg/dl) x mean OGTT insulin concentration (mU/l)]. Univariate linear models were used to assess the association between BMI and ISI, after logarithmic transformation. The linear correlation between log-BMI and log-ISI observed in men (b=-1.77, p<0.0001) was significantly steeper than that in women. (b=-1.34, p<0.0001) with a p value for logBMI-by-sex interaction equal to 0.0009. Very similar data were obtained after propensity score matching in which analyses were restricted to only 244 men (b=-1.77, p<0.0001) and 244 women (b=-1.40, p<0.0001) individuals (p for interaction=0.021). In conclusion, the deleterious effect of increasing BMI is stronger among men than women. Further studies are needed to clarify the biology underlying such sexual dimorphic effect and to address whether it makes reasonable using sex specific BMI cut-off values in the clinical set.

PD43 - PRESENCE AND SEVERITY OF OBSTRUCTIVE SLEEP APNEA ARE INDEPENDENTLY ASSOCIATED WITH GLYCOMETABOLIC ABNORMALITIES IN OBESE NON-DIABETIC SUBJECTS

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Obstructive sleep apnea (OSA) is a common underdiagnosed condition in the obese population, and has been associated with worse glycemic control in individuals with type 2 diabetes. The relationship between OSA and glycometabolic parameters was investigated in obese non-diabetic individuals. Ninety-one obese subjects (57% male, mean age 45.3 ± 12 yrs, mean BMI 42.1 ± 9 kg/m2) underwent polysomnography and a 2-h oral glucose tolerance test (OGTT). OSA was identified in 64% of subjects (73% in male, 41% in female, p=0.032 χ2). Obese subjects with OSA showed higher A1c (5.8% vs 5.5%, p=0.009), plasma glucose at 120 min during OGTT (PPG120) (133 mg/dl vs 102 mg/dl, p=0.001), triglyceride (140 mg/dl vs 117 mg/dl, p=0.045) and uric acid (5.8 mg/dl vs 4.9 mg/dl, p=0.035) levels than obese subjects without OSA. A1c levels and PPG120 were found to be significantly correlated with raised apnea-hypopnea index (AHI) (p=0.007 and p=0.004, respectively), oxygen desaturation index (p=0.002 and p=0.04, respectively), and percent of sleep time with oxyhaemoglobin saturation at <90% (ST90) (p=0.002 and p=0.004, respectively). Furthermore, increasing quartiles of ST90 and AHI were associated with increasing levels of A1c and PPG120 (p=0.015 and p=0.003, respectively, for ST90; and p=0.035 and p=0.014, respectively, for AHI). Multiple regression analysis showed that ST90 was the strongest independent determinant of A1c, after controlling for sex, age, BMI, waist circumference, CRP, HOMA-IR (β=0.442, p=0.007). Similarly, AHI persisted as an independent determinant of A1c (β=0.414, p=0.005). Both ST90 and AHI persisted as determinants of PPG120, albeit not significantly (β=0.377, p=0.095; and β=0.358, p=0.119, respectively). In conclusion, OSA acts as an independent factor exacerbating the metabolic risk attributed to obesity. Recognition and treatment of OSA may decrease the progression toward type 2 diabetes in obese subjects.

PD44 - SILENCING VIMENTIN DECREASES THE NUMBER OF BREAST CANCER PULMONARY METASTASIS IN A HYPERINSULINEMIC MOUSE MODEL

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Women with Type 2 diabetes (T2D) have an increased risk of breast cancer and a higher mortality rate compared with women without T2D. We used the female MKR mouse to study the effects of hyperinsulinemia on breast cancer progression. Compared to control mice, the MKR mice have a significant increase in tumor size and lung metastasis. We found that the primary tumors from the MKR mice have significantly higher vimentin protein expression compared to primary tumors from control mice. In this study, we aimed to determine if silencing vimentin in the tumor cells would lead to either decreased tumor growth or pulmonary metastasis. Lentiviral shRNA of target vimentin and non-coding sequence was used to generate the vimentin knockdown (KD) and control MVT-1 (c-Myc/vegf overexpressing) cells, respectively. Successful knockdown of vimentin was validated by analyzing gene expression by qRT-PCR (90% KD) and analyzing protein expression by Western Blotting (70% KD). Both the control MVT-1 cells (Ctrl) and the vimentin knockdown MVT-1 cells proliferated at the same rate over a 72 hour time course. Both the knockdown and control MVT-1 cells had similar signaling responses to 15min 10nM insulin stimulation, with an increase in phosphorylation of AKT, a downstream target of the insulin signaling pathway. In order to assess the effects of these cells in vivo 100,000 MVT-1 control and 100,000 MVT-1 vimentin knockdown cells were injected orthotopically into 8-10 week old control and MKR mice. MVT-1-Ctrl primary tumors were larger in MKR mice (249.68±28mm3) compared to WT mice (183.17±7mm3), $p<0.05$. There was no difference between the primary tumor volumes of the MVT-1-KD tumors in MKR and WT mice. As expected based on previous published work, the MKR mice with MVT-1-Ctrl tumors had increased pulmonary metastases (22.9±3.5 surface metastases/lung) compared to WT mice with MVT-1-Ctrl tumors (7.75±3.6 surface metastases/lung), $p<0.05$. The MKR mice with MVT-1-KD tumors had significantly decreased number of pulmonary metastasis (10.2±2.6 surface metastases/lung) compared to the MKR mice with MVT-1-Ctrl tumors. These results demonstrate that silencing vimentin in the MVT-1 cell line leads to a decrease in pulmonary metastasis in the hyperinsulinemic mice. These observations provide insight into vimentin, a possible downstream element of the insulin signaling pathway, which could be used as a potential target for cancer therapy in hyperinsulinemic patients.

PD45 - THE IDENTIFICATION OF WRIST CIRCUMFERENCE CUT OFF FOR INSULIN RESISTANCE PREDICTION IN A POPULATION OF CHILDREN/ADOLESCENTS

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Insulin resistance plays a central role in the pathogenesis of the metabolic syndrome and its prevalence in the paediatric population is increasing, particularly among obese children/adolescents. In a previous study we observed a close relationship among wrist circumference, its bone component, and insulin resistance in overweight/obese children/adolescents. The aim of this study was to identify the wrist circumference cut off for the prediction of insulin resistance in a population of children/adolescents. We recruited n=1134 (M=583, F=551; mean age: 10.3±2.9) overweigh/obese children/adolescents in the Department of Pediatrics Sapienza University and n= 114 (M=58, F=56; mean age: 11.8±2.9) normal weight controls in CTO Hospital in Rome. We evaluated the following anthropometric and biochemical parameters: body weight (kg), height (cm), wrist circumference (cm), BMI-z score, fasting glucose (ml/dl), fasting insulin levels (mU/ml). The wrist circumference was measured using a tension-gated tape measure positioned over the Lister tubercle of the distal radius and over the distal ulna. The subjects were divided into two groups according to Tanner stage (TS): prepubertal (TS 1), pubertal (TS from 2 to 5). Insulin resistance was estimated according to the homeostasis model assessment of insulin resistance (HOMA-IR). HOMA-IR cut off values for insulin resistance in the pre-pubertal period were: 2.22 in females and 2.67 in males; in the pubertal period were: 3.82 in females and 5.22 in males. The data were analyzed through logistic regression and ROC curves, with SAS software 9.3. We observed that the cut-off of wrist circumference to predict insulin resistance in the pre-pubertal subjects were: ≥15.8 cm in males (sensitivity 0.70, specificity 0.73), ≥15.4 cm in females (sensitivity: 0.36, specificity: 0.82); in the pubertal subjects were ≥17.7 cm in males (sensitivity: 0.52, specificity: 0.84) and ≥15.2 cm in females (sensitivity: 0.85, specificity: 0.40). The linear regression demonstrated a positive correlation between wrist circumference and HOMA-IR (R^2=0.09, p<0.0001). The wrist circumference, an easy method to detect anthropometric parameter, could be utilized to identify children/adolescents with increased risk for insulin resistance, thus avoiding testing the entire population of overweight/obese children.

PD46 - VITAMIN D DEFICIENCY IN OBESITY: EFFECTS OF CHOLECALCIFEROL SUPPLEMENTATION

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Obesity is associated with an almost twofold increased risk for vitamin D (Vit-D) deficiency. The term, Vit-D refers to a group of fat-soluble compounds that play a significant role in calcium homeostasis and bone metabolism. Vit-D is stored in adipocytes and is available for conversion to its active form, 1,25-dihydroxyvitamin D [1,25(OH)2D]. Besides its effects on calcium and bone homeostasis, vitamin D affects many other cellular regulatory functions. Vit-D deficiency is diagnosed when serum 25-hydroxyvitamin D [25(OH)D], the major circulating form of vitamin D, is ≤20-30 ng/mL.

Here we evaluated the vit-D status in a population of 1188 overweight-obese patients and the relationships between 25(OH)D levels with cardio-metabolic risk factors, bone mineral density (BMD), fat distribution and echocardiographic parameters. A subpopulation of 160 patients with vitamin D deficiency, homogeneous in terms of age, sex, BMI and all the parameters investigated, was randomized to receive or not a cholecalciferol supplementation for 1 year (50.000 UI/month for the first 2 months and 25.000 UI/month thereafter). The skeletal and extra skeletal effects of this treatment were investigated.

1013 (85.3%) patients showed 25(OH)D levels less than 30 ng/ml and 720 (60.6%) less than 20 ng/ml. 25(OH)D correlated negatively with BMI, waist circumference, total fat mass (Kg) and trunk fat mass (Kg), systolic and diastolic blood pressure, epicardial fat, indexed ventricular mass, diastolic left ventricular diameter, systolic left ventricular diameter, fasting glucose, insulin, HOMA-IR and HbA1c and positively with HDL-cholesterol. No correlations between 25(OH)D and BMD were seen. After 1 year, the treated patients showed an improvement of insulin resistance compared with the untreated group, higher levels of HDL-cholesterol and reduced epicardial fat thickness.

In conclusion, our results support an association between 25(OH)D levels and cardio metabolic risk factors and a positive effect of vitamin D supplementation on glucose tolerance, serum lipids and visceral fat mass distribution.

PD47 - EMPTY SELLA IN OBESITY: ASSOCIATION WITH CARDIO-METABOLIC RISK FACTORS, BODY COMPOSITION AND CARDIAC MORPHOLOGY

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Background: The decline of spontaneous and stimulated growth hormone (GH) secretion and the related increase of cardio-metabolic risk factors in obese patients are generally functionally attributed to fat accumulation. An organic hypothalamic–pituitary (HP) damage may contribute to GH deficiency (GHD) in some obese individuals.

Objective: To ascertain whether structural hypothalamic–pituitary (HP) lesions associate with acquired GH deficiency (GHD) in obese patients.

Patients: 447 adults (372 females and 75 males) with symptoms and signs of HP disease out of 906 consecutive outpatients. Short stature or decreased growth rate issues in childhood, pregnancy, lactation, or drugs known to affect pituitary function were exclusion criteria.

Measurements: Anthropometric data, blood pressure, lipid profile, glycaemic parameters, pituitary hormones and IGF-1 values, echocardiography, carotid intima/media thickness, nuclear magnetic resonance of the HP region, body composition, GHRH plus arginine test in patients with a clinical context suggestive of GHD.

Results: 103 patients had normal pituitary (NP), 244 empty sella (ES), 61 microadenoma (MA) and 38 other pituitary lesions (OPA). 184 patients (131 ES, 37 NP, 11 MA and 5 OPA) with hormonal and clinical characteristics/circumstances suggestive of GHD underwent GHRH plus arginine test. GHD was detected in 70 patients (GH peak values <4.2 µg/ml; 69 ES, 1 normal pituitary). ES was a significant independent predictor of GH secretory capacity. The comparison between ES patients and the other groups (NP, MA, OPA) showed: increased prevalence of metabolic syndrome ($p<0.05$), higher BMI ($p<0.001$); altered fat distribution (higher Upper body fat deposition index; $p<0.01$), increased left ventricular mass index ($p<0.01$) and epicardial fat thickness ($p<0.02$). The more reduced the GH secretion was, the more severe the obesity related cardio-metabolic alterations were.

Conclusion: The prevalence of ES and the close association among pituitary morphology, metabolic phenotype and GHD suggest an organic basis for adult onset GHD in a portion of obese patients. This structural/functional relationship could limit the effectiveness of dietary and lifestyle interventions in obese GHD patients with ES and sustain part of their cardio-metabolic phenotype.

PD48 - GLYCEMIC VARIABILITY IN NON-DIABETIC SUBJECTS, ROLE IN ENDOTHELIAL FUNCTION IN A METABOLIC SYNDROME POPULATION.

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Introduction: Metabolic syndrome (MS) is a clinical condition characterized by an increase risk of cardiovascular disease and diabetes. The aim of our study is to evaluate the possible role of glycemic variability (GV) in endothelial disfunction in patients with MS but with no diabetes. **Materials and methods:** Twenty three patients affected by MS according the IDF criteria were recruited. After excluding diabetes through an oral glucose tolerance test, 2 groups were created (MS with normal glycemia (MSNG) and MS with impaired glycemia (SMIG)). All patients underwent continuous glycemic monitoring for 72 hours and the following GV indices were calculated: standard deviation (SD), mean amplitude of glycemic excursions (MAGE), continuous overall net glycemic action (CONGA) and coefficient of variation (Co.Var). EndoPat 2000 was used to evaluate the endothelial function (Reactive hyperemia index (lnRHI)) and arterial stiffness (Augmentation index (AI)). **Results:** SD and Co.Var were negatively correlated with lnRHI (r=-0.615, p=0.033; r=-0.671, p=0.017 respectively) in the MSIG group. No correlations were observed in the MSNG group. AI was statistically higher in the MSIG than the MSNG group (5.6±11.2%; 18.43±11.7%; p=0.017). No statistically significant difference was observed for lnRHI between groups. **Conclusions:** In patients with metabolic syndrome, glycemic impairment was associated with increased arterial stiffness however the endothelial function was not altered compared to MS patients with no glycemic impairment. Although the absence of diabetes, increase of glycemic variability was associated with worsening of endothelial function in MS patients with impaired fasting glucose or impaired glucose tolerance. These preliminary data suggest a role of glucose variability in the pathogenesis of endothelial damage in metabolic syndrome patients, before the onset of overt hyperglycemia.

PD49 - INVOLVEMENT OF ACTIN CYTOSKELETON IN FREE FATTY ACID-INDUCED BETA-CELL DEATH

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The role of actin cytoskeleton in regulation of pancreatic beta-cell survival has not been investigated. In this study, actin cytoskeleton remodeling was examined in the context of beta-cell apoptosis induced by free fatty acids (FFA). Exposure of human 1.1B4 pancreatic beta-cells to 0.5 mM palmitate resulted in a 10- to-14-fold increase in cell apoptosis ($p<0.05$). The organization of actin filaments was then examined in 1.1B4 beta-cells following exposure to palmitate. Palmitate induced a typical peripheral distribution of actin filaments consistent with their mechanical supporting function for shrinking of apoptotic cells. Treatment of beta-cells with cytochalasin D (CD) (0.01 µM, 2 h) led to collapse of the filamentous actin structures and a reduction of actinin-SNAP25 association, and inhibited the effect of palmitate on apoptosis ($p<0.05$). On the other hand, 1.1B4 beta-cells treated with jasplakinolide (25 nM, 2 h), a potent inducer of actin polymerization showed large aggregations of actin filaments, and enhanced palmitate-induced apoptosis ($p<0.05$). In addition, palmitate-induced phosphorylation of the pro-apoptotic stress kinases JNK and p38 MAPK was reduced (by 45% and 90%) or increased (by 47% and 35%) after treatment with CD or jasplakinolide, respectively ($p<0.05$). The reduction of palmitate-induced phosphorylation of stress kinases is caused by a decreased association between JNK and p38MAPK with a-actinin 4, a protein binding both actin fibers and signaling molecules. Finally, palmitate induced a decrease in Akt phosphorylation, and this was prevented by CD ($p<0.05$); preincubation of beta-cells with the PI 3-kinase inhibitor LY294002 abrogated the ability of CD to restore Akt phosphorylation and to inhibit palmitate-induced apoptosis ($p<0.05$). In conclusion, disruption and stabilization of actin cytoskeleton inhibit and enhance, respectively, FFA-induced beta-cell death. The essential role of actin cytoskeleton in FFA-induced apoptosis is coupled with activation of the pro-apoptotic JNK and p38 MAPK and inhibition of the anti-apoptotic Akt kinase.

PD50 - THE TRANSCRIPTION FACTOR PREP1 REGULATES THE NITRIC OXIDE (NO) PRODUCTION IN THE ENDOTHELIUM

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Prep1 is an homeodomain transcription factor belonging to the TALE class of proteins, which plays an important role in organogenesis and in the regulation of energy homeostasis and metabolism. It has been demonstrated that Prep1 hypomorphic heterozygous mice ($Prep1^{i/+}$), which express 55-57% of the protein, display a better insulin sensitivity in several tissues such as skeletal muscle and liver. Insulin-resistance contributes to the pathogenesis of atherosclerosis and cardiovascular disease impairing endothelial cell function and altering Nitric Oxide (NO) bioavailability. NO production is induced by endothelial nitric oxide synthase (eNOS), which can be regulated on multiple phosphorylation sites, and, in particular, phosphorylation on Threonine495 residue by several protein kinases inhibits eNOS function.

In this study we have focused our attention on the role of the transcription factor Prep1 in the regulation of NO production in the mouse aorta.

Prep1 hypomorphic heterozygous mice feature a 20% increase of serum NO production and a 50% decrease of eNOSThr495 phosphorylation in the aortic endothelial tissue respect to the Wt animals. Furthermore a 70% decrease of protein kinase C alpha and delta activation, which are known to induce eNOS phosphorylation on this site, are reduced in the $Prep1^{i/+}$ mice. To better clarify the role of Prep1 in endothelial function, Mouse Aortic Endothelial Cells (MAEC) have been transiently transfected with the full-lenght Prep1 cDNA. Nitric oxide production is 30% decreased in Prep1 overexpressing MAEC cells medium and this effect is paralleled by a significant increase of eNOSThr495, PKCalpha and PKCdelta phosphorylation. A 65% inhibition of these PKC isoforms by staurosporine reverts the Prep1-mediated eNOSThr495 phosphorylation.

These data indicate that Prep1 may be involved in endothelial dysfunction impairing NO production and eNOS function through a PKC mediated pathway.

PD51 - ISLET NEOGENESIS PRESERVES BETA-CELL MASS BUT DOES NOT COMPENSATE FOR LOSS OF GLUCOSE SENSITIVITY

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Progressive deterioration in β cell function and decrease in β cell mass represent the main mechanisms involved in type 2 diabetes. To investigate if the deterioration of the β cell function corresponds to a loss of β cell mass, we performed oral glucose tolerance tests (OGTT), hyperglycemic clamps (HC) and followed by arginine stimulation in 16 patients undergoing pancreatoduodenectomy (PD), pre- and post-surgery. To further explore whether Islet features could be justified by in vivo beta cell function, we explored neogenesis from duct cells, islet size and trans-differentiation of α cells to β cells.

Based on post-surgery OGTT, subjects were divided into 3 groups of glucose tolerance: normal (NGT, n=5), impaired (IGT, n=4) or diabetes (DM, n=7) (8 F/8 M, 51±15 yrs.). To evaluate β cell function, β cell glucose sensitivity (GS) during HC was calculated as the ratio of insulin secretion and glucose increments. During surgery, pancreas samples were collected for IHC for glucagon, insulin and somatostatin+ cells to assess islet morphology. Ductal cells were stained by CK19.

Before surgery, Arginine-stimulated Insulin Secretion (AIS) was similar across groups, whereas GS was lower in IGT and DM as compared with NGT subjects (62.9±23.1 and 45.5±11.2 vs 90.6±18.7 pmol•min-1•m-2, respectively). Following 50% PD, GS decreased in all patients ($p<0.01$ for all groups), but the reduction was greater in DM compared to IGT and NGT patients (ΔGS: NGT -0.20±0.19 vs. IGT -0.27±0.11 vs. DM 0.37±0.08; $p<0.003$). A similarly scaled reduction was observed in ΔAIS (NGT -0.38±0.13 vs. IGT -0.76±0.06 vs. DM -0.90±0.04; $p<0.01$) and in 2nd phase insulin secretion. IHC demonstrated an increase in islet size, insulin+ CK19+cells ($p<0.05$) and scattered islets (<8 cells) ($p=0.01$) in DM patients, as compared with NGT and IGT.

In this study, loss of GS was the most reliable parameter for predicting the appearance of diabetes after 50% PD. Increased islet size and neogenesis could be compensatory mechanisms to cope with reduced function (GS) and to preserve β cell mass, at least as estimated by AIS. However, increased islet size does not seem to be able to compensate for the loss of glucose sensitivity.

PD52 - CARBOHYDRATE COUNTING COMBINED WITH AUTOMATED BOLUS CALCULATOR USE: EFFECTIVENESS AND USEFULNESS IN A POPULATION OF TYPE 2 DIABETES MELLITUS (T2DM) SUBJECTS TREATED WITH MULTIPLE DAILY INSULIN INJECTIONS. A SINGLE CENTER EXPERIENCE.

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Background: Carbohydrate counting (CarbC) is a meal planning approach for patients treated with multiple daily insulin injections (MDI) which focuses on carbohydrate as the primary nutrient affecting postprandial glycemic response: by calculating the carbohydrates amounts in each meal and snack, the insulin doses required to preserve postprandial blood glucose within normal limits can be predicted. New technology provides the capability to automatically calculate bolus insulin dosages to cover carbohydrates intake and address out-of-range blood glucose levels. Aim of the study was to investigate the effect of carbC with the aid of bolus calculator in T2DM patients treated with MDI therapy. **Materials and methods:** we recruited 32 T2DM caucasian subjects (18M/14F) aged 40-60, disease duration > 5 years. The observational period lasted 9 months. A diabetologist and a dietician delivered a carbC training to patients who were invited not to change life style during that period. Demographic information, weight, waist circumference, height, blood pressure, heart rate, glycated haemoglobin were recorded at baseline and after 9 months. Participants completed a form with number of Carbohydrates in their meals, prandial glycaemic profiles and bolus doses over 3 consecutive days. Carb factor and Correction factor were calculated for each patient and were estimated individual target blood glucose. Patients were provided with Accu-Chek® Aviva Expert BG meter (Roche Diagnostics) and trained in its use. **Results:** At baseline, age, BMI, HbA1c, blood pressure were similar for all patients. Changes of BMI and Blood pressure after 9 months weren't significantly vs. baseline. HbA1c, instead, improved from baseline patients using CarboC and bolus calculator: HbA1c 7.42%±1.05 DS at 9 months vs. 8.45%±1.70 DS at baseline (P=0.0084). **Conclusions:** CarbC helped to improve glycaemic control in T2DM using multiple daily injections. The use of bolus calculator has different advantages:prandial insulin can be calculated more accurately, which may improve postprandial glucose control; patients may remain for more time within target glucose range.

PD53 - SODIUM-GLUCOSE TRANSPORTER 1 AND 2 ARE INVOLVED IN THE GLP-1 RELEASE FROM PANCREATIC ALPHA CELLS

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It has been recently reported that human alpha cells express the sodium-glucose co-transporters and that the diabetic condition is associated with reduced expression of SGLT2 and compensatory increased expression of SGLT1 and glucagon genes. Moreover, SGLT2 silencing or inhibition by the SGLT-2 inhibitor dapagliflozin was associated with increased expression of the glucagon gene. We have tested whether this system also is involved in the recently reported regulation of GLP-1 secretion by the alpha cell. Moreover, since glucagon/GLP-1 secretion by the alpha cell has been claimed to be under the control of the TCF7L2 signaling pathway, we have also evaluated its interaction of the two systems in the alpha cell.

We have studied TC1/6 (TC1/6) alpha cells from a mice pancreatic cell line in the presence of low (LG, 5.5 mM) or high (HG, 16.7 mM) glucose concentrations. Experiments were repeated with and without phloridzin (50 **m**M), a non-selective SGLT inhibitor. mRNA and protein expressions of SGLT1, SGLT2 and TF7L2 were determined by RT-PCR and Western blot. Total GLP-1 secretion in culture medium was measured by ELISA.

At LG both mRNA and protein expressions of SGLT1 and 2, and TCF7L2 were all detectable. HG incubation was associated with an increase of both mRNA and protein expression of TCF7L2 (protein: +46±10%, $p<0.001$; mRNA 2.79±0.47 folds, $p<0.001$). Concomitantly, SGLT1 mRNA expression increased (2.31±0.49 folds, $p<0.001$) while SGLT2 decreased (0.48±0.09, $p<0.001$). Changes in mRNA expressions were associated with -34±11% ($p<0.01$) reduction in SGLT1 protein expression with no significant changes for the SGLT2 protein. GLP-1 concentration in the medium increased by +25±3% ($p<0.001$) as compared to LG. SGLT1/2 inhibition by phloridzin did not affect GLP-1 release at LG (-10±6%, p=NS), while it was associated with a -30±3% reduction ($p<0.001$) as compared to LG.

These data suggest that GLP-1 release from alpha cells in response to high glucose is mediated by SGLT expression independently of TCF7L2 activation.

PD54 - INFLAMMATION INDUCES LEPTIN SECRETION BY HMGB1 MEDIATED PATHWAY.

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Type 2 diabetes (T2D) has been defined as a chronic low grade inflammatory disease; among cytokines involved in T2D induced inflammation, HMGB (High-Mobility Group Box)-1 and Leptin are thought to play a pivotal role: monocytes and adipose tissue can secrete HMGB1 during inflammation in T2D patients. Leptin has a role in T2D pathogenesis and could modulate CD4+ T lymphocytes and NK cells.Leptin and HMGB1 can interact with both Peripheral blood Mononuclear Cells (PBMCs) and adipocytes. We hypothized HMGB1 can modulate Leptin secretion.To avoid anti inflammatory effect of T2D therapy, human healthy PBMCs were isolated by ficoll gradient; Low density (LD)-PBMCs cells (NK, NK-T, B cells and monocytes) and high density (HD) PBMCs subpopulations (T-cells) were isolated using percoll gradient. NK-T, CD56+ and CD14+ cells were isolated by magnetic sorting. Human adipocytes were differentiated from human pre-adipocytes. Inflammatory stimuli were obtained with IL-2 (200u/ml) for 72 hour with or without HMGB1 (2ug/ml). After 48 hours, supernatants were collected and cytokines levels were evaluated by western blot or ELISA. Results were analyzed utilizing t-test and a P values <0.05 was considered statistically significant. HGMB1 increases Leptin secretion in IL-2 activated PBMCs: afterPBMCs subpopulation isolation, we determine that Leptin secretion it's a LD-PBMCs prerogative: no variations were detected in HD-PBMCs supernatant. Among LD-PBMCs, we investigated leptin secretion in each single cell population. Our treatment induces an increase in Leptin secretion in NK cells but not in NK-T cells. HMGB1 seems to induce Leptin secretion in IL-2 activated CD14+monocyte. On human adipocytes the effect of HMGB1 with IL-2 slightly increases Leptin secretion. The amount of Leptin secreted is 8-15 pg/ml more than controls, and were used to stimulate CD14+ cells to release IL-1b. We found increased levels of IL-1b in supernatants of CD14+ cells. We showed: a) Leptin can be secreted by NK cells, monocytes and adipocytes in response to HMGB1 and IL-2, b) the amount of Leptin secreted has a biological effect. We can speculate Leptin can represent the molecular link between immunity and metabolic diseases.

PD55 - LIVER PROTEOME AND MICRORNAS IN INSULIN RECEPTOR KNOCKOUT MICE REVEAL NOVEL MOLECULES INVOLVED IN THE DIABETES PATHOPHYSIOLOGY

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Type 2 Diabetes Mellitus (T2DM) is a disease characterized by alteration of insulin signaling in specific target tissues, such as skeletal muscle, adipose tissue, and liver. Dysfunction and later failure of insulin-producing pancreatic beta cells (β-cells) and peripheral insulin resistance induce hyperglycemia in pre-diabetes conditions. Fundamental is discovering early biomarkers to delay or prevent onset of T2DM. Recently several studies are seeking for biomarkers through proteomic and microRNA (miRNA) approaches. To understand the pathophysiologic mechanisms underlay T2DM and metabolic-liver disease, we analyzed protein and miRNA patterns by proteomic and miRNA arrays in insulin receptor knockout ($IR^{-/-}$) and heterozygous ($IR^{+/-}$) mice as a murine model of liver metabolic dysfunction associated with diabetic ketoacidosis and insulin resistance. We evaluated protein expressions by using protein 2-DE MALDI-TOF/TOF and peptic nLC-MS/MS shotgun profiling. Twenty-eight proteins identified by 2-DE analysis and 24 identified by nLC-MS/MS shotgun, were differentially expressed among the 3 genotypes. Bioinformatic analysis revealed a central role of High Mobility Group Box 1/2 and huntigtin (HTT) never reported to be associated with metabolic and related liver disease.

MiRNAs array identified only 4 miRNA differently expressed between $IR^{+/+}$, $IR^{+/-}$ and $IR^{-/-}$: miR-376b, miR-154, miR-543, and miR-199b. Quantitative Real time polymerase reaction (qRTPCR) confirmed these results, and bioinformatic analysis reveals interesting mRNA targets involved in metabolic pathways linked with proteomic analysis. These results provide new insight into pathophysiology of T2DM and non alcoholic fatty liver disease, and could be useful in identifying novel biomarkers to predict risk for diabetes and its complications.

PD56 - A SINGLE BOUT OF AEROBIC EXERCISE IMPROVES 1-HOUR POSTLOAD GLUCOSE TOLERANCE AND INSULIN SENSITIVITY WITHOUT AFFECTING INSULIN SECRETION

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While the direct effect of physical activity on glucose metabolism in skeletal muscle is well established, the potential effects on insulin secretion and on 1-h post-load plasma (1-PL) glucose are still debated.

We therefore investigated the effect of a single bout of moderate aerobic exercise on 1-PLG and insulin secretion in 32 (18 M) young, healthy, normal weight volunteers with a sedentary/moderately active life-style. The oral glucose tolerance (OGTT) was performed before and on the day-after a bout of aerobic exercise (a single workout of jogging or running for 30-40 minutes, or until exhaustion). Insulin secretion and sensitivity were estimated using OGTT-derived indices.

Even after a single session of exercise, fasting and 1-PL glycemia significantly decreased (82±5 vs. 78±7 mg/dl, $p<0.005$; 123±34 vs. 112±25 mg/dl, $p<0.03$, respectively), as well as 1-PL insulinemia (57.4±36 vs. 43.5±19 mUI/ml, $p<0.02$); AUC of glycemia and insulinemia, as well as fasting insulin, were slightly, and non-significantly, decreased. Insulin sensitivity significantly improved after exercise (OGTT-derived Matsuda Index, 7.79±3.2 vs. 9.02±3.6; $p<0.03$), whereas there was no significant change in insulin secretion (insulinogenic index, p=NS). Interestingly, the improvement of 1-PL glycemia is not paralleled by a similar amelioration of the first phase insulin secretion (Delta InsAUC30/GluAUC30 and Stumvoll-first phase are slightly but non-significantly increased). The change from baseline in 1-PL glucose is well correlated with the improvement of Matsuda ($p<0.02$; $r=-0.4$). In young, healthy, normal weight subjects, even a single bout of aerobic exercise significantly increases insulin sensitivity. The improvement of 1-h post-load glycemia seems to predict the amelioration of the insulin sensitivity and is independent of the insulin secretion.

PD57 - A CASE OF FACTITIOUS HYPOGLYCEMIA: CONSIDERATIONS ON METHODS FOR INSULIN ASSESSMENT.

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We report a case of 57 years old nurse admitted to the hospital for an evaluation of recurrent episodes of spontaneous hypoglycemia referred in the last 7 years. Physical assessment revealed an underweight phenotypic female with normal blood pressure, heart rate and without any pathological finding at thoracic or abdominal examination. Having excluded other causes of hypoglycemia, and with the clinical suspicion of insulinoma, we performed a fasting test. After 8 hours from the beginning of this test, the patient showed a quick onset of tremor, palpitations and neuroglycopenic symptoms such as difficult speech and partially loss of consciousness. On that occasion, we performed a capillary glucose stick which showed 20 mg/dl of glycaemia. Than a venous blood sample for glycaemia, insulin and C-peptide measurement was done. This episode was managed with 33% dextrose boluses. Then, we applied an infusion of 10% dextrose at 500 mL/h to prevent recurrent hypoglycemia overnight. Insulin measurement of the same blood sample was performed with two insulin assays, RIA and non-RIA (chemiluminescence, Beckman). A striking difference was observed between the two test responses. C-peptide during the test was low (<1 ng/ml) excluding an endogenous hyperproduction of insulin. Insulin levels were low for non-RIA assay (<1µU/ml) and conversely elevated in RIA assay. For this reason, we supposed that the RIA-insulin assay might show crossreactivity with the insulin synthetic analogs.

We confirmed that hypothesis obtaining vials of two short-acting recombinant insulin: Aspart insulin (Novo-LogTM; Novo Nordisk Pharmaceuticals) and Lispro insulin (Humalog®; Eli Lilly and Company). Each of the two insulin analogs, with a nominal concentration of 100 UI/mL and suitable for injection, was diluted volumetrically with a random human serum to final insulin concentrations of 10, 100, and 1000 µUI/ml. All dilutions of each insulin preparation were analyzed in duplicate by RIA and non-RIA immunoassay. We observed that RIA insulin immunoassay recognized Lispro and Aspart analogs with a crossreactivity of about 81% and 100% respectively. Instead, the non-RIA insulin assay did not recognize exogenous insulin preparations. This allowed us to indicate exactly which exogenous insulin the patient assumed autonomously. The patient was then referred to our psychiatrist colleagues.

PD58 - THE WNT/B-CATENIN PATHWAY MIGHT PLAY A ROLE IN REGULATING THE EXPRESSION OF EARLY EMBRYONIC STEM CELL GENES IN HUMAN PARATHYROID TUMORS

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The Wnt/ß-catenin pathway is deregulated in parathyroid tumors, though constitutive accumulation of ß-catenin was not detected. Investigating unphosphorylated active ß-catenin distribution by western blot in 16 typical parathyroid adenomas (PAds), we detected a great variability among PAds samples in the nuclear ß-catenin accumulation with a subset of PAds showing levels similar to that in Caco-2 cells with constitutively active Wnt signaling (3 PAds). Nuclear ß-catenin accumulation positively correlated with *AXIN2* mRNA levels (r=0.546, P=0.03). The Wnt/ß-catenin pathway is connected to the embryonic pluripotent core circuitry. Therefore, we treated PAds-derived cells (n=3) with 10-20 mM lithium chloride for 72 hours: nuclear accumulation of ß-catenin and concomitant increases in mRNA levels of *NANOG*, decreases of *SOX2* and no changes of *POU5F1/OCT4* were detected. In PAds nuclear ß-catenin levels positively correlated with the *NANOG* mRNA levels and negatively with the *SOX2* mRNA levels. Immunostaining of 11 PAds and 8 carcinomas (PCas) tumor sections identified positivity for the stem cell genes in the nucleus of a subset of tumor cells; in particular, NANOG-expressing cells were more abundant in PCas (40.0±5.8%; range 30-70%) than in PAds (11.4±4.5%; range 1-40%; P=0.01). OCT4 was similarly detected in 5-20% of cells in both PAds and PCas. Forty-two percent of PAds showed SOX2-expressing cells, that were more abundant in PAds than in PCas (15.8±5.9% vs 7.0±3.6%; P=0.05). Primary hyperparathyroidism at diagnosis was more severe in patients harboring the PAds with detectable *SOX2* mRNA (n=22): serum PTH and calcium levels were higher than that in patients with *SOX2*-undetectable PAds (n=14; PTH: 324.2±248.0 *vs* 150.2±56.0 pg/ml; P=0.014; calcium: 11.9±1.2 *vs* 11.2±0.7 mg/dl, P=0.06). Immunofluorescence detected few cells coexpressing SOX2 and NANOG or OCT4, while cells expressing PTH were negative for all the stem genes. In conclusion, we firstly showed that ß-catenin might be involved in the regulation of the stem-like phenotype acquisition by a subset of parathyroid tumor cells.

PD59 - GLOMERULAR FILTRATION RATE ESTIMATED USING SERUM CYSTATIN C IN PATIENTS WITH PRIMARY HYPERPARATHYROIDISM: CORRELATIONS WITH BIOCHEMICAL, CARDIOMETABOLIC FEATURES AND CARDIOVASCULAR DISEASE.

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Patients with primary hyperparathyroidism (PHPT) are at risk of chronic kidney disease (CKD). PHPT patients (n=190, 146 females, 44 males, aged 59.7±14.2 years) and non-hypertensive, non-diabetic age- and sex-matched healthy controls were evaluated for serum cystatin C and creatinine. PHPT patients and controls with established CKD were excluded. Serum cystatin C was measured by immunonephelometric assay and calculation of estimated glomerular filtration rate (eGFR) was based on serum creatinine and cystatin C (eGFRcr-cys) using the CKD-EPI equation. Serum cystatin C well correlated with serum creatinine in PHPT patients (r=0.594, P=0.0001), while mean cystatin C level was significantly higher in PHPT patients than in healthy controls (0.93±0.02 *vs* 0.78±0.01 mg/L, P=0.001). In particular, among PHPT patients with eGFRcr > 60 ml/min/1.73m2, 18.4% had cystatin C levels >1.03 mg/L (95° percentile of controls' values), consistent with a "preclinical kidney disease". These patients had higher HOMA-IR values and were more hypertensive. In PHPT patients, cystatin C levels positively correlated with total and ionized calcium (r=0.151, P=0.024, r=0.259, P=0.004) and with PTH (r=0.176, P=0.01). No significant correlation with the occurrence of kidney stones could be detected. Using the eGFRcr-cys equation, CKD (stages G3a, 3b, 4) was diagnosed in 13.7% of PHPT patients. CKD-PHPT patients had higher total and ionized calcium, were older, more frequently males, heavier, more insulin-resistant and more frequently affected with hypertension. Regression analysis identified hypertension and HOMA-IR values as the independent variables able to predict eGFRcr-cys in PHPT patients. Considering the occurrence of cardiovascular disease (CVD)(coronaropathy, arithmopathy, cerebral vasculopathy), after adjustment for age and sex, CVD was positively correlated with cystatin C levels (β 0.305±0.109; P=0.006) and negatively with eGFRcr-cys values (β -0.005±0.002; P=0.011). In conclusion, elevated cystatin C levels were cross-sectionally associated with key CVD risk factors and with CVD events in PHPT patients as in the general population.

PD60 - DIFFERENTIAL DIAGNOSIS OF A LARGE ITALIAN SERIES OF PATIENTS AFFECTED WITH ALBRIGHT HEREDITARY OSTEODYSTROPHY AND/OR PSEUDOHYPOPARATHYROIDISM

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Pseudohypoparathyroidism (PHP) is a heterogeneous group of rare genetic metabolic disorders due to molecular defects at the GNAS locus, that encodes also for the α-subunit of the stimulatory G protein (Gsα), causing end-organ resistance to the actions of PTH.

The classification of the different subtypes of PHP is based on the presence of specific somatic and developmental abnormalities, referred to as Albright hereditary osteodystrophy (AHO), and the resistance to other hormones acting via GPCRs.

Recently, mutations in genes encoding proteins crucial for cAMP-mediated signalling different from Gsα and deletions of chromosome 2q37.2 have been detected in a small subset of patients with PHP with no GNAS defects, showing a phenotypic overlap with Acrodysostosis (ACRDYS) and brachydactyly-mental retardation syndrome (BDMR), also called AHO-like syndrome.

Despite the high detection rate of genetic and epigenetic defects by currently available molecular approaches, about 30% of PHP patients still lack a molecular diagnosis, hence the need to screen patients negative for GNAS genetic or epigenetic defects also for chromosomal regions and genes associated to diseases that undergo differential diagnosis with PHP.

In this study, we screened by Sanger sequencing and multiplex ligand-dependent probe amplification (MS-MLPA) our series of AHO/PHP patients negative for GNAS locus genetic and imprinting defects (sporadic or genetic-based) (n=78), for the presence of mutations at PRKAR1A and PDE4D gene, as well as for deletions affecting the chromosome region 2q37.

We detected 3 and 4 different missense mutations at the PRKAR1A gene and PDE4D gene, respectively, and 4 heterozygous deletions of 2q37, overlapping with previously described rearrangements affecting this subtelomeric region. In silico analysis predicted a pathological effect for all genetic defects found in our patients.

In conclusion, our data further confirm the molecular and clinical overlap among these disorders and highlight the complexity in performing an accurate diagnosis of PHP, as well as the pivotal role of the cAMP pathway in the development of the AHO phenotype.

PD61 - EFFECTS OF PROINFLAMMATORY CYTOKINES ON BONE HOMEOSTASIS IN SAOS-2 OSTEOBLAST-LIKE CELLS

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Proinflammatory cytokines mediate the effects of inflammation on bone tissue, where they regulate remodeling processes, thus acting as bone homeostasis modulators. In fact, osteoblasts respond to IL-17, which, in turn, acts redundantly with other cytokines, such as TNF-a. Moreover, evidence showed that the potent proinflammatory/proapoptotic cytokine TRAIL, a member of the TNF superfamily, promotes osteoclast differentiation. We investigated possible interaction of IL-17, TRAIL, and TNF-a within bone homeostasis in vitro, in the human differentiated osteosarcoma cell line Saos-2. Effects of IL-17, TNF-a and TRAIL on cell viability were first assessed. IL-17 alone, or in combination with TNF-a, had no effect on Saos-2 cell viability. On the other hand, TRAIL exhibited time- and concentration-dependent cytotoxicity. Then, the effects of IL-17 and TNF-a on bone remodeling markers were assessed by means of western blot and ELISA in tissue culture media. Both IL-17 and TNF-α, either alone or in combination, were able to increase soluble RANKL and OPG release. Real time RT-PCR for RANKL or OPG mRNAs indicated an increase of their respective expression, ranging from low to substantial levels. Finally, the effects of IL-17 and TNF-α on pro-inflammatory molecules, such as chemokines CXCL-1 and -5, COX-2, and IL-6, assessed by real time RT-PCR, indicated synergistic effects of the combination. COX-2 mRNA levels augmented either after treatment with IL-17 alone, or combined with TNF-α. In addition, whilst both IL-17 and TNF-α alone induced minimal IL-6 release, combined treatment caused robust release of the latter. Overall, results suggest that IL-17, TRAIL and TNF-α sustain bone tissue inflammation associated with loss of calcified component. To do so, they act redundantly each other, to amplify the inflammatory response in the bone. In conclusion, unraveling novel molecular targets within the bone-cytokine network represents a platform for innovative treatment of bone diseases.

PD62 - EVALUATION OF NEW CYTOKINES (OPG, A-KLOTHO AND FGF-23) IN PHPT PATIENTS AND POSSIBLE THERAPY EFFECTS

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Background. Recent studies have shown that several cytokines (osteoprotegerin (OPG), α-klotho, fibroblast growth factor 23 (FGF23)) and parathyroid hormone (PTH) are the main players in the parathyroid-bone-kidney axis in secondary hyperparathyroidism (HPT). In patients with primary HPT (pHPT), data on the relationship between PTH and these cytokines are scarce and not concordant. The aim of this study was to evaluate the relationships among PTH, serum calcium (S-Ca), vitamin D (VitD) and other cytokines in patients with pHPT. In addition, the possible influence of their levels on pHPT medical therapy was studied. Methods. We enrolled 66 patients diagnosed with pHPT (mean age 64.6±14 yrs; range 29-91 yrs) including 55 women (2 pregnant). We considered 3 groups of patients: control group (patients disease-free after parathyroidectomy), cinacalcet group, and bisphosphonate group. All subjects underwent physical examination, bone densitometry, neck ultrasound and blood tests to assay OPG, α-Klotho, FGF23, PTH, s-Ca, serum phosphorous (s-P), alkaline phosphatase (ALP), vitD and creatinine (Cr), free-T4 and TSH. Results. All subjects had adequate thyroid and renal function. Most patients were overweight (BMI 27.1±0.6 kg/m2). The mean duration of disease was 5.7±4.1 yrs (median 5.0 yrs). The mean FGF23 level in all pHPT patients was 77.2±14.8 ng/L (median 27.2; n.r. 10-50) and was found to be negatively correlated with ALP levels (Sr -0.32; P<0.05); the mean α-Klotho level was 412.0±58.9 pg/ml (median 354.2; n.r. 250-500) and was found to be negatively correlated with s-P levels (Sr -0.36; P<0.05). Alpha-Klotho and FGF23 were significantly correlated (Sr 0.32; P<0.01), and were found to be positively correlated with s-Ca levels (FGF23: Sr 0.36, P<0.005; α-Klotho: Sr 0.26, P<0.05), but neither was correlated with age, BMI, or levels of PTH, Cr and vitD. The levels of FGF23 were lower in the cinacalcet group than in the other two groups, but not significantly. By contrast, the levels of α-Klotho differed among the groups (ANOVA P<0.01). The levels of both cytokines were lower in both the cinacalcet and control groups than in the bisphosfonate group (data in table; data are expressed as mean±ES and median).

	cinacalcet group (N=12)	control group (N=23)	bisphosphonate group (N=31)
FGF23	28.4±3.1 (27.9)	42.6±18.5 (22.6)	32.8±5.6 (28.9)
α-klotho	394.7±40.1 (432.7)	256.1±38.2 (217.7)	586.9±184.0 (394.9)

Conclusion. The α-klotho levels were higher in pHPT patients with active disease on drug treatment than in controls. By contrast, FGF23 levels were lower in controls

than in pHPT patients on cinacalcet or bisphosphonate therapy. A larger study population might provide more conclusive results.

PD63 - EFFICACY AND SAFETY OF TERIPARATIDE IN BETA-THALASSEMIA INDUCED OSTEOPOROSIS

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Teriparatide is a bone anabolic agent used to treat patients with severe osteoporosis. Efficacy and safety of teriparatide in beta thalassemia-induced osteoporosis have not been investigated. In our medical centre, 12 patients affected by beta thalassemia (8F,4M; mean age=45±4.4;11 major,1 intermedia) are or were treated with teriparatide for established osteoporosis (all had at least one vertebral or rib fracture). All patients had been previously treated with bisphosphonates and were on vitamin D replacement therapy; half of them had a history of untreated hypogonadism. The first target of our analysis was to evaluate safety of teriparatide. 58,3% of patients (7/12) had side effects such as bone and muscle pain (7/12) and fever (1/12); 2 patients discontinued therapy after one month because of important bone pain; 10 patients continued therapy for 6-24 months (4 for 6 months, 3 for 12 months, 2 for 18 months and 1 for 24 months); 2 of them temporarily withdrew the drug because of side effects; only 1 patient reported an improvement in bone pain in contrast with the reduction in back pain usually described in the non thalassemic population. The second aim of our study was to evaluate efficacy of teriparatide in patients treated for at least 12 months (50%, 6/12). The mean BMD T and Z scores measured by DEXA at baseline were -3.8 and -3.5 at the lumbar spine (LS) and -2.8 and -2.3 at the femoral neck (FN), respectively. After one year therapy, mean BMD T and Z scores were -2.6 and -2.3 at the LS and -2.3 and -1.9 at the FN, respectively, with an improvement in T-score of 31% at LS and 15.4% at FN and in Z-score of 36% at LS and 16.9% at FN, respectively. None of them experienced a new fracture during teriparatide treatment. Mean serum calcium and vitamin D levels at baseline were 2.25±0.07 mmol/l and 23.6±10.6 ng/ml, respectively with no significant difference after 12 months of therapy; similarly, no significant difference was found between urinary calcium at baseline (8.02±4.6 mmol/24h) and after 12 months. On the contrary, a significant reduction of 39% in parathormone levels was found between the baseline (25.2±5.7 pg/ml) and after 12 months. The preliminary results of our ongoing observational study suggest that teriparatide can be an effective treatment for beta thalassemia-induced osteoporosis by preventing fractures and improving BMD especially at lumbar spine; the multifactorial pathogenesis of bone disease in these patients may explain the greater incidence of bone pain in contrast with the non thalassemic population. Further studies on a large scale are required to confirm long-term effects of teriparatide therapy for thalassemic patients and to explain the physiopathological basis of our findings.

PD64 - ENDOCRINOLOGICAL ASPECTS OF HYPOPHOSPHATEMIA IN HIV INFECTION: AN OBSERVATIONAL STUDY

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Hypophosphatemia is an emerging problem among patients with HIV infection, likely due to HIV infection itself or to renal loss due to antiretroviral therapy (ART), and in particular tenofovir (TDF). The endocrinological aetiology of hypophosphatemia in HIV patients has not been fully explored, despite the high prevalence in HIV population hypovitaminosis D and low bone mass density (BMD). Our aim was to analyse endocrinological aspects and bone metabolism of HIV hypophosphatemic patients. We performed an observational study on 50 HIV-infected subjects (mean age: 53.3±10; 46M, 4F; average period from diagnosis: 14 years) with at least one documented hypophosphatemia event between Jan 2013 and May 2014 at the HIV centre. All patients, except one, were on ART and 80% of them had been exposed to TDF. Hypophosphatemia was classified as mild (2-2.4 mg/dl), moderate (1-1.9 mg/dl) and severe (<1mg/dl). Biochemical evaluation, including serum calcium, parathormone (PTH), vitamin D, bone turnover markers (osteocalcin and ß-crosslaps) was performed in every patient. BMD was measured by phalangeal quantitative US (according to WHO, osteopenia was defined by T-score < -1 S.D. and osteoporosis by T-score < -3.2 S.D) in 40 patients and by DEXA in 3 patients. Moderate hypophosphatemia was found in 72% of the subjects. Vitamin D was normal (>30 ng/ml), insufficient (10-30 ng/ml) and deficient (<10 ng/ml) in 18%, 58%, 24% of the patients, respectively. We found hyperparathyroidism in 20% of subjects, 30% of which primary. ß-crosslaps and osteocalcin were increased in 24% and 16% of the patients, respectively. Prevalence of low BMD was 72%. We found that mild hypophosphatemia occurred more frequently in subjects with low BMD and TDF exposure without reaching statistical significance. BMD was significantly inversely associated with age (p= 0.005) and severely symptomatic disease (CDC C class, p=0.05). T-score was significantly correlated with vitamin D (p= 0.001) and PTH (p=0.03) levels. We also found a correlation between nadir phosphate and vitamin D levels (p=0.05). Our study confirms a high prevalence of hypophosphatemia among HIV patients on ART. A significant correlation was found between hypophosphatemia and low BMD, TDF exposure and vitamin D levels. Despite the high prevalence of hypophosphatemia and low vitamin D levels, hyperparathyroidism was found only in 20% of the subjects. We therefore plan to investigate the effects of 1-year replacement therapy of hypovitaminosis D on BMD and phosphatemia in this population in order to assess the role of endocrinologic aspects on HIV-related hypophosphatemia.

PD65 - ASSOCIATION BETWEEN VERTEBRAL FRACTURES AND ABNORMALITIES OF TRABECULAR BONE STRUCTURE IN ACROMEGALY

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Vertebral fractures (VFs) are an emerging complication of acromegaly, but it is still unclear how we could predict the fracture risk in this clinical setting. As a matter of fact, DXA measurement of bone mineral density (BMD) does not provide reliable information on fracture risk in acromegaly, since patients may fracture even in presence of normal BMD. The aim of this cross-sectional study was to investigate whether high-resolution Cone Beam computed tomography (CBCT) may provide information on the skeletal abnormalities associated with VFs in acromegaly. Twenty-two patients with acromegaly (12 females, 10 males; mean age 62 years, range 25-72) were evaluated for VFs using a quantitative morphometric evaluation of spine X-ray. Geometric trabecular parameters and cortical thickness were measured at the distal radius using a high resolution CBCT system (Newtom 5G; QR, Verona, Italy) with an isotropic voxel size of 75 microns. After measurement of the cortical thickness, an irregular volume of interest (VOI) containing only trabecular bone was defined through segmentation and exclusion in every slice of the cortical cross-sectional area. A fixed threshold value equal for every patient was applied in order to separate trabeculae from bone marrow in the VOI. Bone volume fraction (BV/TV), mean trabecular thickness (Th.mean) and mean trabecular separation (Sp.mean) were measured by means of BoneJ plugin for ImageJ software version 1.47v (Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, MD, USA). All patients were also evaluated for DXA BMD at lumbar spine, total hip, femoral neck and distal radius.

VFs were found in 12 patients with acromegaly (8 with active and 4 with controlled/cured disease). Patients with VFs had significantly lower BV/TV (0.68±0.14% vs. 0.85±0.09%; p=0.008) and greater Sp.mean (0.46±0.11 mm vs. 0.33±0.008 mm; p=0.008) as compared with non fractured patients, without statistically significant differences (p=0.1) in Th.mean and cortical thickness (p=0.55). Fractured and non-fractured acromegaly patients did not have significant differences in BMD at distal radius (p=0.14), lumbar spine (p=0.75), femoral neck (p=0.38) and total hip (p=0.12).

This study shows for the first time that abnormalities of bone trabecular structure are associated with morphometric VFs in acromegaly. This study provides also evidence that high resolution CBCT at the distal radius may be an useful tool to evaluate and measure the deleterious effects of acromegaly on trabecular bone.

PD66 - SERUM SCLEROSTIN AND BONE TURNOVER IN RELATION TO METABOLIC SYNDROME IN PATIENTS WITH TYPE 2 DIABETES OR LADA: ACTION LADA AND NIRAD GROUPS

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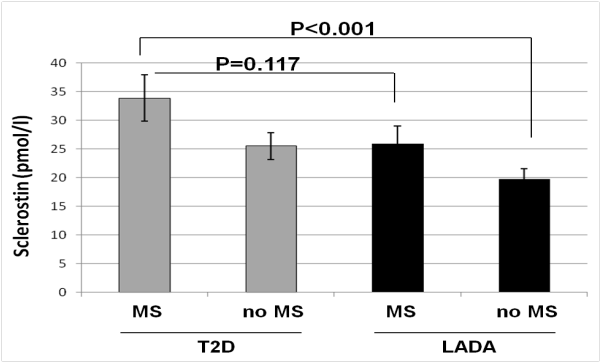
Diabetic patients may suffer of a low bone turnover condition. Several factors, including insulin deficiency and suppression of Wnt signaling may be involved. Sclerostin is a circulating Wnt antagonist and a main negative regulator of bone formation.

The aim of this study was to evaluate serum sclerostin levels and bone turnover in relation to metabolic syndrome (MS) in subjects with Type 2 Diabetes (T2D) or Latent Autoimmune Diabetes inAdults (LADA).

This was a cross-sectional study including 60 subjects with T2D and 89 with LADA recruited through the ACTION LADA and NIRAD cohorts and further divided according to diagnosis of MS (NCEP criteria). Serum sclerostin, bone formation (P1NP) and bone resorption (serum CTx) were analyzed by ELISA.

Serum sclerostin was significantly higher in T2D compared with LADA (29.7±2.4 vs 22.6±1.8 pmol/l, P=0.04). T2D with MS had almost double levels of sclerostin in comparison with LADA without MS (33.9±4 vs 19.7±1.8 pmol/l, P<0.001). P1NP levels were lower in T2D compared to LADA (P<0.038) while CTx levels were similar between the two groups. In the all groups of subjects, sclerostin levels were significantly correlated with BMI (r=0.17; *P*=0.047).

In conclusion, our findings indicate that T2D patients present a greater suppression of Wnt signaling than LADA ones which may cause low bone formation. MS may further play a negative effect on bone health.


P<0.001
P=0.117
Sclerostin (pmol/l)
40
35
30
25
20
15
10
5
0
MS
no MS
MS
no MS
T2D
LADA

PD67 - METHYLATION STATUS OF VITAMIN D-RECEPTOR GENE PROMOTER IN BENIGN AND MALIGNANT ADRENOCORTICAL TUMORS

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Vitamin D receptor (VDR) and its ligand 1,25-Dihydroxyvitamin D3 play, in general, an inhibitory activity on tumor cell proliferation. We previously showed a decreased expression of VDR mRNA/protein in a small group of adrenocortical carcinoma (ACC) tissues, suggesting the loss of a protective role of VDR against malignant cell growth in this cancer type (Pilon et al, JSBMB, 2014). Down-regulation of VDR gene expression may result from epigenetics events, i.e., methylation of cytosine nucleotide of CpG islands in the VDR-gene promoter. We analyzed methylation of CpG sites in the VDR-gene promoter of various adrenocortical tumor samples, including 3 normal adrenals, 15 benign tumors (3 non-functioning adenomas, 10 aldosterone-producing adenomas, 2 cortisol-producing adenomas) and 8 carcinomas (5 cortisol-producing carcinomas and 3 non-functioning carcinomas). Methylation of CpG-rich 5' regions was assessed by bisulfite sequencing PCR using bisulfite-treated DNA from archival microdissected paraffin-embedded adrenocortical tissues. A high methylation level (arbitrary cut-off of 30% or more) was found in the promoter region of VDR gene in 3/8 (2 cortisol-producing and 1 non-functioning) ACCs, while no VDR-gene methylation was observed in normal adrenals and adrenocortical adenomas. VDR mRNA expression was lower in ACCs than in benign tumors and normal adrenals ($P < 0.05$), and VDR immunostaining was weak or negative in ACCs, including all 3 methylated samples. Conclusions: The association of VDR-gene promoter methylation with reduced VDR gene expression is not a rare event in ACC, suggesting that VDR epigenetic inactivation may have a role in adrenocortical tumorigenesis. Other epigenetic mechanisms involved in silencing VDR, i.e. histone modifications, should be investigated.

PD68 - PDCD4 AND MIR-21: NEW POTENTIAL PHARMACOLOGICAL TARGET IN ADRENOCORTICAL TUMORS

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Adrenocortical tumors (ACT) include benign and malignant forms. Adrenocortical carcinomas (ACC) are highly malignant neoplasms with poor prognosis and strong metastatic potential. Pdcd4 is a tumor suppressor involved in invasion, transformation, intravasation and apoptosis. Among translational regulators of Pdcd4, miR-21 seems to play a fundamental role, as it is frequently overexpressed in many malignancies.

Aim of this study is to evaluate the expression of Pdcd4 and miR21 in ACT samples and their interaction.

Expression of miR-21 was evaluated by qRT-PCR in cell lines (SW13 and H295R cells) and in 39 ACT samples: 21 ACC, 6 Aldosterone Producing Adenoma (APA) and 12 Non Aldosterone Secreting Cortical Tumors (NACA) and 6 Normal adrenal (NA). Expression of Pdcd4 was analyzed in 20 ACC, 4 APA, 8 NACA, 3 NA. Also 2 ACC, 2 APA, 2 NACA with their healthy counterparts were analyzed by Western blot.

miR21 resulted upregulated in 71% of ACC and 27% of ACA (APA+NACA) and more expressed in SW13 cells. In ACC samples, Pdcd4 resulted statistically different if compared to miR-21 expression ($P<0.046$). WB analysis showed that Pdcd4 reactivity decreased in 2 ACC tissues compared to healthy parts, while in other samples no appreciable difference was perceived.

Pdcd4 and miR-21 are involved in ACT progress. Increase in miR-21 and loss of Pdcd4 expression are common in many tumors, such as thyroid, colon, ovarian carcinomas. Our results provided evidence of Pdcd4 and miR-21 involvement in ACC tumorigenesis. Their interaction may be a potential target for future therapies.

PD69 - 2D-DIGE PROTEOMIC ANALYSIS IDENTIFIES NEW POTENTIAL BIOMARKERS IN ADRENOCORTICAL CARCINOMA

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Adrenocortical carcinoma (ACC) is a rare and aggressive tumor with poor prognosis when metastatic at diagnosis. The biology of the tumor is still mostly unclear, thus justifying the limited specificity and efficacy of the anti-cancer drugs currently available. The present study reports the first proteomic analysis of ACC by using the two-dimensional-difference-in-gel-electrophoresis (DIGE) technique to evaluate a differential protein expression profile between adrenocortical carcinomas and normal adrenals. Mass spectrometry associated to DIGE analysis of carcinoma (n=10) and normal (n=8) adrenal specimens identified 22 proteins which are differentially expressed (fold variation <-2 or >2, $P<0.05$) between pathology and normal condition. All proteins appear to be overexpressed in ACC, except one which was downregulated. Among the overexpressed proteins, the differential expression obtained by DIGE analysis for ALDH6A1, Transferrin, Fascin-1, Lamin A/C, CAP1 and Adrenodoxin Reductase was validated by Western Blot analysis on the tissue samples of the same cohort (fold increase±SE7.5±1.4, 3.6±1.2, 2.9±0.2, 2.6±2.1, 1.9±1.4, 1.6±0.8, $P<0.05$, respectively) and by immunohistochemistry only for ALDH6A1, Transferrin and Fascin-1 on paraffin-embedded ACC and normal adrenal specimens of the same patients. In conclusion, our preliminary findings reveal a different proteomic profile in adrenocortical carcinoma compared to normal adrenals, identifying 6 proteins significantly overexpressed in the tumor. These proteins could represent novel valid protein ACC biomarkers if further validated in a larger cohort of patients.

PD70 - INHIBITOR OF APOPTOSIS PROTEINS LIVIN AND XIAP IN ADRENOCORTICAL TUMORS.

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Introduction: Adrenocortical tumors comprise frequent benign adenoma (ACA) and rare highly malignant carcinoma (ACC). Livin/ML-IAP/BIRC7 and XIAP/BIRC4 genes are two important members of the inhibitors of apoptosis proteins (IAP) family, which are involved in cancer development and progression, mostly through the inhibition of caspase-3. Aim of the study was to evaluate the expression of livin/BIRC7, its isoforms livin α and β, XIAP/BIRC4 and caspase-3 in normal and neoplastic adrenal glands.

Methods: Total *BIRC7, livin α, livin β, BIRC4* and *caspase-3* mRNA expression was evaluated by quantitative real-time PCR in 84 fresh-frozen tissue samples (34 ACC, 25 ACA, and 25 normal adrenal glands=NAG), including 19 paired samples of tumor and surrounding NAG (13 ACA and 6 ACC). The correlation between mRNA levels and several clinical parameters was also investigated. Additionally, livin protein expression was assessed by Western Blot analysis in a subgroup of 14 paired samples (8 ACA and 6 ACC).

Results: *BIRC7* mRNA expression was similar between ACAs and NAGs, but significantly higher in ACCs (*P<0.005 vs* both ACAs and NAGs). Both isoforms *α* and *β* were detected, *livin β* having constantly higher expression than *livin α* (*P=0.07* in ACCs; *P=0.10* in ACAs; *P=0.02* in NAGs). These results were also confirmed at the protein level. In contrast, *BIRC4* mRNA levels were lower in ACC compared to NAGs and ACAs (*P=0.07* for trend), while *caspase-3* was higher expressed in benign tumors than in ACCs and NAGs (*P=0.05* and *P=0.03*, respectively). There was an inverse correlation between *caspase-3* levels and tumor size (*P=0.005*, R=0.36) in all tumors. However, no impact on overall and progression free survival was found for all investigated genes.

Conclusion: Our study demonstrates that livin/BIRC7 is specifically over-expressed in ACC, suggesting that it may be involved in adrenocortical tumorigenesis, may represent a novel marker for malignancy and may be used as a potential target for therapeutic approaches in ACC.

PD71 - EFFECT OF THE SWITCH FROM CONVENTIONAL GLUCOCORTICOIDS TO "DUAL RELEASE HYDROCORTISONE" IN ADULT PATIENTS WITH PRIMARY AND SECONDARY ADRENAL INSUFFICIENCY: A SIX-MONTHS MULTICENTER STUDY

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Adrenal Insufficiency (AI) requires life-long glucocorticoid (GC) treatment, which is associated with an increased risk of metabolic syndrome (MS), probably due to cortisol overexposure for multiple drug daily doses, together with an impairment of Quality of life (QoL). Moreover treatment compliance (TC) is reported to be suboptimal in AI patients. The current study aimed at investigating the impact of the switch from twice/thrice daily conventional GCs to once daily dual-release-hydrocortisone (DR-HC) treatment on metabolic profile, QoL and TC in patients with primary AI (PAI) and secondary AI (SAI). Thirty-five patients [12 with PAI (7F, 5M, 33-60 yrs), 8 treated with cortisone acetate (37.5-75 mg/day) and 4 with hydrocortisone (20-30 mg/day), and 23 patients with SAI (9F, 14M, 20-77 yrs), 15 treated with cortisone acetate (18.75-37.5 mg/day) and 8 with hydrocortisone (15-20 mg/day) entered the study and were evaluated before and 6 months after the switch to DR-HC (PAI: 20-60 mg/day; SAI: 20-40 mg/day). At 6-month-follow-up, in PAI patients, body weight (BW) (p=0.036) significantly improved and a trend to a significant improvement was also found for waist circumference (WC) (p=0.086). A diagnosis of MS, performed in 2 patients (17%) at baseline, was not confirmed after 6 months. In SAI patients, BW (p=0.001), BMI (p=0.003) and WC (p=0.007) significantly improved. A clear diagnosis of MS, performed in 7 patients (30%) at baseline, was confirmed only in 4 (17%) of these patients after 6 months. In a subgroup of 12 patients with AI, visceral adiposity index (VAI), an indicator of adipose function and distribution, which seems to indirectly express the cardiometabolic risk, significantly improved (p=0.05) while an improvement in glucose levels (p=0.064) and in Insulin Sensitivity Index (ISI 120) (p=0.052) was reported 120 minutes after glucose load. In the subgroup of 10 patients considered for the evaluation of QoL and TC, working ability ameliorated in 6 patients (60%), vitality in 5 (50%), general health perception and depression in 3 patients (30%) and body pain perception in 2 (20 %) patients. Moreover, nine (90%) of these 10 patients improved TC, changing from low to medium adherence. In conclusion, the switch from conventional GCs to DR-HC in patients with AI improved BW, BMI, WC, prevalence of MS, glucose tolerance and insulin sensitivity, QoL and TC.

PD72 - SIMPLE AND HIGH-THROUGHPUT DETERMINATION OF SERUM STEROIDS BY LIQUID CHROMATOGRAPHY – TANDEM MASS SPECTROMETRY (LC-MS/MS): DEVELOPMENT AND VALIDATION OF A ROUTINE-SUITED METHOD BASED ON MINIMAL SAMPLE PRE-TREATMENT.

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Steroid measurement is mandatory for the management of diseases such as hypercortisolisms, female hyperandrogenism, male hypogonadism or inborn disorders of steroid synthesis. LC-MS/MS largely proved its superiority to automated immunoassays (IA) in accurately and sensitively measuring low level steroids. However, the introduction of LC-MS/MS platforms in routine laboratories is still limited by the need for sample preparation requiring operator handwork, large volume and long runtime. Aiming at reconciling reliability and practicability, we developed a LC-MS/MS method based on minimal sample preparation for the simultaneous determination of cortisol (F), testosterone (T), androstenedione (A), 17OHprogesterone (OHP) and the non-routinely assayed 17OHpregnenolone (OHp).

One hundred µl of serum were treated by protein precipitation, diluted with H2O and injected into the analytical system Prominence UFLC – electrospray - LCMS-8050 (Shimadzu). Samples underwent on-line purification on perfusion column (6ml/min, 3min) and separation on Shim-pack XR-ODS-50x3mm,2µm column (Shimadzu) in 5min H2O/acetonitrile gradient, before 7min clean-up and reconditioning program. Total runtime was 15min. Quantitative and qualitative transitions were monitored. Isotopic dilution quantitation was performed by using d4-F, d5-T, d5-A, d8-OHP and $13C_3$-estrone as internal standard for F, T, A, OHP and OHp, respectively.

Lower limits of quantitation (pg on column) assessed in calibrators diluted in bovine serum albumin (4%) were 122.1pg/ml (1.5pg), 9.77pg/ml (0.12pg), 19.5pg/ml (0.24pg), 39.1pg/ml (0.49pg) and 312.5pg/ml (3.9pg), and functional sensitivity in serum matrix was 122.1, 19.5, 19.5, 39.1 and 312.5pg/ml for F, T, A, OHP and OHp, respectively. Intra and inter-assay CV ranged between 3.4-7.2% and 5.8-16.7%, respectively. Performance was stable along 150 consecutive samples. A comparison with an established extractive LC-MS/MS assay for F, T, A, and OHP revealed optimal correlation (r:0.9933-0.9996) and slope coefficients (0.810-1.060).

These preliminary data showed that our LC-MS/MS method is able to measure with optimal sensitivity and robustness five key steroids needed for the clinical assessment of a wide spectrum of endocrine disease. Moreover, the practicability and high-throughput of this novel LC-MS/MS approach promotes its application in routine laboratories.

PD73 - IS THE DEGREE OF URINARY FREE CORTISOL AN EXHAUSTIVE PARAMETER FOR DEFINING CUSHING SYNDROME SEVERITY?

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Background: Cushing syndrome (CS) is characterized by increased morbidity and mortality compared to the general population. However, there are patients who have more aggressive forms than others. To date, there is an arbitrary classification in mild, moderate and severe hypercortisolism, based on the times of exceeding the upper limit of normal (ULN) of urinary free cortisol (UFC).

Aim: To evaluate by a for trend analysis whether the degree of hypercortisolism, defined by the times of exceeding the ULN of UFC levels, is related to the worsening of phenotypic, cardiovascular and metabolic parameters, in a cohort of CS patients.

Materials and methods: We conducted a cross-sectional study on 192 patients with CS, at diagnosis, consecutively presenting at the outpatients' clinic of the Universities of Ancona, Naples and Palermo. Patients were grouped into mild (UFC not exceeding twice the ULN), moderate (2-5 times the ULN) and severe (more than 5 times the ULN) hypercortisolism.

Results: 37 patients (19.3%) had mild, 115 (59.8%) moderate and 40 (20.9%) severe hypercortisolism. A significant trend of increase among the three groups was demonstrated for cortisol at 08.00, 16.00 and 24.00 h levels and cortisol after dexamethasone (all $p < 0.001$). No significant trend of increase was found regarding phenotype [(moon face p=0.416, facial plethora p=0.978, buffalo hump p= 0.148, purple striae p=0.148, central obesity p= 0.524)] and comorbidities [(coronary heart disease p=0.648, coagulopathy p=0.180, peripheral vascular disease p=0.072, cerebral vascular disease p=0.757, depression p=0.231, osteoporosis p=0.291, diabetes mellitus p=0.797, hypercholesterolemia p= 0.873, arterial hypertension p=0.773 and metabolic syndrome p=0.540)].

Conclusions: Our findings show that the degree of hypercortisolism (evaluated by the times of exceeding the ULN of UFC or by serum cortisol levels) does not express the severity of the disease. Indeed, cortisol secretion variability and the exact clinical onset of CS and consequently the duration of disease make it difficult to find adequate parameters to define CS severity and identify aggressiveness at an early stage.

PD74 - IMPAIRMENT OF INNATE IMMUNITY OF ADRENAL INSUFFICIENT PATIENTS TREATED WITH CONVENTIONAL GLUCOCORTICOID THERAPY IS RESTORED BY SWITCHING TO ONCE-DAILY DUAL RELEASE HYDROCORTISONE: A MULTICENTER CONTROLLED TRIAL

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CONTEXT: Adrenal insufficient (AI)patients treated with conventional glucocorticoid therapies(CGC)have increased morbidity and mortality rate, with infections, cardiovascular diseases and malignancies reported as major causes of death.CGC fail to recapitulate cortisol circadian rhythm.We hypothesized that a defect in the immune system due to inappropriate CGC exposure-time might be a contributing factor.We carried out a multicenter trial(NCT02277587)to test the effect of shifting from CGC to once-daily oral hydrocortisone dual-release(DR-HC)mimicking a more physiological cortisol profile.**AIM:**To study the immune cell profile of conventionally treated primary and secondary AI pts and the changes induced by shifting to once daily DR- HC.**PATIENTS:**The trial enrolled 58 adults:43(m.a.46.1±13.7; F=20)with AI due to primary(n= 21)or secondary(n=22) causes,and 15 controls(age 39.9±11.6; F=7).Duration of disease was 63±76 months.At baseline,all AIs were on cortisone acetate(65%,mean equivalent HC dose 28±9 mg)or HC(35%, mean dose 21±3 mg)and,after providing informed consent,were randomly assigned to continue their own CGC(n=16) or shift to an equivalent dose of DR-HC(n=27,pAI=13) in an open label 6-month trial.At 0,3 and 6 months all pts had clinical, biochemical,hematological and metabolic assessment plus comprehensive immune cell flow cytometry analyses.**RESULTS:**All 58 pts completed the first 3-month assessment.Compared to healthy controls,AIs exhibited a baseline marked reduction of NKs (10.9±4.2 vs 5.5±5.7% of $CD16^{+}CD56^{+}$,p<0.01),and a tendency toward increased classical monocytes (21.2±3.5 vs 28.9±17.0% of $CD14^{++}CD16^{-}$,p=0.08).No differences in T lymphocytes and granulocytes were found.After 3 months,a normalization of cytotoxic NK $CD16^{+}CD56^{+}$cells was observed in DR-HC shifted (D=5.2±7.4,P<0.01),but not in CGC treated patients(D=0.8±5.9,P=NS)or healthy controls(D=1.0±3.4,P=NS).DR-HC shift also produced a significant change in classical monocytes(P<0.01).The NKs and monocytes variation produced by DR-HC switch were similar between subjects previously on cortisone acetate or conventional hydrocortisone.NK changes didn't correlate to disease duration or equivalent HC doses.Body weight(P<0.01)and systolic blood pressure(P<0.05)improved to a greater extent in DR-HC than CGC.A trend toward Hb1Ac reduction was also observed (p=0.07)in DR-HC shifted pts only.**CONCLUSION:**CGC treated AI is associated with severely impaired NKs and classical monocytes profile,compromising early innate immune host defense against infections and malignancies.Switch to DR-HC was associated with a recovery of NKs.

PD75 - LIMITATIONS OF LATE NIGHT SALIVARY CORTISOL BY AUTOMATED ASSAY IN THE DIAGNOSIS OF PSEUDO-CUSHING'S STATES

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CONTEXT Late-night salivary cortisol (LNSC) measurement has been promoted as screening test for the diagnosis of Cushing's Syndrome (CS). However, normal reference ranges and diagnostic cutoff should be validated in each laboratory before being applied in clinical practice. In addition, only few studies have compared prospectively different screening tests for hypercortisolism whitin the same population and in patients with Pseudo-Cushing states (PC).

AIM To prospectively evaluate the usefulness of LNSC measured by automated assay as chemiluminescence (CLIA) in a wide cohort of ambulatory patients with suspect of hypercortisolism and compare the results with other tests current used to diagnose CS (1mg dexamethasone suppression test DST; urinary free cortisol UFC; MSC midnight serum cortisol).

METHODS We selected a total of 281 patients including 117 with euthyroid goiter (Volunteers subjects VS) and 164 patients with suspect of CS . All patients, provided one salivary sample (h 23:00) for cortisol measurement by CLIA (Access Beckman Coulter) in an outpatient setting. If LNSC was increased the patients were invited to a brief hospitalization for the further investigations.

RESULTS LNSC in outpatients was significantly higher ($P< 0.005$) in suspected CS (1.02 ± 0.69 mcg/dl) compared with volunteer subjects (0.27 ± 0.14 mcg/dl). Among subjects with abnormal LNSC, two were VS, 35 were PC and 46 were confirmed CS. Only one patient with normal LNSC but with a strong clinical suspect, was affected by CS. In overall population the sensitivity (SE) was 97,9% and specificity (SP) 84,2% confirming the good performance of LNSC. However, in patients suspect for CS, the SE was 97,9% but the specificity was low 41,2%. The difficulties in distiguishing PC patients was confirmed when other tests were used. In these patients the rate of false positive tests was elevated: 44% had increased value of UFC, 26.6% had a increased MSC and 14.6 % had a unsuppressed serum cortisol after DST. No statistically differences were observed in weight, age and gender in patients with or without abnormal tests. On the contrary, patients with PC were often diabetics and obese.

CONCLUSIONS This study confirms the utility of LNCS measured by routinary automated assay as a screening test for CS. Although a normal LNSC value is useful to exclude Cushing's Syndrome, the specificity of LNSC is low in patients with PC who have a functional hypercortisolism. These data confirm that the differentiation between Cushing's syndrome and pseudo-Cushing's states is very difficult and requires additional tests.

PD76 - ESTROGEN AND PROGESTERON RECEPTORS EXPRESSION IN PAPILLARY THYROID CANCER: A ROLE IN THE RISK STRATIFICATION AND PRE-GRAVIDIC COUNSELLING

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Thyroid cancer is highly prevalent in women during the fertile age indicating a possible role of sex hormones in the pathogenesis and development of thyroid tumors. We studied the expression of Estrogen receptor alpha (ER alpha) and Progesteron receptor (PR) in 182 female and male patients with papillary thyroid cancer (PTC) and correlated it to clinical and molecular features. ER alpha and PR expression was found in 66.5% and 75.8% of patients, respectively, and significantly correlated with larger tumor size and with the presence of metastatic neck lymph-nodes at diagnosis. Interestingly, the occurrence of the "receptor conversion" phenomenon, already reported to have a negative prognostic effect in breast cancer, has been demonstrated for the first time in thyroid tumors. Indeed, almost all the ER alpha positive primary tumors analyzed had ER alpha negative metastatic lymph-nodes. BRAFV600E mutation was detected in 23.2% of the tumors, with a higher prevalence in larger tumors and in those with a stronger ER alpha or PR staining. Finally, the follow-up of a woman with persistent PTC during pregnancy, highlighted the role of estrogens in the progression of the disease.

In conclusion, the expression of ER alpha and PR is frequent in differentiated thyroid tumor tissues and it is significantly associated with a more aggressive presentation. Although their expression did not seem to influence the outcome of the disease, the evaluation of sex hormone receptors could be an additional tool in the post-operative risk stratification and might be useful in the pre-gravidic counselling of fertile women affected with persistent thyroid cancer.

PD77 - CHARACTERIZATION OF NUCLEOPORIN 153 IN AGGRESSIVE PROSTATE CANCER: CONSEQUENCES OF THE INTERPLAY WITH ENDOTHELIAL NITRIC OXIDE SYNTHASE UPON ESTROGEN STIMULATION

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It has been recently demonstrated that Nucleoporin 153 (Nup153), a component of the Nuclear Pore Complex, represents a major new class of global chromatin-binding proteins regulating the spatial organization of chromosomes and associating at very high density with transcriptionally active regions. From an independent line of research it emerged that nitric oxide (NO) and its endothelial synthase (eNOS), in association with the Estrogen Receptors (ERs), play a key role in tumor maintenance and progression. In this regard, our recent data indicated that: i. eNOS exerts a critical epigenetic function in prostate cancer (PCa); ii. a significant number of eNOS-containing nuclear complexes by ChIP-Seq exhibit a specific pattern upon estrogen (E_2) treatment, directly affecting gene expression.

While eNOS usually associates to caveolae on the plasma membrane trans-locating to the nucleus upon hypoxia or E_2, Nup153 is placed in the inner part of the nuclear envelop and, upon still unknown signals, it is the only member of the nucleoporins family capable to move to the nucleoplasm and interact with nuclear matrix and chromatin, thus contributing to nuclear import/export and cell migration and metastasis. Notably, Nup153 has recently revealed oncogenic properties in pancreatic tumor cells although its role has not yet been investigated in PCa.

First we found increased expression of eNOS and Nup153 and stronger nuclear interaction between these proteins in PCa as compared to normal prostate epithelial cells. Of interest, E_2 induced an increase of Nup153 expression and of eNOS/Nup153 nuclear interaction in PCa cells, by Co-IP and confocal analysis, suggestive of a hormone-dependent mechanism. These data were substantiated by results from a ChIP-Seq analysis on chromatin-associated eNOS, revealing an exclusively hormone-dependent localization of eNOS-DNA peaks along Nup153 promoter region.Intriguingly, after Nup153 RNA interference (siNup153), eNOS nuclear localization was lost as assessed by confocal analysis, indicating that Nup153 is crucial for the eNOS nuclear translocation in both E_2-treated or untreated cells. In addition, upon Nup153 depletion, we also observed a loss of E_2–dependent nuclear localization of other eNOS partners, such as ERb. To explore the role of Nup153 in the E_2 signaling we validated the hormone-responsiveness of VEGF Type2 Receptor (KDR), an E_2-target gene, upon siNup153. Nup153 depletion virtually causes loss of the E_2-responsiveness of KDR. Notably, in the same condition we found a reduction of migration capacity in both primary and metastatic PCa cells by scratch test.

Our findings reveal Nup153 as a novel partner of eNOS also contributing to its nuclear translocation and a potential estrogen and nitric oxide therapeutic target.

PD78 - HYPERINSULINISM AND POLYCYSTIC OVARY SYNDROME (PCOS): ROLE OF INSULIN CLEARANCE.

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The evidences favour insulin resistance and compensatory hyperinsulinism as the predominant metabolic defects in polycystic ovary syndrome (PCOS). However, hyperinsulinism, as well as being compensatory, can also express a condition of reduced insulin clearance in the kidney, liver and insulin-sensitive tissues. The aim of the present study was to evaluate the differences in insulin action and metabolism between women with PCOS (but normal glucose tolerance) and age- and BMI-matched women with prediabetes (without hyperandrogenism and ovulatory disorders).

Using a cross-sectional study design, 22 PCOS (Rotterdam criteria) and 21 age/BMI-matched women with prediabetes (impaired fasting glucose and/or impaired glucose tolerance), after one month's withdrawal of insulin sensitizers and oral contraceptive pills, were subjected to an Euglycaemic–hyperinsulinaemic clamp and an Oral Glucose tolerance Test. Insulin sensitivity was assessed by the glucose infusion rate during clamp (M-value); insulin secretion by Insulinogenic index, fasting insulin and $AUC_{2h\text{-}insulin}$ during OGTT; insulin clearance by the metabolic clearance rate of insulin (MCRI) during clamp; adipose tissue distribution and function by Visceral Adiposity Index (VAI).

Obviously, women with PCOS, compared to women with prediabetes, showed significantly higher levels of fasting insulin ($p<0.001$) and $AUC_{2h\text{-}insulin}$ ($p=0.011$) and significantly lower levels of fasting glucose ($p<0.001$) and $AUC_{2h\text{-}glucose}$ ($p=0.008$). Between the two groups, no difference was found regarding insulin-sensitivity (M-value) and glucose-induced insulin secretion (Insulinogenic Index). By contrast, lower levels of MCRI were found in women with PCOS [420 (IQR:227-588) vs. 743 (IQR:597-888) $ml \cdot m^{-2} \cdot min^{-1}$: $p< 0.001$]. Furthermore, in a multiple linear regression analysis, only in the PCOS group there was an independent inverse correlation observed between MCRI and both fasting insulin (β:-0.540; $p=0.006$) and $AUC_{2h\text{-}insulin}$ (β:-0.858; $p<0.001$).

Our study suggests that in women with PCOS there is peripheral insulin sensitivity similar to that found in other disorders characterized by insulin resistance, such as prediabetes. What characterizes PCOS is hyperinsulinism, which is simplistically defined "compensatory"; actually, this is related to a decreased insulin clearance whose specific causes and dynamics have yet to be studied.

PD79 - PRIMARY OVARIAN INSUFFICIENCY CANDIDATE GENE IDENTIFICATION THROUGH THE TRANSCRIPTOME PROFILING OF HUMAN GRANULOSA CELLS INDUCED BY THE OOCYTE PARACRINE FACTOR BMP15.

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Primary Ovarian Insufficiency (POI) is an inheritable disease with a strong genetic component but characterized by a highly variable expressivity and penetrance. To date, several mutations in the X-linked gene *BMP15* have been identified in association with POI and this oocyte-derived growth factor plays an essential role as a local regulator of the ovarian folliculogenesis in animal models and in humans, also controlling the ovulation quota. Nevertheless, the already known genetic alterations may explain only few cases. The identification of novel molecular mechanisms and genes involved in the pathogenesis of POI is mandatory to clarify the unknown etiopathogenesis underlying this fertility disorder. For this purpose, we investigated pathways and molecular events induced by treatment with recombinant human BMP15 (rhBMP15) in human Granulosa cells (hGCs) through a global approach for large-scale gene-expression profiling. The hGCs were obtained from fertile women undergoing in vitro fertilization and then stimulated in triplicates with rhBMP15. Treated and untreated hGCs were harvested after 0, 2 and 6 hours, to evaluate early and late regulated genes. RNAs from each condition were processed for hybridization on the Illumina BeadChips. A differential statistical analysis was performed by applying the Linear Model for Microarray Data (Limma): stimulation, time and pool variables were considered. Gene Ontology pathways were also tested for differential expression. After the quantile normalization, 4 groups of samples emerged, pool and time dependents (6h vs. 0h/2h), by applying the Principal Component Analysis (PCA) of the means of technical replicates. The differential analysis of stimulated vs non-stimulated samples over time shows 34 differentially expressed probes after 2h and 46 probes after 6h (Benjamini-Hochberg (BH)-adjusted P-value < 0.05). Moreover, 21 probes were differentially expressed both after 2 and 6 hours. The following GO gene sets differential analysis was performed by ROAST test (Rotation gene set tests for complex microarray experiments). The rhBMP15 stimulus modulates the BMP signaling pathway, cell fate commitment, proliferation, apoptosis and estrogen response. Several of the pathways here identified have already been described in previous studies dissecting the mechanisms of folliculogenesis in models of altered fertility, confirming BMP15 as a master regulator of folliculogenesis processes. These findings should prompt future analysis of BMP15-regulated genes as novel candidates in POI cohorts.

PD80 - STRUCTURAL ALTERATION OF FMR1 GENE IN TWO SISTERS WITH SECONDARY AMENORRHEA AFFECTED BY PRIMARY OVARIAN INSUFFICIENCY (POI)

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Primary ovarian insufficiency (POI) is defined as the development of hypergonadotropic hypogonadism before the age of 40 years in women who have a normal karyotype. The clinical manifestations include oligomenorrhea or amenorrhea, estrogen deficiency symptoms, elevated serum gonadotropin and low serum estradiol concentrations. POI can have different causes which include autoimmunity, drugs, infections and genetic defects although in many –women affected it remains an idiopathic disease. Genetic causes comprise chromosomal or single genes alterations: X-chromosome abnormalities represent 13% of the cases followed by the FMR1 gene premutation that account for up to 6% of POI with positive family history. We present a case of a 29-year-old woman with secondary amenorrhea. Her family history revealed that her 28-year-old sister was in oligomenorrhea with a full-term pregnancy three years before and her mother had menopause at 35 years of age. Her medical history was positive for autoimmune thrombocytopenia and Hashimoto thyroiditis and negative regarding smoking, chemotherapy or radiation. Her physical examination showed normal body weight, no galactorrhea, no clinical signs of hyperandrogenism but symptoms/signs of hypoestrogenism such as hot flashes, decreased libido and vaginal dryness. Hormonal blood tests documented high levels of pituitary gonadotropins (FSH 84.19 mU/ml and LH 48.06 mU/ml) with low levels of estradiol (E2 < 20 pg/ml) and normal values of prolactin and thyroid hormones. Transvaginal ultrasound showed a normal volume uterus with a reduced endometrial thickness, suggesting a low estrogen secretion and ovaries at the lower limits of normal size. Low levels of anti-Mullerian hormone (AMH 0.1 pmol/l) and inhibin B (6.9 ng/ml) indicated reduced ovarian reserve. Genetic counseling suggested molecular investigation of the FMR1 gene whose full mutation (>200 CGG repeats) causes Fragile X Syndrome while premutation (55-200 CGG repeats) is associated with POI in 20 to 28% of carrier women. This analysis revealed an FMR1 premutation of 81 CGG repeats in our patient and 87 CGG in her sister. Hormonal replacement therapy with estro-progestinic was started and ovodonation has been proposed for reproductive aim. The interdisciplinary endocrine and clinicl genetic management of familial case of POI may allow the diagnosis with implications in terms of prediction of the POI phenotype in relatives at-risk and of fertility perspectives at young age.

PD81 - IGF-II MRNA EXPRESSION IN BREAST CANCER: PREDICTIVE VALUE AND RELATIONSHIP TO OTHER PROGNOSTIC FACTORS

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OBJECTIVES: IGF-II is an important regulator of neoplastic growth and it is stromal in origin. The aim of the study was to evaluate the impact of IGF-II mRNA expression in clinical outcome of breast cancer (BC).

METHODS: the expression of IGF-II mRNA was compared to several prognostic parameters such as node metastases, oestrogen (ER) and progesterone (PR) receptors, ki-67 protein expression (ki-67) and p53 oncogene protein expression (p53). IGF-II mRNA was examined using *in situ* hybridization method.

RESULTS: The study group included 68 women (mean age ± SD = 52.2 ± 15.7 yrs) submitted to radical mastectomy for ductal infiltrating BC. 37 out of this 68 patients (54.4%) had axillary node metastases (N+), 33 (48.5%) were ER+, 30 (44.1%) PR+, 11 (16.2%) p53+ and 12 (17.6%) Ki-67+. Positive IGF-II mRNA expression (IGF-II +) was detected in 33/68 (51.6%) BC. After a follow-up of 5 years 50/68 BC patients (73.5%) were alive and relapse free. Survival rate was significantly lower in N+ than in N- (20/37, 54% vs 30/31, 96.7%; $p<0.0001$) and in p53+ than in p53- (5/11, 45.4% vs 44/56, 78.6%; $p=0.002$). No relation was found between ER and PR status and survival.

Survival was not significantly different between IGF-II+ and IGF-II- patients (23/33, 69.6% vs 24/31, 77.4%; p=NS). IGF-II mRNA expression did not affect the prognosis in N- and in p53- BC patients, while the majority of p53+/IGF-II+ patients (5/8, 62.5%) died within two year from diagnosis. Ki-67 did not affect survival, but a significant poorer prognosis was observed in IGF-II+/ki-67+ than in IGF II+/Ki-67– BC patients (7/33, 21.2% vs 26/33, 78.8%; $p=0.04$).

CONCLUSIONS: Our data indicate that in BC: 1) node metastases and p53 expression are independent poor predictive factors; 2) ER, PR and ki-67 expression have no impact on survival; 3) IGF-II mRNA expression *per se* is not connected to five years survival but in association with p53 or ki-67 may contribute to select a group of patients with particular poor clinical outcome.

PD82 - ANDROGENS POSITIVELY REGULATE NO-MEDIATED RELAXANT PATHWAY IN RAT CLITORIS

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Female sexual response is the result of a complex interplay between central and peripheral mechanisms. Hormonal regulation of female sexual excitement is poorly understood.

To evaluate sex steroid regulation of the NO-dependent relaxant and RhoA/ROCK contractile pathways in clitoris, subgroups of ovariectomized rats were or left untreated or supplemented with estradiol, progesterone, testosterone (T) and T plus the aromatase inhibitor, letrozole. mRNA expression (qRTPCR) of genes of the relaxant NO-signaling, and genes of the contractile RhoA/ROCK pathway were studied in rat clitoris.

In- vivo treatment with T increased clitoris eNOS, nNOS, sGC1a3, sGC1b3, PDE5, PKG1 mRNAs, that were all further increased by cotreatment with letrozole. T also increased ROCK2mRNA. E2-supplementation increased RhoA and ROCK2 expression. All NO-signaling genes, and ROCK2 resulted positively associated with T plasma level, while E2 level was positively associated with RhoA , ROCK2 and sGC1a3. When T and E2 (ROCK2 determinants at univariate analysis) were introduced as covariates in a multivariate model, only the association between E2 and ROCK2 was confirmed. To further investigate the effect of T and E2, in isolated rat clitoris smooth muscle cells (clitSMC) we studied migration, as a read-out of RhoA/ROCK activity. E2 increased clitSMC migration. Also T increased clitSMC migration. Letrozole pretreatment abrogated T-induced migration. The non aromatizable androgen, DHT, reduced clitSMC chemotaxis even below untreated cells.

Our data demonstrate that T improves the NO-mediated signaling, whilst E2 stimulates the contractile RhoA/ROCK signaling in clitoris.

PD83 - MOLECULAR DISSECTION OF ESTROGEN RECEPTOR SIGNALING IN HORMONE-DRIVEN CANCER: NOVEL ROLE OF LONG NON CONDING RNAS, HOTAIR AND MALAT1

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Breast and prostate cancer are hormone-driven cancers controlled by sex steroid hormones and their receptors. We established that in this type of cancers estrogen receptors (ERs) interact with other transcription factors and co-factors, like the endothelial nitric oxide synthase (eNOS) and the hypoxia inducible factors (HIFs). Recent, discoveries enlightened a key role for long noncoding RNAs (lncRNAs) in several human cancers including hormone-dependent cancers. The deregulated expression of lncRNAs marks disease-progression and may alter chromatin state promoting metastatization ultimately functioning as independent predictors for the patient outcome.

Our recent study by ChIP-Seq approach, indicates that, in prostate cancer (PCa), the estrogen-dependent eNOS-containing complexes formed preferentially at genomic regions in which several cancer-associated lncRNAs are placed (e.g. H19, HOTAIR and MALAT1). The level of these transcripts were first evaluated in a large population of breast and prostate cancer cell lines and a prevalence in terms of expression in breast as compared to prostate cancer-derived cells has been observed. These lncRNAs were consistently induced by estradiol (E_2, 10^{-7}M) with an intensity and dynamic profile comparable to that of classical estrogen-responsive genes including PS2 or the catalytic subunit of telomerase. ChIP analysis confirmed that this regulation is mediated by the estrogen-dependent recruitment of ERs and eNOS onto the promoter region of those lncRNAs. Notably, RNA-ChIP assays showed that HOTAIR and MALAT1 are capable to directly interact *in vivo* with ERs or eNOS on chromatin, being this phenomenon intriguingly modulated by estrogen in both breast and prostate cancer cells. Specifically, the interaction of HOTAIR/ERs and MALAT1/ERs has been strongly enhanced by estradiol treatment in breast and prostate environment. On the other hand, interaction of HOTAIR/eNOS and MALAT1/eNOS has been regulated by estrogen although in opposite direction in breast compared to prostate. Depletion of HOTAIR or MALAT1 transcripts by antisense gap-mers oligonucleotides significantly impaired response of classical target genes, such as PS2, to estradiol. Conversely, HOTAIR interference caused a delay of PS2 estrogen response, while the interference of MALAT1 caused an increase in PS2 basal level, thus determining a virtual abrogation of estrogen responsiveness, suggestive of a ordered functional hierarchy between the two lncRNAs in response to estrogens.

In summary, our data indicate that the interaction of lncRNAs MALAT1 and HOTAIR with ERs or eNOS are necessary to achieve a complete estrogen responsiveness.

PD84 - CLINICAL, BIOCHEMICAL AND HORMONAL CHARACTERISTICS OF OVARIAN AND ADRENAL ANDROGEN SECRETING TUMOURS

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Background: Ovarian (OAST) and adrenal (AAST) androgen secreting tumours are very rare, accounting for only 0,2% of all causes of hyperandrogenism. Clinical manifestation and biochemical and hormonal presentation, even if potentially similar, have never been compared.

Design: Retrospective comparison of clinical signs, and biochemical and hormonal baseline levels between female with OAST and with AAST

Methods: Data from 18 consecutive female patients, 9 with OAST and 9 with AAST presenting at Endocrinology Unit of Bologna from 2000-2014, were reviewed. Clinical, biochemical and hormonal characteristic were compared between the groups. Of these 18 subjects, 17 had histological confirmation (9 AAST: 8 adrenal cortical cancer and 1 adenoma; 8 OAST: 3 Leydig cell tumours, 2 Sertoli-Leydig cell tumours, 1 fibrothecoma, 1 stromal luteoma and 1 hilar cells hyperplasia). One patient refused surgical treatment, however her clinical presentation and biochemical, hormonal and radiological analysis were compatible with OAST and therefore included in the study.

Results: Patients with OAST were older (59 vs 40 years p=0,02), and had higher BMI (vs 29,9±4,7 vs 23,5±4,8 kg/m2, p=0,01) than AAST. The prevalence of hirsutism and alopecia was higher in the OAST (100% and 77%, respectively) compared with the AAST group (44% and 22%, respectively). Libido was increased in 4 patients with OAST and in only one with AAST. Two patients with OAST had clitoridomegaly and one AAST had acne. Seven of the AAST patients were of reproductive age (3 had amenorrhea, 4 AAST were eumenorrheic), whereas all the remaining were post-menopausal women. The OAST group had higher haemoglobin and haematocrit values respect to the AAST group (14,6 ±1,5 vs 12,8±0,6 g/dl, p=0,04; 43± 5 vs 39±2 %, p=0,02). Testosterone levels were higher (4,2±2,9 vs 1,8±0,9 ng/ml p=0,01, respectively) whereas DHEA-S, (0,7±0,5 vs 4,8±3,5 mcg/ml, p=<0,001) androstenedione (383±281 vs 903±440 ng/ml, p=0,006) and cortisol levels (136±30 vs 190±52 ng/ml, p=0,01) were lower in the OAST group with respect to AAST.

Conclusion: To our knowledge this is the first study comparing adrenal and ovarian androgen tumours. Our study suggests that the clinical and biochemical signs of hyperandrogenism are more frequently manifested in the OAST group compared with the AAST. These findings might be the consequence of the higher testosterone

levels and the slow growth of the OAST.

PP001 - DIABETES MELLITUS-ASSOCIATED FUNCTIONAL HYPERCORTISOLISM IMPAIRS SEXUAL FUNCTION IN MALE LATE-ONSET HYPOGONADISM

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Functional hypercortisolism is generated by conditions able to chronically activate hypothalamic-pituitary-adrenal axis and has been proven to have a negative role in several complications. However, no study has evaluated the possible influence of diabetes mellitus-associated functional hypercortisolism on male hypogonadism and sexual function. We aimed to identify any association of hypothalamic-pituitary-adrenal axis dysregulation measures with testosterone and sexual function in men simultaneously affected by diabetes mellitus and late-onset hypogonadism. Fifteen diabetes mellitus and late-onset hypogonadism subjects suffering from functional hypercortisolism and fifteen diabetes mellitus and late-onset hypogonadism subjects who were free of functional hypercortisolism were retrospectively reviewed. Clinical, hormonal and sexual parameters were considered. Hypercortisolemic subjects showed higher values of body mass index, waist and glycated haemoglobin and lower ones of testosterone compared to normocortisolemic ones. All sexual parameters, except for orgasmic function, were significantly worse in hypercortisolemic than in normocortisolemic subjects. Hypercortisolemic patients showed higher values of cortisol after dexamethasone and urinary free cortisol as well as a lesser ACTH response after corticotropin releasing hormone test (ACTH area under curve) compared to normocortisolemic ones. No significant association was found at Poisson regression analysis between hormonal and sexual variables in normocortisolemic patients. Conversely, in hypercortisolemic subjects, negative and significant associations of cortisol response after corticotropin releasing hormone (cortisol area under curve) with erectile function and total international index of erectile function score were evident. This study suggests for the first time the impairing influence of the dysregulated hypothalamic-pituitary-adrenal axis on sexual function in diabetes mellitus-associated late-onset hypogonadism.

PP002 - SYNERGISTIC EFFECT OF ANDROGEN RECEPTOR (CAG REPEAT LENGTH) AND ENDOTHELIAL NITRIC OXIDE SYNTHASE (GLU298ASP VARIANT) GENE POLYMORPHISMS ON SEMINAL PARAMETERS IN MEN WITH IDIOPATHIC OLIGOASTHENOZOOSPERMIA.

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Many studies have shown the very contrasting associations of androgen receptor (AR) gene CAG repeats and endothelial nitric oxide synthase (eNOS) (Glu298Asp Variant) with male fertility. We therefore hypothesized that the simultaneous presence of the two mutations could be the real factor to affect seminal parameters. In this study we aimed to assess the association between AR and eNOS gene polymorphisms with male fertility, considering them both separately and combined. Oligoasthenozoospermic (n=34) and normozoospermic men (n=43) were studied. Hormonal, seminal and genetic analyses (AR gene CAG repeat and eNOS-Glu298Asp variant) were carried out. AR and eNOS gene polymorphisms were not clearly and independently associated with worse seminal parameters. Interestingly, when the six possible genotypes deriving from the combination of AR and eNOS genotypic variants were considered, the subjects who simultaneously had short CAG repeat and $eNOS_{tt}$ genotype presented significantly worse sperm parameters than the other five groups. Our findings suggest that the effect of AR and eNOS gene polymorphisms on sperm parameters could occur not independently, but through a synergistic action of the two genetic variants.

PP003 - PROTECTIVE EFFECTS OF COENZYME Q10 AND ASPARTIC ACID ON OXIDATIVE STRESS AND DNA DAMAGE IN SUBJECTS AFFECTED BY IDIOPATHIC ASTHENOZOOSPERMIA.

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Objective: To assess the effects of Coenzyme Q_{10} (CoQ_{10}) and Aspartic acid (D-Asp) on some previously untested parameters of sperm oxidative stress and DNA damage.

Design: Observational longitudinal study.

Setting: Division of Endocrinology, Ancona, Italy.

Patients: Twenty patients affected by idiopathic asthenozoospermia.

Intervention: Administration of oral dietary supplement including CoQ_{10} and D-Asp.

Main Outcomes: CoQ_{10} and D-Asp levels, superoxide dismutases (SOD) activity, nitric oxide (NO) and peroxynitrite levels and DNA damage (comet assay) in sperm.

Results: NO and peroxynitrite levels decreased, whereas SOD activity increased significantly after therapy. Furthermore, tail intensity, a marker of DNA damage, diminished significantly after treatment. Correlation analysis revealed a negative relationship between the increase of CoQ_{10} and the decrease of NO and tail intensity and a positive one between the increase of CoQ_{10} and the rise of SOD activity; no significant correlation was found between the increment of D-Asp and the changes of markers of oxidative stress and DNA damage. Increase of SOD activity and decrease of NO levels were negatively and positively correlated with the diminishment of tail intensity, respectively.

Conclusions: Only CoQ_{10}, and not D-Asp, seems to play a protective role against oxidative stress and DNA damage in sperm.

PP004 - PREVALENCE OF ENDOCRINE AND METABOLIC DISORDERS IN SUBJECTS WITH ERECTILE DYSFUNCTION: A COMPARATIVE STUDY.

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Alterations of gonadal, thyroid and pituitary hormones, along with metabolic disorders, might be involved in causing erectile dysfunction (ED).

The prevalence of endocrine abnormalities in two different cohorts from the general and the symptomatic populations of Florence were compared.

The first group is a general population sample derived from a Florentine spin-off of the European Male Aging Study (EMAS cohort; n=202); the second group is a series of n=3847 patients attending our clinic for ED (UNIFI cohort).

Both primary and secondary hypogonadism were more often observed in the UNIFI than in the EMAS cohort (2.8 vs. 0%; $p<0.05$ and 18.9 vs. 8%; $p<0.001$, respectively). However, only the second association retained statistical significance after adjusting for age. Compensated hypogonadism was more common in the EMAS cohort (4.4 vs. 8.1%; $p<0.05$). No statistically significant difference in the prevalence of overt thyroid disorders was observed. Conversely, subclinical hyperthyroidism was more prevalent in the EMAS cohort (2 vs. 4.1%, $p<0.05$). No significant difference in the prevalence of hyperprolactinemia was detected, while the prevalence of hypoprolactinemia was significantly higher in the UNIFI than in the EMAS cohort (28.2% vs. 17.8%, $p=0.001$), even after the adjustment for age, BMI and testosterone ($p=0.001$). Central obesity (waist ≥102 cm), impaired fasting glucose (IFG) and type 2 diabetes mellitus (T2DM) were more often detected in UNIFI patients (31.7 vs. 22.8%, $p<0.05$; 44.5 vs. 33.3%, $p<0.05$; 20.1% vs. 1.0%, $p<0.001$ in the UNIFI and EMAS cohort, respectively), even after adjusting for age. In contrast, the prevalence of overweight and obesity did not differ between the two groups.

T2DM, IFG, central obesity, secondary hypogonadism and hypoprolactinemia are more frequent in subjects consulting for ED than in the general population of the same geographic area. Our data suggest that these conditions could play a central role in determining consultation for ED.

PP005 - DEREGULATION OF SERTOLI AND LEYDIG CELLS FUNCTION IN PATIENTS WITH KLINEFELTER SYNDROME AS EVIDENCED BY TESTIS TRANSCRIPTOME ANALYSIS

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Background: Klinefelter Syndrome (KS) is the most common abnormality of sex chromosomes (47,XXY) and represents the first genetic cause of male infertility. Mechanisms leading to KS testis degeneration are still not completely defined but considered to be mainly the result of germ cells loss. In order to unravel the molecular basis of global testis dysfunction in KS patients, we performed a transcriptome analysis on testis biopsies obtained from 6 azoospermic non-mosaic KS patients and 3 control subjects.

Results: The analysis found that, compared to controls, KS patients showed the differential up- and down-expression of 656 and 247 transcripts. The large majority of the deregulated transcripts were expressed by Sertoli cells (SCs) and Leydig cells (LCs). Functional analysis of the deregulated transcripts indicated changes of genes involved in cell death, inflammatory response, lipid metabolism, steroidogenesis, blood-testis-barrier (BTB) formation and maintenance, as well as spermatogenesis failure.

Conclusions: Taken together, the present data highlight the modulation of hundreds of genes in the somatic components the testis of KS patient. The increased LCs steroidogenic function together with the impairment of inflammatory pathways and BTB structure, result in increased apoptosis. These findings might represent a critical roadmap for therapeutic intervention and prevention of KS-related testis failure.

PP006 - CIRCULATING LEVELS OF FSH IN MEN ARE GENETICALLY DETERMINED: STUDY OF THE COMBINED EFFECT OF POLYMORPHISMS IN FSHR AND FSHB GENES

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Introduction

Recent studies showed that the following polymorphisms in the gene for FSH receptor (*FSHR*) and FSH beta subunit (*FSHB*) might modulate FSH circulating levels: rs6166 (c.2039 A>G, Asn680Ser) and rs1394205 (c.-29 G>A) in *FSHR*, and rs10835638 (-211 G>T) in *FSHB*. However, studies considering the combined effect of these three polymorphisms have not yet been conducted.

Materials and methods

We studied 572 consecutive infertile males (including 93 with azoospermia-cryptozoospermia, 231 with oligozoospermia and 248 with normozoospermia) by means of semen analysis, FSH, LH, and T levels, testicular volume, and rs6166, rs1394205 and rs10835638 genotyping by RFLP and direct sequencing.

Results

The *FSHB* promoter polymorphism 211 G>T is significantly associated with FSH levels (9.6±7.6, 7.2±5.8 and 2.6±1.9 IU/L respectively in men GG homozygotes, GT heterozygotes and TT homozygotes, P<0.001). The polymorphisms -29 G>A and Asn680Ser in *FSHR* taken alone were not associated with different FSH concentrations. Combined analysis of the three polymorphisms showed again that the major determinant of FSH levels is the -211 G>T polymorphisms, only slightly modulated by the -29G>A polymorphism. Polymorphism Asn680Ser in *FSHR* had no effect neither alone nor in combined analysed. The three polymorphisms had no effect on LH and T levels, and in accordance in these data the total number of sperm and testicular volume are modulated by the genotype. Men TT homozygotes for the -211 polymorphism are invariably azoo-oligozoospermic with low testicular volume and FSH concentration <8 UI/L.

Conclusions

This is the first study analysing the combined contribution of the most common polymorphisms in *FSHR* and *FSHB* genes in influencing male reproductive system and showed that polymorphism -211 G>T in *FSHB* has a determinant role, only slightly modulated by *FSHR* polymorphisms, in determining FSH levels, sperm count and testicular volume. This polymorphism alters the transcriptional activity of the gene, and therefore it determines a sort of isolated FSH deficiency with azoo-oligozoospermia and represents the ideal pharmacogenetic marker of FSH treatment

response.

PP007 - FSH SERUM LEVEL PREDICTED THE APPEARANCE OF EJACULATED SPERMATOZOA AFTER EMBOLIZATION OF LEFT SPERMATIC VEIN IN NON-OBSTRUCTIVE AZOOSPERMIC MEN WITH VARICOCELE

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Introduction. Varicocele repair was offered in the management of non-obstructive azoospermia (NOA) to improve spermatogenesis and to obtain ejaculated spermatozoa but success predictors have not been identified. Aim of our study was to determine the predictive value of baseline variables for occurrence of ejaculated spermatozoa after varicocele repair in NOA. **Materials And Methods.** We performed a prospective study in men affected by NOA and left side varicocele, consulting a University clinic due to couple infertility. Azoospermic men with grade II and grade III varicocele were submitted to hormone analysis and to scrotal color Doppler ultrasound (CDU) and re-evaluated after 3 months for semen analysis. NOA was confirmed in 19 patients aged 28 to 47 years, who were then submitted to retrograde embolization of left internal spermatic vein. Patients were re-evaluated 6 months after varicocele repair for semen analysis and for CDU. **Results.** 6 months following internal spermatic vein embolization, 9 patients (47.4%) were still azoospermic (Group 1) while 10 patients (52.6%) reported ejaculated spermatozoa (Group 2) (sperm count: $1.2x10^6$/mL, 0.5 $x10^6$/mL- 1.3 $x10^6$/mL (median, 25^{th} – 75^{th} centiles); forward sperm motility: 9%,3.5%-13.5%). Serum baseline level of FSH was lower in Group 2 (p=0.037 vs Group 1), while serum level of LH and of Testosterone, age at embolization, baseline testicular volumes, the distribution of cases with degree II or degree III of left side varicocele, the percentage of patients with a mean diameter of the venous vessels of the pampiniform plexus (MVD) >3mm, and a continuous spermatic venous reflux (SVR) velocity were not different in the two groups. A reduced right testis volume was observed in six cases in Group 2 and in one case in Group1 (p=0.057). No differences between groups were observed in the rate of SVR disappearance or in the velocity of SVR after embolization. ROC analysis indicated that baseline FSH serum level predicted the appearance of ejaculated spermatozoa after treatment (AUC=0.811; 95% CI 0.6 to 0.9; p=0.0027). A cut-off level of FSH < 10.3 mIU/mL identified 80.00% of cases with ejaculated spermatozoa after embolization with a specificity of 80.0% and a sensitivity of 88.9%. **Conclusion**. FSH serum level significantly predicted the appearance of ejaculated spermatozoa in NOA associated to left side varicocele, after a non-invasive embolization of left spermatic vein.

PP008 - LOW TESTOSTERONE AND NON-ALCOHOLIC FATTY LIVER DISEASE: EVIDENCE FOR THEIR INDEPENDENT ASSOCIATION IN MEN WITH CHRONIC SPINAL CORD INJURY

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Non-alcoholic fatty liver disease (NAFLD) is regarded as a liver phenotype of metabolic syndrome, which in turn is associated with male hypogonadism. Therefore, an association between NAFLD and androgen deficiency would be expected. We tested this hypothesis in men with chronic spinal cord injury (SCI), who exhibit a very high prevalence of biochemical androgen deficiency and a combination of risk factors for metabolic syndrome. NAFLD was ultrasonographically diagnosed in 27 out of 55 consecutive men with chronic SCI (49.1%), admitted to a rehabilitation program. Patients with NAFLD were older and exhibited significantly higher BMI, insulin, HOMA-IR, triglycerides and gamma-glutamyl transpeptidase values, lower total and free testosterone levels and they were engaged in a significantly lower number of hours/week of leisure time physical activity (LTPA). At the multiple logistic regression analysis, only total and free testosterone levels exhibited a significant independent association with NAFLD: the risk of having NAFLD increased of 1% for each decrement of 1 ng/dL of total testosterone and of 3% for each decrement of 1 pg/mL of free testosterone, after adjustment for confounders. In men with total testosterone <300 ng/dL (36.4%) the prevalence of NAFLD reached 85%: they had a risk of having NAFLD significantly higher (12-fold) than those with total testosterone ≥ 300 ng/dL, after adjustment for confounders. In conclusion, the evidence of an independent association between NAFLD and low testosterone is strongly reinforced by its demonstration in men with chronic SCI, in spite of the many confounders peculiar to this population.

PP009 - SPERMATOGENESIS IN KLINEFELTER SYNDROME AND SPERM RETRIEVAL IN RELATION TO TESTOSTERONE REPLACEMENT THERAPY

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Background: Klinefelter syndrome (KS) shows a 47,XXY karyotype and clinical manifestations characterized by male hypergonadotropic hypogonadism and infertility. Despite most patients are azoospermic and hypogonadal, some cases have severe oligozoospermia or focal spermatogenesis in the testis with normal or reduced testosterone (T) levels. Aims: to evaluate sperm retrieval in the ejaculate and testis of KS subjects, to look for predictive parameters of sperm production and to compare patients who received and who did not receive testosterone replacement therapy (TRT). Subjects and Methods: 353 patients with 47,XXY KS were evaluated with semen analysis, reproductive hormones, 25-OH vitamin D, parathormone (PTH), Ferriman and Gallwey score, testicular and prostate ultrasonographic scanning, prostate specific antigen (PSA), weight and height, waist and body mass index (BMI), homeostasis model assessment (HOMA) index, bone mass density (BMD) by dual X-ray absorptiometry (DEXA). Furthermore, 98 patients underwent testicular sperm extraction (TESE). Results of sperm retrieval were compared in KS patients who received TRT (at least 5-years of treatment, with different T formulations, at different doses) and in untreated. Statistical differences between groups were evaluated by unpaired two-sided Student's t-test. Comparisons of means of testicular volumes and sperm retrieval were analysed by Wilcoxon rank sum test. Results: Out of 353 patients, 29 (8.2%) had sperm in the ejaculate. Out of the 98 TESE 30 (30.6%) had successful sperm recovery. The comparison of age (27.9+7.7 and 29.4+8.0), reproductive hormones, 25-OH vitamin D, PTH, Ferriman and Gallwey score, prostate volume, PSA, weight and height, waist and BMI, HOMA index and BMD was not different between patients with and without sperm recovery in semen analysis or TESE. Patients with sperm recovery, had significantly higher bitesticular volume (2.4+1.1 vs 2.0+0.7, p=0.04). In 127 patients (29 with sperm in the ejaculate and 98 who underwent TESE), comparison of sperm retrieval in those with or without TRT (respectively 39 and 88 subjects) showed a significantly higher retrieval rate in untreated ones (57.9% vs 20.5%, p<0.001). In this group no difference was found in patients with normal (>10.4 nmol/L) or reduced (<10.4 nmol/L) T plasma level (retrieval rate 58.1% and 57.8% respectively). In TRT group, patients with normal T and suppressed LH (<1.0 U/L) had lower retrieval rate than patients with both normal T and LH (>1U/L) levels (retrieval rate 35.0 and 5.3% respectively). Conclusions: Men with KS have about 8 and 30% chance to have sperm in the ejaculate or in the testis respectively. This study, performed in a large number of KS patients provides three interesting and new insights: i) the probability to retrieve sperm in the ejaculate and testis is related to testicular volume; ii) the retrieval rate is higher in KS patients with no TRT; iii)

patients whith TRT and suppressed LH levels had the worse chance to have sperm.

PP010 - HEALTH-RELATED QUALITY OF LIFE IN KLINEFELTER SYNDROME: THE ROLE OF PSYCHOPATHOLOGY AND PERSONALITY.

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Background: Klinefelter syndrome (KS, 47,XXY) is associated with an increased prevalence of psychiatric disorders, although the neuropsychological phenotype shows great variability. Prospective studies of KS also reported higher rates of psychiatric referral among boys with KS, and in adolescence, 54% of KS males had mild to moderate psychiatric disorders.The present study aimed at assessing whether psychological factors, specifically psychopathology and personality, together with clinical and sociodemographic factors, were independent predictors of quality of life (QoL) in patients with KS.

Methods: A cohort of 20 male patients (age ranging from 19 to 52 years) with KS was enrolled in the study. Clinical and sociodemographic variables were recorded. QoL was assessed by the Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q-735); personality traits were assessed by means of the Temperament and Character Inventory Revised (TCI-R) and psychopathology was evaluated by Self Report Symptom Inventory Revised (SCL-90) and Mini Mental State Evaluation (MMSE). Moreover, the Coping Orientation to the Problems Experienced (COPE) was used to evaluate the influence of stressfull-life events. To explore the relation of variables on the QoL, univariate analyses were performed for each variable. Factors statistically significant at these analyses were included in a multiple linear regression analyses with dimensional and global scores as dependent variables.

Results: Some personality traits (avoidant behavior, fears of criticism, disapproval and rejection) and psychological factors (depressed mood, anxiety) were related with QoL; the strongest predictors of its impairment were represented by psychological distress and intimate relationships. On a cognitive level, instead, the great majority of subject consistently showed a normal range of intelligence, with the exception of 2 patients who reported a moderate impairment.

Conclusions: Psychological distress in KS patients contributes to poor QoL along with the presence of depressive symptoms and personality disorders such as the avoidant type.

PP011 - ACUTE ENDOTHELIAL RESPONSE TO TESTOSTERONE ADMINISTRATION IN NAÏVE MEN WITH HYPOGONADISM IS INFLUENCED BY ANDROGEN RECEPTOR POLYMORPHISM

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Introduction: The possibility that testosterone (T) may have detrimental effects on cardiovascular system is receiving increasing attention. We investigated the acute effects of T on vascular function in men with severe hypogonadism.

Materials and Methods: 10 men (18-40 years age) with severe hypogonadism (mean T = 0.6 ng/mL ± 0.3 SD; 2.1 nmol/L ± 1 SD) were enrolled after a 4-weeks washout run-in. This was a 4-days, double blind, randomized placebo-controlled crossover study with 80 mg fixed dose of transdermal-T gel. Primary endpoints were variations from baseline of the hyperaemic response (Reactive Hyperemia Index, RHI) as calculated by fingertip peripheral arterial tonometry (PAT) and of the Augmentation Index (AI); androgen receptor polymorphism was evaluated by CAG repeat polymorphism in exon 1 of the androgen receptor (AR) gene.

Results: 4 and 96 hours after transdermal-T gel administrations, T serum levels strongly increased reaching mean plasma levels of 4 ng/mL ± 2.1 SD, 13.9 nmol/L ± 7.3 SD ($p<0.0001$) and 2 ng/mL ± 1.48 SD, 6.9 nmol/L ± 5.1 SD ($p<0.001$), respectively; with no variation in 17β-estradiol and SHBG levels. RHI significantly improved at time 4h ($p<0.05$), while AI improvement was recorded at 4h at 96h, respectively ($p<0.01$ and $p<0.001$). Interestingly, a direct relationship between Δ-T and ΔRHI variations ($p<0.01$ $r=0.37$) as well as between "CAG repeats" length and natural logarithmical variations of RHI (ΔLnRHI) at 96 hours ($p<0.03$ $r^2=0.47$) were found. Accordingly, an inverse relationship between Δ-T and ΔAI ($p<0.01$ $r=-0.35$) was found, which was maintained even when adjusted for heart rate (ΔAI@75 $p<0.01$ $r=-0.38$). No adverse events were reported.

Conclusions: Acute administration of fixed dose of transdermal-T causes an acute vasodilation and improvements in arterial stiffness probably due to non-genomic actions of testosterone. The endothelial response is more pronounced depending on higher plasma T levels attained and longer CAG repeat lengths.

PP012 - FACTORS AFFECTING SPERMATOGENESIS UPON GONADOTROPIN-REPLACEMENT THERAPY: A META-ANALYTIC STUDY

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A meta-analysis was performed to systematically analyse the results of gonadotropin and GnRH therapy in inducing spermatogenesis in subjects with hypogonadotropic hypogonadism (HHG) and azoospermia. An extensive Medline and Embase search was performed including the following words: 'gonadotropins' or 'GnRH', 'infertility', 'hypogonadotropic', 'hypogonadism' and limited to studies in male humans. Overall, 44 and 16 studies were retrieved for gonadotropin and GnRH therapy, respectively. Of those, 43 and 16 considered the appearance of at least one spermatozoa in semen, whereas 26 and 10 considered sperm concentration upon gonadotropin and GnRH, respectively. The combination of the study results showed an overall success rate of 75% (69–81) and 75% (60–85) in achieving spermatogenesis, with a mean sperm concentration obtained of 5.92 (4.72–7.13) and 4.27 (1.80–6.74) million/mL for gonadotropin and GnRH therapy, respectively. The results upon gonadotropin were significantly worse in studies involving only subjects with a pre-pubertal onset HHG, as compared with studies involving a mixed population of pre- and post-pubertal onset [68% (58–77) vs. 84% (76–89), $p = 0.011$ and 3.37 (2.25–4.49) vs. 12.94 (8.00–17.88) million/mL, $p < 0.0001$; for dichotomous and continuous data, respectively]. A similar effect was observed also upon GnRH. No difference in terms of successful achievement of spermatogenesis and sperm concentration was found for different FSH preparations. Previous use of testosterone replacement therapy (TRT) did not affect the results obtained with gonadotropins. Finally, a higher success rate was found for subjects with lower levels of gonadotropins at the baseline and for those using both human chorionic gonadotropin and FSH. Gonadotropin therapy, even with urinary derivatives, is a suitable option in inducing/restoring fertility in azoospermic HHG subjects. Gonadotropins appear to be more efficacious in subjects with a pure secondary nature (low gonadotropins) and a post-pubertal onset of the disorder, whereas previous TRT does not affect outcome.

PP013 - SIAMS-SURVEY ON THE SEXOLOGICAL SCREENING DURING THE PRAXIS OF MEDICALLY ASSISTED REPRODUCTION IN ITALY

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Introduction. The assessment of sexual functioning into is fundamental during the praxis of Medically Assisted Reproduction (MAR). Nevertheless, it is not still a consolidate clinical routine among the centres of infertility. The aim of this survey is describe the main aspects of sexological screening that are considered in the Italian centres of MAR.

Methods. After the consensus of the Italian Society of Andrology and Sexual Medicine (SIAMS), a mailing list of reproductive medicine centres was created. Then, we have sent a questionnaire concerning the essential characteristics of sexological screening. The compilers of questionnaire sent back the information from their centres and an analysis of absolute frequencies and percentages was performed.

Results. Firstly, we noticed that 16 centres have compiled and return back the questionnaire, while 5 ignored the invitation. The main findings regard the large consideration of Vardenafil 10mg (68.75%; 11/16) for the treatment of erectile dysfunction in comorbidity with reproductive problems, the diffuse administration of IIEF (68.25%; 11/16) and Siedy (50%; 8/16) as psychometric tools and a minor consideration of FSFI (31.25%; 5/16) for the evaluation of female sexuality in the infertile couple.

Conclusion. To conclude, we noticed a major attention to the male sexuality and to the eventual treatment or evaluation of sexual dysfunction compared to the female sexuality. This aspect opens an important issue on the clinical praxis to take in major consideration and eventually to reinforce. In this regard, an improvement of the assessment and of treatment of possible female sexual problems in the reproductive medicine seems necessary.

PP014 - PRESENCE AND SEVERITY OF OBSTRUCTIVE SLEEP APNEA ARE INDEPENDENTLY ASSOCIATED WITH PITUITARY-GONADAL AXIS ABNORMALITIES IN OBESE NON-DIABETIC MALE SUBJECTS

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Background and aim: Obstructive sleep apnea (OSA) is strongly associated with obesity and is characterized by changes in the serum levels or secretory patterns of several hormones. The relationship between OSA and hormones of the pituitary–gonadal (HPG) axis was investigated in morbid obese non-diabetic male individuals.

Materials and methods: Fifty-three obese male subjects (mean age 48.1 ± 10 yrs, mean BMI 38.8 ± 7 kg/m2) underwent polysomnography, antropometric and hematochemical evaluation.

Results: OSA was identified in 85 % of subjects. Obese subjects with OSA showed higher waist circumference (127.2 cm vs 118.1 cm, p=0.02) and neck circumference (44.2 cm vs 40.9 cm, p=0.01) than obese subjects without OSA. Subjects with OSA displayed lower level of total testosterone (TT) (4,8 ng/ml vs 3,2 ng/ml, p<0,001) and higher percentage of hypogonadism (83 % vs 37 %, p=0.035) as compared to subjects without OSA. TT, LH and FSH levels were found to be negatively correlated with raised apnea-hypopnea index (AHI) (r=-0.228, p=0.17; r=-0.441, p=0.028; r=-0.442, p=0.027, respectively), oxygen desaturation index (r=-0.296, p=0.07; r=-0.390, p=0.05; r=-0.409, p=0.042, respectively), and percent of sleep time with oxyhaemoglobin saturation at <90% (ST90) (r=-0.359, p=0.027; r=-0.439, p=0.028; r=-0.321, p=0.117, respectively). Furthermore, increasing tertiles of ST90 were associated with decreasing levels of TT (I tertile 4.2 ng/ml; III tertile: 2.9 ng/ml; III tertile: 2.7 ng/ml; p=<0.01), LH (I tertile: 3,9 mcUI/ml; II 3,17 mcUI/ml; III 2,1 mcUI/ml; p<0.05) and SHBG (I tertile 36,633 nmol/l; II tertile 26,788 nmol/l; III tertile 14,75 nmol/l; p=0.02). Multiple regression analysis showed that ST90 was the strongest independent determinant of TT, after controlling for age, neck circumference and waist circumference (β=-0.02, p=0.03). Moreover, ST90 persisted as an independent determinant of FSH (β=-0.11, p=0.03), LH (β=-0.05, p=0.04) and SHBG (β=-0.52, p=0.01). Interestingly, indexes of sleep fragmentation (AHI, sleep efficiency) appeared not as critical as hypoxia index (ST90) in affecting TT level (β=-0.02, p=0.23; β=0.05, p=0.05).

Conclusions: OSA-induced nocturnal hypoxia acts as an independent negative predictor of pituitary-gonadal axis function exacerbating the detrimental risk attributed to obesity. Recognition and treatment of OSA may counteract the central suppression of nocturnal total testosterone in OSA obese male subjects.

PP015 - RELATIONSHIP BETWEEN GONADAL HOMEOSTASIS AND METABOLIC PARAMETERS IN A POPULATION OF HOSPITALIZED DIABETIC MALES.

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INTRODUCTION: The 2011 update of AACE reported that a biochemical assessment of hypophysis-testis axis in male patients with type 2 Diabetes Mellitus (T2DM) has to be performed. It is well known that low testosterone is commonly associated with adipose tissue dysfunction in T2DM and metabolic syndrome.

AIM: To investigate in a cross-sectional study gonadal function in T2DM patients with poor metabolic control.

METHODS: We performed a physical and hormonal assessment of the gonadal state in a population of 70 T2DM males (mean age 54.81 ±14.22) hospitalized from emergency areas for dehydration, hyponatremia and hyperglycemic state. Patients were divided into tertiles of serum total testosterone (TT) levels (tertile I: TT < 2.62 ng/ml; tertile II: TT 2.62-5.13 ng/ml; tertile III: TT > 5.13 ng/ml). Data were analyzed by ANOVA and χ^2 for trend.

RESULTS: The study showed that the levels of TT are inversely correlated with older age (p=0,041), disease duration (p=0.012), waist circumference (WC) (p=0.003), BMI (p<0.001), triglycerides (p=0.03), hsCRP (p<0.001), fibrinogen (p<0.001) and visceral adiposity index (VAI) (p=0.023). A direct correlation was found with HDL-cholesterol (p<0.001). No significant correlations were found among TT levels and HbA1c, GOT/GPT, gonadotropin, estradiol and testis volume.

CONCLUSIONS: Our data show no correlation between gonadal function and glycemic control, while by contrast TT levels decrease when disease duration increases. The overlap in estradiol levels between the TT-tertiles suggests no role for adipose aromatase activity leading to the hypothesis that there exists a direct effect of adipocytokines on gonadal function. Moreover, the atherogenic lipid profile, inflammation markers and visceral adipose tissue are intimately associated with lower TT. In this light low testosterone concentration in men can be considered as a marker of cardiovascular risk in T2DM male patients.

PP016 - BIOAVAILABILITY OF TESTOSTERONE GEL IN YOUNG AND OLDER HYPOGONADAL MEN

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Background Topical gel formulations have been able to mimic the circadian pattern of testosterone (T) release showing a safe profile. However, gel bioavailability seems to be impaired by different agents and serum total testosterone (TT) levels may change following the same dose of T gel administration.

Aim To investigate the bioavailability of a 2% T gel formulation (80 mg T contained in 4 g gel) in young and older hypogonadal men undergoing T replacement therapy.

Methods Thirteen male hypogonadal patients were enrolled in our study and divided in two groups according to their chronological age: 7 younger men (Group A; 31,4±6,55 yrs.) and 6 older men (Group B; 67,6±8,35 yrs.). After an adequate wash-out period, blood samples were collected in the morning immediately before (T0) and four hours after (T4) a self administration of 4 g of gel (80 mg T). All samples were assayed for TT concentration by using LC-MS/MS.

Results Group A showed a significantly lower serum TT level at T0 with respect to group B (2,22±1,95 *vs*. 7,61±2,86 nmol/L; p.<0,01). At T4, no significant differences were observed between groups in TT concentration (16,16±2,86 *vs*. 30,33±14,36 nmol/L; p.>0,05). Interestingly, Group A showed a more marked, but not significant, percentage of increase (+1102% *vs.* +388%; p.>0,05). All the subjects of Group A reached a physiological TT concentration whereas in group B two subjects (33%) showed TT levels beyond the normal range.

Discussion Our preliminary results show a greater increase and a reduced variability in TT concentration in young hypogonadal men treated with T gel compared to older men undergoing the same treatment. Indeed, the increase in Group B was inferior and less predictable. Several factors may explain such a difference: young men may show a better compliance in applying the gel according to the correct instructions. We hypothesized that older men may apply gel on a small part of the body in a shorter time, thus decreasing the absorption. Besides, age-related changes in hydration and lipidic structure, such as a less hydrated skin and reduced quantity of stratum corneum intercellular lipids compared to younger men, result in an increased barrier function of the stratum corneum. These factors decrease percutaneous absorption of T gel, which involves diffusion through the intercellular lipids of the stratum corneum, also necessary to trap water. If confirmed in a larger number of subjects, such results reinforce that a treatment with T gel should be

carefully personalized.

PP017 - TESTICULAR CANCER IMPACT ON MALE SEXUALITY

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The diagnosis of a testicular cancer (TC), as well as its location, may have a considerable impact on the patient's sexuality, resulting in the onset of sexual dysfunctions. Great interest has recently risen in its implications over the quality of life due to the high prevalence in young adults and the good prognosis. The aim of this study is to evaluate the impact of the testicular cancer diagnosis and of the orchifunicolectomy in the patients sexual life. For this purpose, we recruited 161 TC patients (seminoma and non-seminoma) attending the Seminology laboratory sperm bank at the University of Rome "La Sapienza" Department of Experimental Medicine - Medical Pathophysiology Section for cryobanking of semen (Group A, 31.2 ± 7.1 years) and 253 cancer-free men who were undergoing a preventive andrological investigation in the same Department (Group B, 32.4 ± 7.3 years). The IIEF-15 test has been administered to all patients to evaluate the following domains: erectile function (EF), orgasmic function (OF), sexual desire (SD), intercourse satisfaction (IS), general satisfaction (GS). By comparing Group A and B, a statistically significant difference has been found between the scores of all domains, but the OF (EF_A vs EF_B = 22.4 ± 9.3 vs 27.9 ± 3.0, p<0,0001; OF_A vs OF_B = 8.3 ± 2.7 vs 8.9 ± 1.7, p=0,11; SD_A vs SD_B = 7.5 ± 1.8 vs 8.0 ± 1.5, p=0,017; IS_A vs IS_B = 7.7 ± 4.4 vs 11.8 ± 2.2; p<0,0001; GS_A vs GS_B = 7.4 ± 2.6 vs 8.6 ± 2.5; p<0,0001). Finally TC patients have been divided in two groups on the basis of the IIEF score: 101 patients with normal IIEF score (32.0 ± 6.9 years) and 60 patients with IIEF score suggestive for erectile dysfunction (29.7 ± 7.1 years). No statistically significant difference was found between gonadotropin and testosterone values in the two groups. In conclusion TC has significant effect on the patients sexuality, however it has not been possible to attribute an hormonal origin to these symptoms, suggesting a greater impact of either the surgical procedure or the psychological impact (anxiety, fear of death) following the communication of a cancer disease.

PP018 - EVALUATION OF CYTOSTATIC THERAPY EFFECTS ON THE MALE GAMETE GENOME

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Background: The most frequent malignancies among men in reproductive age are testicular cancer, Hodgkin's and non-Hodgkin's lymphoma. The relatively low gonadotoxicity of therapies allows a rapid recovery of spermatogenesis and the future welfare of the offspring conceived by a father treated with cytotoxic therapy remains a major concern. The right timing for natural conception is still uncertain and based only on few studies.

Aim: To evaluate the effects of cytostatic therapies on the integrity/instability of the sperm genome

Methods: 47 patients affected by testis cancer (n=33) and lymphoma (n=12) were analysed prior to and after cytotoxic therapy. Sperm DNA fragmentation analysis: based on terminal-uridine nick end assay (TUNEL) coupled to Flow Cytometry. Microsatellite instability (MSI) assessment: 7 selected loci (AR, ER, BAT25, BAT26, D2S123, D5S346, D17S250) of mono-, di- and tri-nucleotide tandem repeats were amplified by fluorescent PCR and analyzed on ABI Prism 310 sequence analyzer (GeneScan software). For comparison of the tested parameters, data on 90 fertile controls were used.

Results: A significantly higher DNA fragmentation was found after: i) 1 years from the cytostatic therapy in the lymphoma group (50.03±16.20% vs 34.04±14.51%, p=0.001); ii) 2 years testis cancer group (46.63±18.91% vs 34.04±14.51%, p=0.003). No microsatellite instability was observed.

Conclusion: Our pilot study shows that cytotoxic therapies do affect DNA integrity up to two years suggesting that natural conception should be avoided for a longer time than it was advised before. For the first time in the literature we evaluated the stability of microsatellites which resulted not affected indicating that genomic instability is unlikely to occur after 1-2 years from chemotherapy. Further enlargement of the study population will allow to stratify data according to the type of therapies while long term follow up is needed in patients presenting high DNA fragmentation after 2 years.

PP019 - CLINICAL DIFFERENCES BETWEEN TREATED AND NOT TREATED HYPOGONADAL MEN: RESULTS FROM THE SIAMO-NOI STUDY

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Background: Whether low testosterone (T) related symptoms improves upon treatment for hypogonadism (HG) is under debate. **Aim:** To evaluate differences in clinical and biochemical features between subjects who start therapy for HG and those who do not. **Methods:** This is a prospective study involving 420 HG subjects [median age 51(39-63)] participating to SIAMsO-NOI, a multicenter observational longitudinal study enrolling HG patients (T<12 nmol/L) in 15 Italian Endocrinology and/or Andrology centres. Decisions on treatment were made according to physician and patient choice, as in clinical practice. Each patient underwent 4 visits every 4 months for a total time of 1 year. Questionnaires on sexual, physical, psychological and urinary symptoms (IIEF-15, AMS and IPSS) were completed at each visit. Hormonal and biochemical parameters were also registered. **Results:** Of the 420 enrolled patient, 373, 346 and 381 attended the first (V1), second (V2) and third (V3) follow-up visit, respectively and 329 attended all the scheduled visits (V1, V2 and V3). Among the patients studied, 71 (16.9%) started therapy for HG within the study period and 55 (13.1%) did not start at all. In a between centre-adjusted multilevel logistic model, younger age, presence of at least one sexual symptom, greater severity of urinary symptoms and baseline T<8 nmol/L were all predictors for starting therapy (OR=0.57(0.35-0.92) for each year, OR=11.55(2.01-66.35), OR=11.55(2.01-66.35), OR=1.27(1.01-1.60) for each unitary increase in IPSS score, and OR=4.69(1.59-13.81), respectively). For outcome analyses, men who started therapy immediately after enrolment (period between enrolment and V1) and maintained treatment until study end (n=55, 13.1%) were compared with untreated men (n=55, 13.1%). After adjusting for confounders, during all the study duration treated subjects showed significantly higher scores on each IIEF-15 domain (erectile function, orgasm, desire, intercourse and overall satisfaction) as compared with untreated group. Furthermore, glycaemia and triglycerides significantly decreased during the study only in the treated group (both p<0.05). **Conclusion:** Younger age, presence of sexual complains, severity of urinary symptoms and lower baseline testosterone levels are predictors for starting therapy in HG subjects. Sexual symptoms and metabolic profile got better upon treatment for HG.

PP020 - ERECTILE DYSFUNCTION IS COMMON AMONG MEN WITH ACROMEGALY AND IS ASSOCIATED WITH MORBIDITIES RELATED TO THE DISEASE

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Introduction: The prevalence of erectile dysfunction (ED) and its correlates in men with acromegaly has never been investigated. The aim of this study is to evaluate sexual function in acromegalic men.

Methods: Multicenter-based, retrospective analysis of a non-selected series of 57 acromegalic subjects (mean age: 52.7±14.2 years) was performed. Patients were interviewed using SIEDY structured interview, a 13-item tool for the assessment of ED-related morbidities. Several clinical and biochemical parameters were taken. Penile colour-Doppler ultrasound (PCDU) was performed in a subgroup of 37 acromegalic subjects. Acromegalic subjects reporting ED (n=24) were compared with matched ED-patients without acromegaly or pituitary disease (controls), selected from a cohort of more than 4000 subjects enrolled in the Florence Sexual Medicine and Andrology Unit.

Results: ED was reported by 42.1% of acromegalic subjects. After adjusting for age and testosterone, acromegalic subjects with ED had a higher prevalence of hypertension and impairment of sleep-related erections and a longer smoking habit. Accordingly, acromegaly-associated ED was characterized by a higher organic component and worse PCDU parameters. No relationship between ED and testosterone levels or other acromegaly-related parameters was found. However, acromegalic subjects with severe ED reported a longer disease duration. In a case-control analysis, comparing acromegalic subjects with ED-matched-controls free from acromegaly (1:5 ratio), acromegalic men had a worse ED problem and a higher organic component of ED, as derived from SIEDY score. In line with these data, acromegalic patients with ED had a higher prevalence of major adverse cardiovascular events (MACE) history at enrolment and lower PCDU parameters.

Conclusions: Subjects with complicated acromegaly are at an increased risk of developing ED, especially those with cardiovascular morbidities. Our data suggest including a sexual function evaluation in routine acromegaly follow-up.

PP021 - DNA FRAGMENTATION IN TWO CYTOMETRIC SPERM POPULATIONS: RELATIONSHIP WITH CLINICAL AND ULTRASOUND CHARACTERISTICS OF THE MALE GENITAL TRACT

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Introduction: Evaluating sperm DNA fragmentation (sDF) we previously reported the identification of two subpopulations, PIdimmer and PIbrighter. The possible anatomic origin of these two subpopulations is presently unknown. The aim of this study is to investigate the possible anatomic origin of PIdimmer and PIbrighter sDF evaluating their correlations with clinical and colour-Doppler ultrasound (CDUS) parameters of the male genital tract in males of infertile couples.

Methods: A consecutive series of 160 males with couple infertility (36.3±9.2 years) were evaluated, during the same session, for clinical, scrotal and transrectal CDUS characteristics, hormonal parameters, presence of prostate-related symptoms (evaluated by National Institutes of Health-Chronic Prostatitis Symptom Index [NIH-CPSI]) and sDF (evaluated with TUNEL/PI coupled to flow cytometry method). Significant correlations were adjusted for age (Model 1) along with waist circumference, testosterone levels and smoking habit (Model 2).

Results: According to the statistical Model 2, PIdimmer sDF was significantly associated with testicular abnormalities, including lower clinical and ultrasound volume (adj. r = -0.198 and adj. r = -0.199, respectively; both $p<0.05$), higher FSH levels (adj. r = 0.354, $p<0.0001$), occurrence of a higher frequency of inhomogeneity ($p<0.05$), hypoechogenicity ($p<0.05$), and positive history of cryptorchidism ($p<0.02$). Conversely PIbrighter sDF was mostly related to prostate abnormalities, including prostate-related symptoms (NIH-CPSI total score; r=0.183, $p<0.005$) and CDUS alterations, such as detection of a higher prevalence of macro-calcifications, severe echo-texture inhomogeneity, hyperemia (all $p<0.05$) and higher arterial peak systolic velocity (adj. r = 0.214, $p <0.05$).

Conclusions: This is the first study reporting correlations between sDF and clinical parameters of the male genital tract. Our results indicate that PIdimmer sDF mainly originates in the testis, likely due to testicular apoptosis, and at epididymal level, whereas DNA damage in the PIbrighter population appears to originate downstream, during sperm transit through the prostate, likely due to the presence of an inflammatory status.

PP022 - PREVALENCE OF TESTICULAR ADRENAL REST TUMORS IN SUBJECTS AFFECTED BY CONGENITAL ADRENAL HYPERPLASIA: ULTRASOUND, HORMONAL AND SEMINAL CHARACTERISTICS

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OBJECTIVE: Testicular Adrenal Rest Tumors (T-ART) have already been described in subjects suffering from Congenital Adrenal Hyperplasia (CAH). The aim of this work was to: a) evaluate the prevalence of T-ART in subjects with CAH; b) define the ultrasound, hormonal and seminal profile of such subjects; c) continue a longitudinal study in order to evaluate the possible role of ACTH plasma levels in the induction and persistence of T-ART.

MATHERIAL AND METHODS: N^{O} 21 subjects affected by CAH (aged 21 to 41 years) were studied. These were all patients referred to our Endocrinology Unit for the first time to undergo a clinical evaluation. All the subjects were taking long-term cortisone acetate and fludrocortisone replacement therapy. The study included: a) andrological clinical examination; b) testis ultrasound (US); c) hormonal profile (ACTH, 17-α-Hyroxyprogesterone, cortisol, LH, FSH, testosterone); d) standard semen analysis.

RESULTS: The physical examination of the scrotum showed a palpable testicular mass only in one subject (4.8%). Another subject had had left orchiectomy for Leydig cell tumor. The remaining subjects were negative.US showed a T-ART in 12/21 (57.1%) subjects (10 cases bilaterally and 2 case unilaterally). The maximal diameter of the lesions varied from 4 mm to 38 mm. In 10/12 (83.3%) subjects, the lesions were mainly hypoechoic; in the remaining 2/12 (16.7%) subjects, the lesions were mainly hyperechoic.All 12 subjects with US positive for areas of T-ART, despite long-term cortisone therapy, showed high ACTH plasma levels. The other hormone levels were normal.Semen Analysis showed azoospermia in 2/12 (16.7%) cases, oligo-astheno-terato-zoospermia in 7/12 (58.3%) cases, normo-zoospermia in the remaining 3/12 (25.0%) subjects. These seminal alterations could be due to mechanical problems (compression on the *rete testis*) and/or ACTH/CRH interference in the spermatogenetic process.The longitudinal study has shown, to date, 6/12 (50.0%) patients with a disappearance or reduction of testicular T-ART after 6 months of modified cortisone therapy after an improvement in ACTH levels.

CONCLUSIONS: This study confirms the high prevalence of T-ART in subjects with CAH and the major role played in the pathogenesis by high ACTH plasma levels. Therefore, all male CAH patients need to undergo periodic US evaluation of the testis and ACTH plasma levels must be maintained within the normal range, differently from what is usually suggested in the treatment of CAH patients.

PP023 - EVALUATION OF METHYLATION STATUS OF GLUTATIONE-S-TRANSFERASE GENE PROMOTER IN PLASMA SAMPLES AS MARKER OF PROSTATE CANCER RISK

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Currently, a widely used marker for the diagnosis and follow-up of prostate cancer (PCa) is the prostate specific antigen (PSA). Furthermore, in order to ensure an efficient monitoring of the patients at risk of PCa, there is a growing need of new tools able to early detect cancer. Molecular analysis of neoplastic prostate tissues shows the inactivation of the glutatione-S-transferase gene (GSTP1), due to promoter hypermethylation. This features could be a potential biomarker for PCa. The aim of this study is the specific and sensitive detection of the methylation status of GSTP1 gene in plasma samples of patients with either prostate benign prostatic hyperplasia (BPH) or PCa, and in healthy controls . The methylation status of 5' promoter region of GSTP1 gene was investigated by methylation sensitivity-PCR (MS-PCR). The test was optimized in terms of the specificity, and sensitivity. The diagnostic efficacy of the test was tested on the DNA from 20 healthy donors, 57 benign prostatic hypertrophy (BPH), and 57 patients with PCa. GSTP1 promoter gene hyper-methylation was detected in 43.9% of patients with BPH (25/57 mean age 60.5 years) and in 57.6% of patients with PCa (34/57 mean age 67.8 years), whereas was absent in all normal controls. In particular, 81.8% of patients with PCa, with age >65 years and total PSA ≤ 4 ng/ml, were positive for the hyper-methylation status of GSTP1 gene, suggesting a correlation between age and carcinogenesis process in the prostate. In conclusion our study demonstrates that specific detection of methylation status of GSTP1 gene may be an useful tool for the prediction of patients at risk of PCa particularly in advanced age. In addition the test is cost-effectiveness and could be used extensively in the clinical setting for cancer prevention.

PP024 - BIOLOGICAL EFFECTS OF PHYSIOLOGIC AND SUPRA-PHYSIOLOGIC PULSED VERSUS CONTINUOUS TESTOSTERONE SUPPLEMENTATION IN ANDROGEN SENSITIVE HUMAN PROSTATE CANCER CELLS GROWN UNDER DIFFERENT TESTOSTERONE CONCENTRATIONS

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To evaluate whether continuous or pulsed testosterone (T) supplemented to Pca cell lines, grown in culture conditions mimicking hypogonadal conditions, affect biological parameters associated with aggressive behavioural properties. Two cellular models of androgen-sensitive Pca cells (LnCaP and 22rv1) were used. Tumour cells were cultured in a medium mimicking the intraprostatic T concentrations measured in subjects with hypogonadal condition. T, irrespective of concentrations or supplementation regimens used, did not adversely affect Pca biology. Interestingly, pulsed T supplementation decreased both tumour growth rate and the invasive propensity of Pca cells. Reduced expression of c-Myc, Cyclin-D1, hTERT and PCNA paralleled with senescence induction and growth arrest in the G1-phase of the cell cycle. Pulsed and continuous T supplementation, in the range of physiological and supra-physiological intraprostatic concentrations may not adversely affect Pca biology providing biological evidence supporting the use of TRT in men suffering from hypogonadal condition and successfully treated Pca.

PP025 - PREMATURE EJACULATION REDUCES THE INTENSITY OF AUTO-PERCEIVED ORGASMIC SENSATION

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Introduction: A limited body of evidence suggests that premature ejaculation (PE) might be characterized not only by early ejaculation with reduced control over ejaculation and consequent negative emotions, but also by subjective perception of reduced orgasmic intensity. To our knowledge no psychometric tools has been developed to measure this important subjective parameter. **Objective:** The primary end-point was to create and validate the *orgasmomether*, a new, single-item psychometric tool to measure orgasmic intensity. The secondary end-point was to verify if orgasmic intensity was different between men with PE, erectile dysfunction (ED), and PE with ED. **Methods:** A sample of 268 men was studied. The total population was divided in four sub-groups as follow: (i) 98 sexually healthy males (Control Group); (ii) 113 males suffering from PE (PE Group); (iii) 35 males suffering from ED (ED Group); (iii) 22 males suffering from PE and ED (PE/ED Group). Men were requested to fill out the orgasmomether, a visual tool recording the orgasmic intensity on a Likert scale ranging from 0 to 10, the IIEF-15 and the PEDT. **Results:** The psychometric tool was validated on a population of men with PE. This population was compared with sexually healthy group. Comprehensively, the orgasmomether was well understood. Test-retest reliability was 0.91 (95% CI 0.8715 to 0.9390) and 0.92 (95% CI 0.8912 to 0.9521) in the PE-group and the controls, respectively. The orgasmomether revealed a high AUC (0.946; 95% 0.906 to 0.972). The ROC curve analysis showed that a cut off $\leq$ 6 had 86.73% sensitivity (95%CI 79.1 – 92.4), 93.88 specificity (95% CI 87.1 – 97.7), 94.2% positive predictive value and 86% negative predictive value in discriminating PE subjects with low orgasmic intensity. Men with PE experienced a significant lower orgasmic intensity with respect to men with PE and ED. These two groups significantly differed from controls and ED group. Men with ED did not significantly differ from controls. Multivariate Ancova analysis adjusted for age, weight, height and BMI revealed that these variables did not influence the differences in the variance observed among groups. **Conclusions:** This study suggests that premature ejaculation is characterized by a subjective perception of a lower orgasmic intensity. Orgasmomether configure as an easy-to-perform, user-friendly tool for measuring orgasmic intensity.

PP026 - METABOLIC SYNDROME AND BENIGN PROSTATIC ENLARGEMENT: A SYSTEMATIC REVIEW AND META-ANALYSIS

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INTRODUCTION: Metabolic syndrome (MetS) is a complex and worldwide epidemic disorder including abdominal obesity, impaired glucose metabolism, hypertriglyceridaemia, low serum HDL cholesterol and arterial hypertension. Evidence suggests an association between MetS and lower urinary tract symptoms (LUTS) due to benign prostatic enlargement (BPE).

MATERIALS AND METHODS: We summarized and meta-analyzed the current literature concerning MetS and BPE, focusing on all the components of MetS and their relationship with prostate volume, transitional zone volume, PSA and urinary symptoms.An extensive PubMed, Embase, and Cochrane Library search was performed including the following keywords: “metabolic syndrome”, “MetS”, “diabetes”, “hypertension”, “obesity”, “waist circumference” (WC) and “dyslipidaemia” combined with “lower urinary tract symptoms”, “LUTS”, “benign prostatic enlargement”, “BPE”, “benign prostatic hyperplasia”, “BPH”, “prostate” and “prostate volume”.

RESULTS: Subjects with MetS had significantly higher total prostate volume when compared to those without MetS (+1.8 [95% CI: 0.74;2.87] ml; $p<0.001$). Conversely, no differences were observed between subjects with or without MetS for IPSS total or LUTS subdomain scores. Meta-regression analysis showed that differences in total prostate volume were significantly higher in older (adj $r=0.09$; $p=0.02$), obese patients (adj $r=0.26$; $p<0.005$, Figure 1A) and low serum HDL cholesterol concentrations (adj $r=-0.33$; $p<0.0001$, Figure 1B)

CONCLUSIONS: Our results underline the positive role for MetS-induced metabolic derangements in the development of BPE. Obese, dyslipidemic, and aged men have an higher risk of having MetS as a determinant of their prostate enlargement.

PP027 - IS SERUM ESTRADIOL (E2) REALLY INCREASED IN PATIENTS WITH KLINEFELER SYNDROME (KS)? RESULTS FROM A META-ANALYSIS STUDY

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INTRODUCTION: KS has been classically described as characterized by hyperestrogenism and elevated serum E2 together with increased gonadotropins and low-to-normal serum testosterone (T). In literature, data on increased serum E2 are not solid.

AIM: The aim of this study is to meta-analyse data from studies evaluating serum E2 in both KS and healthy subjects (HS) in order to verify if E2 is increased in KS.

METHODS: An extensive MEDLINE was performed using 'PubMed' with the following key words: 'KS' and 'E2' or 'T' or 'sex steroids' from 1946 to January 2015 (Current Contents-ISI was used for searching oldest studies). All studies (case-control, case-series, case-reports) reporting E2 measurement were considered. Controlled-studies were used for meta-analysis, the others only for reviews. Only serum E2 at baseline (no ongoing treatments) was included. Meta-analysis was conducted according to the PRISMA statement using RevMan.

RESULTS: Out of 956 articles, 26 case-control studies, 15 case-series and 21 case-reports had data on serum E2. A total of 878 KS and 1000 HS were included in the meta-analysis. Serum E2 was significantly higher in HS than in KS, with a mean difference of 7,93 pg/mL (CI:2,24,13,61;p=0,006), with a chi-squared=688,32 (I-square=97%). Serum T was significantly lower in KS than in HS, with a mean difference of -2,79 ng/mL (CI:-3,46,-2,11;p<0,001), with a chi-squared=198,29 (I-square=89%). Data from case-series and case-reports confirmed that E2 is not above the normal range in KS.

CONCLUSIONS: Serum E2 is not increased in KS and is significantly lower than in HS in this meta-analysis. The limits of this study are the heterogeneity of methods for steroids measurement and the lack of studies having the comparison of serum E2 between KS and HS as primary endpoint. The traditional belief that KS is associated to elevated E2 should be reconsidered together with some pathophysiological and clinical issues.

PP028 - INJECTABLE TESTOSTERONE UNDECANOATE FOR THE TREATMENT OF HYPOGONADISM.

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INTRODUCTION:

Injectable testosterone undecanoate (TU) is a long-acting testosterone (T) formulation available for the treatment of male hypogonadism (HG) since 2003.

METHODS:

The efficacy and safety of injectable TU are assessed, as obtained by meta-analyzing available evidence. An extensive Medline, Embase and Cochrane search was performed.

RESULTS:

All uncontrolled and placebo-controlled randomized clinical trials (RCTs), evaluating the effect of injectable TU on different outcomes, were included. Of the 98 retrieved articles, 33 were included in the study. Among those, 11 were placebo-controlled RCTs. Injectable TU was significantly associated with a reduction of fat mass and HbA1c in both controlled and uncontrolled trials, in particular when hypogonadal subjects were enrolled. Similar results were observed for the improvement of erectile function. In addition, TU ameliorated several other outcomes, including blood pressure, lipid profile, waist circumference and body mass index in uncontrolled studies, but these data were not confirmed in placebo-controlled trials. The treatment was well tolerated and no risk of prostate cancer or cardiovascular disease was observed.

CONCLUSION:

Injectable TU is a safe and effective treatment for male HG. The possibility of a therapeutic intervention just four to five times per year frees the patient, at least partially, from having a chronic condition, thus maintaining a positive, active role in self-caring.

PP029 - A WORSE ERECTILE FUNCTION IS OBSERVED COMPARING INFERTILE WITH FERTILE MEN AND AZOOSPERMIC WITH INFERTILE NORMOZOOSPERMIC MEN, THE LAST CASE IN RELATION TO PSYCHOPATHOLOGIC TRAITS

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Introduction: Sexual function in infertile men has been poorly investigated. This study is aimed at evaluating possible differences in sexual function-related parameters comparing different categories of infertile men [azoospermic (A), oligo- and/or astheno- and/or terato-zoospermic (OAT) and normozoospermic (N) men] to each other and to fertile men.

Methods: A consecutive series of 407 men with couple infertility (36.7±8.3 years) was studied. Patients were divided in 3 groups: A (n=92), OAT (n=227) and N (n=88) men according to WHO, 2010. A consecutive series of 46 fertile (F) men (35.7±4.7 years) was taken as control group. Sexual and erectile functions and ejaculatory status were assessed using IIEF-15, IIEF-15-erectile function domain (EFD) and Premature Ejaculation Diagnostic Tool (PEDT), respectively. Psychological traits were evaluated using Middlesex Hospital Questionnaire (MHQ). Clinical and biochemical parameters were assessed.

Results: The prevalences of ED (IIEF-15-EFD score<26) and PE (PEDT score>8) in infertile men were 19.5% and 11.8%, respectively. ED frequency in infertile men was significantly higher than that (0%) of F men ($p<0.05$); no difference in PE prevalence was observed. No age difference was observed among A, OAT, N and F groups. A had a higher waist when compared with F men. A, OAT and N had significantly lower calculated free testosterone (cFT) levels and higher pack-years of smoking when compared with F men. A and OAT had a higher MHQ total score when compared with F men. EFD score was significantly lower in A, OAT or N when compared to F men (all $p<0.0001$), while orgasmic and sexual desire scores were significantly lower in A when compared with F men ($p<0.05$). A significant difference in EFD score was also observed comparing A and N men ($p=0.005$). After adjusting for confounders (age, waist, cFT, pack-years of smoking and MHQ total score), at ANCOVA analysis, the differences in EFD score between A, OAT or N and F men were confirmed ($p<0.05$). The comparison between A and N men moderated the association between EFD and MHQ total score. After adjusting for confounders, MHQ total score was significantly associated with EFD score only in A group ($\beta=-0.255, p<0.05$). A significant higher PEDT score was observed in A when compared with F men, however this difference was not confirmed after adjusting for confounders.

Conclusions: Infertile men have a worse erectile function when compared with F men. A have a worse erectile function when compared with infertile N men in relation to psychopathologic traits.

PP030 - CARDIOVASCULAR RISK ASSOCIATED WITH TESTOSTERONE-BOOSTING MEDICATIONS: A SYSTEMATIC REVIEW AND META-ANALYSIS.

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INTRODUCTION:

Recent reports have significantly halted the enthusiasm regarding androgen-boosting; suggesting that testosterone supplementation (TS) increases cardiovascular (CV) events.

METHODS:

In order to overcome some of the limitations of the current evidence, the authors performed an updated systematic review and meta-analysis of all placebo-controlled randomized clinical trials (RCTs) on the effect of TS on CV-related problems.

RESULTS:

Out of 2747 retrieved articles, 75 were analyzed, including 3016 and 2448 patients in TS and placebo groups, respectively, and a mean duration of 34 weeks. Our analyses, performed on the largest number of studies collected so far, indicate that TS is not related to any increase in CV risk, even when composite or single adverse events were considered. In RCTs performed in subjects with metabolic derangements a protective effect of TS on CV risk was observed.

CONCLUSIONS:

The present systematic review and meta-analysis does not support a causal role between TS and adverse CV events. Our results are in agreement with a large body of literature from the last 20 years supporting TS of hypogonadal men as a valuable strategy in improving a patient's metabolic profile, reducing body fat and increasing lean muscle mass, which would ultimately reduce the risk of heart disease.

PP031 - A NEW PROTOTYPE OF PRE-PUBERTAL PORCINE, ARTIFICIAL MICRO-TESTIS FOR THE SELECTIVE EVALUATION OF HUMAN GONADOTROPINS EFFECTS ON RE-ASSEMBLED SERTOLI AND LEYDIG CELLS: AN IN VITRO STUDY

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There is no *in vitro* model, available so far, for a comprehensive study of human gonadotropins on the testis tubular (Sertoli cells-SC) and interstitial (Leydig cells-LD) testis compartments. These studies could help validating future trends for the therapy of male infertility. Gonadotropins (FSH, LH) are the main regulators of testis multiple functions. Pre-pubertal age is notoriously associated with a physiologic hypogonadotropic hypogonadic state, with the exception of anti-mullerian hormone (AMH) that is upregulated by FSH and down-regulated by androgens. That is why AMH has been proposed as a potential marker of SC function in the pre-pubertal age. Furthermore, this is the age when the majority of pathogenic conditions will occur, leading to many forms of male infertility. The aims of our study then were to: a) establish an *"in vitro"* pre-pubertal porcine mini-testis artificial prototype model; b) study the effects of FSH and LH on such cell reassembled mini-testis. We have obtained highly purified pre-pubertal SC and LD cultures, upon their retrieval, by our method, from 15-20 days old neonatal pig testes. SC and LD were isolated and evaluated in terms of purity by AMH (the unique pre-pubertal SC marker), INSL3 (LD marker), ASMI (peritubular cells marker) and PGP9.5 (gonocytes and spermatogonial cells marker). Finally, purified SC and LD were co-cultured so as to obtain the artificial "mini-testis". We have demonstrated that our *"in vitro"* mini-testis is a functional model to study the effects of gonadotropins on these cells. In fact, inhibin B increased after FSH and FSH/LH stimulation and, testosterone production, was selectively increased by LH treatment. AMH secretion was down-regulated upon FSH exposure/treatment. These data seem to preliminarily suggest that ERK1/ERK2 expression was up-regulated by FSH and FSH/LH stimulation while FSH-receptor expression was down-regulated by FSH and increased by FSH/LH treatment; AKT was up-regulated in all conditions. The proposed model, by creating an artificial mini-testis, could help better understanding the complex and still partially unfolded interactions between human gonadotropins, SC and LD possibly creating a novel background to shed light inside a future therapy of male infertility.

PP032 - FUNCTIONAL CHARACTERIZATION OF PLATELETS IN PATIENTS WITH ARTERIAL ERECTILE DYSFUNCTION.

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Arterial erectile dysfunction (ED) is commonly associated with classic cardiovascular and metabolic risk factors, such as smoking, hypertension, diabetes mellitus, dyslipidaemia and obesity. However, some patients with arterial ED do not present any cardiovascular risk factor. As mean platelet volume (MPV) has been shown to be directly related to the cardiovascular risk and the percentage of platelets expressing the vitronectin receptor (αVβ3), involved in the early stages of platelet adhesion, is higher in patients with ED, the present study was undertaken to evaluate MPV and αVβ3 in 15 patients with arterial ED not associated with any cardiovascular risk factor. Their MPV and αVβ3 values were compared with those of men with normal penile haemodynamic. Patients with arterial ED had a mean value of MPV (11.25 vs. 9.88 fL; $p < 0.001$) and a percentage of platelets expressing the αVβ3 (7.39 vs. 2.07%; $p < 0.001$) significantly higher compared to controls. A negative correlation was observed between peak systolic velocity and MPV ($r = 0.916$; $p < 0.001$) or αVβ3 ($r = 0.930$; $p < 0.001$), whereas MPV and αVβ3 correlated positively ($r = 0.908$; $p < 0.001$). In conclusion, this study showed for the first time that MPV and the percentage of platelet expressing αVβ3 are significantly higher in patients with arterial ED compared to controls. We speculate that these parameters of platelet function may be envisaged as markers of cardiovascular risk in patients with arterial ED.

PP033 - HORMONAL AND METABOLIC RESPONSES DURING AN INTEGRATED TRAINING KUMITE AT SUBMAXIMAL AND MAXIMAL INTENSITIES IN INTERNATIONAL KARATE ATHLETES

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Aim of the study was to investigate whether an athlete can differentiate between a fight (integrated training, IA) at different perceived intensities and the impact on the endocrine-metabolic homeostasis. Ten Karate athletes (6 females and 4 males) of International level were recruited as volunteered for this study. Athletes were asked to fight at a their own perceived intensity corresponding to “low” and “highest” compared their maximum. The protocol provided fighting 4 matches of 2 minutes each at the two different intensities, interspersed by 3 min 30 s of rest. The goal was the stimulation of the energetic systems for improving the aerobic profile in kumite by fighting without interruptions using punches, kicks and combination techniques. Hormonal responses were assessed by means of blood samples collected in the basal condition and after the last session. Plasma glucose, plasma insulin, cortisol, epinephrine and norepinephrine were measured. During the two intensities (low and highest) the baseline values of glycaemia (81 ± 7, 78 ± 15 mg/dl), insulin (8.5 ± 3.1, 6.6 ± 3.2 mU/ml), and cortisol (18.7 ± 8.5, 18.9 ± 8.5 mg/dl) were within the normal ranges. Post-hoc analysis revealed that all the hormonal and metabolic parameters changed and were higher at the end of the 4 matches at the highest compared to low intensity (glycaemia 169 ± 25, 112 ± 19 mg/dl, insulin 22.6 ± 6.8, 14.4 ± 5.3 mU/ml, and cortisol 29.1 ± 8.1, 17.5 ± 7.5 mg/dl all values 100% and 50% respectively). A similar pattern was encountered also for the catecholamines. Post-hoc analysis revealed that catecolamines levels were higher at the end of the 4 matches in the 100% condition compared to 50% (adrenaline 77 ± 25, 209 ± 57 pg/ml, noradrenaline 632 ± 324, 2341 ± 797 pg/ml all values 100% and 50% respectively, $p<0.05$). In conclusion the relevant hyperglycemia after fight is due to the activation of counter regulatory hormones of insulin that, in turn, allow glucose release and its consumption primarily by the striated muscles involved during the matches.

PP034 - HEART VALVE CALCIFICATION IN PATIENTS WITH TYPE 2 DIABETES AND NONALCOHOLIC FATTY LIVER DISEASE

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Background & Aims: aortic valve sclerosis (AVS) and mitral annulus calcification (MAC) are two powerful predictors of adverse cardiovascular outcomes in patients with type 2 diabetes, but the aetiology of valvular calcification is uncertain. Nonalcoholic fatty liver disease (NAFLD) is an emerging cardiovascular risk factor and is very common in type 2 diabetes, but whether NAFLD is associated with valvular calcification in this group of patients is presently unknown.

Methods: we undertook a cross-sectional study of 247 consecutive patients with type 2 diabetes without a history of chronic heart failure, moderate-to-severe valvular heart disease and known hepatic diseases, and performed conventional echocardiography and liver ultrasonography.

Results: overall, 139 (56.3%) patients had no heart valve calcification (HVC-0), 65 (26.3%) patients had one valve affected (HVC-1) and 43 (17.4%) patients had both valves affected (HVC-2). 175 (70.8%) patients had NAFLD and the prevalence of this disease markedly increased in patients with HVC-2 compared with either HVC-1 or HVC-0 (86.1% *vs*. 83.1% *vs*. 60.4%, respectively; p<0.001). NAFLD was associated with AVS and/or MAC (unadjusted-odds ratio [OR] 3.51, 95%CI 1.89–6.51, p<0.001). Adjustments for age, sex, smoking, alcohol consumption, blood pressure, hemoglobin A1c, LDL-cholesterol, kidney function parameters, medication use and echocardiographic variables did not substantially attenuate the strong association between NAFLD, AVS and MAC (adjusted-OR 2.97, 95% CI 1.31–6.70, p<0.01).

Conclusions: our results show for the first time that in patients with type 2 diabetes, NAFLD is a strong and independent predictor of cardiac calcification in both the aortic and mitral valves.

PP035 - ITALIAN GUIDELINES FOR GESTATIONAL DIABETES MELLITUS: ADHERENCE IN CALABRIAN POPULATION

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Recent Italian guidelines for diagnosis of gestational diabetes mellitus (GDM) established an early screening (14-18 weeks) for high risk (HR) women (previous GDM, BMI≥30, or fasting plasma glucose between 100 and 125 mg/dl), and a later screening (24-28 weeks) for intermediate risk (IR) women (age≥35 years, BMI=25.0-29.9, previous macrosomy, familiarity for type 2 diabetes mellitus, or ethnic group at risk). Our aim was to examine adherence rate to these new guidelines in the Calabrian population.

Thus, 2104 gravidic women were retrospectively enrolled at Struttura Operativa Complessa Endocrinologia-Diabetologia, Ospedale Pugliese-Ciaccio, Catanzaro, and Policlinico universitario di Catanzaro, Southern Italy, between August 2011 and August 2014. Among them, 333 (15.8%) were HR women, and 1771 (84.2%) IR women. GDM was diagnosed with 2 hour 75-g oral glucose tolerance test (OGTT), following the IADPSG 2010 cut-offs.

Among the 333 HR women, only 122 (36.6%) underwent early screening. Of these, 60 (49.2%) were affected by GDM. The 62 unaffected women and the 211 women not performing early screening, underwent the later screening: GDM was diagnosed in 30 (48.4%) women among the former group and 106 (50.2)% women among the latter group. Overall, 196 (58.9%) HR women were affected by GDM. Also, GDM was diagnosed in 325 (18.4%) women at IR.

In conclusion, in Calabria over 60% of HR women failed the early screening. Although all non adherent women underwent the later screening, for many of them a proper treatment can be delayed, potentially resulting in maternal-fetal complications. Hence the importance of early diagnosis.

PP036 - SHORT-TERM EFFECTS OF GLUCAGON-LIKE PEPTIDE 1 (GLP-1) RECEPTOR AGONISTS ON FAT DISTRIBUTION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS: AN ULTRASONOGRAPHY STUDY

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Aims Glucagon-like peptide 1 receptor agonists (GLP-1 RA) induce weight loss and reduction in adipose tissue, but the effects of GLP-1 RA on the distribution of fat deposits have been poorly investigated. **Methods** In 25 patients with type 2 diabetes (16 females and 9 males, mean age 63.5 ± 8.8 years), treated with GLP-1 RA (exenatide, n. 12; liraglutide, n.13), both before and 3 months after starting treatment, an abdominal ultrasonographic scan, with Doppler of renal arteries, and echocardiography were performed. Subcutaneous fat width (peri-umbilical and sub-xiphoid), deep fat deposits (preaortic, peri-renal, and epicardial), and renal resistive index (RI) were evaluated. **Results** GLP-1 RA induced highly significant ($p<0.001$) decrease in BMI and in fat thickness at all the assessed sites, without differences between exenatide and liraglutide treatment. A slight decrease in RI ($p = 0.055$) was also found. The percent changes of fat thickness was different between sites ($p<0.025$), and the changes in subcutaneous deposits showed no significant correlation ($p = 0.064$) with those of deep fat deposits. **Conclusions** A short course of treatment with GLP-1 RA, besides weight loss, induces a redistribution of adipose tissue deposits, possibly contributing to a better cardiovascular risk profile in patients with type 2 diabetes mellitus.

PP037 - GALNT2 MRNA LEVELS ARE ASSOCIATED WITH SERUM TRIGLYCERIDES IN HUMANS

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Both human and rodent studies point *GALNT2,* which encode for ppGal-NAc-T2 involved in O-linked N-Acetyl glucosamine glycosylation, as a modulator of triglycerides (TG) and HDL-cholesterol levels. To get deeper insights about this subject, *GALNT2* mRNA levels were measured in peripheral whole blood cells (PWBC) from 224 individuals with a wide range of TG and HDL-cholesterol levels, as well as other metabolic parameters. *GALNT2* expression levels were strongly and inversely associated with serum TG concentrations (adjusted r^2=0.044, p=0.001) and directly associated with HDL-cholesterol levels (adjusted r^2=0.014, p=0.042). In a model comprising both TG and HDL-cholesterol, *GALNT2* levels were significantly associated with TG (p=0.008) but not with HDL-cholesterol (p=0.68) concentrations. The association between *GALNT2* expression and TG levels was independent of age, sex, obesity and diabetes. *GALNT2* expression was associated to serum TG also after taking into account either insulin resistance (as indicated by the $HOMA_{IR}$ index) in non-diabetic individuals, or anti-dyslipidemic treatments, among diabetic patients.

In conclusion, our data indicate that *GALNT2* expression in PWBC is associated with TG levels and, taken together with previous findings, reinforce the hypothesis that *GALNT2* plays a role on lipid levels and possibly atherogenic dyslipidemia.

PP038 - LIRAGLUTIDE AND WEIGHT LOSS IN CALABRIAN TYPE 2 DIABETIC PATIENTS

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Several recent evidences reported that the hypoglycemic agent liraglutide, a human GLP-1 analogue, reduces body weight in type 2 diabetic patients. Thus, our aim was to examine this effect in Calabrian patients with type 2 diabetes uncontrolled by metformin alone.

To this end, 101 type 2 diabetic patients attending three outpatient clinics in Calabria, Southern Italy, treated with metformin plus liraglutide (1.2 or 1.8 mg) were retrospectively assessed at baseline and after 18 months of continuous therapy. As a control group, we enrolled, in the same period,150 diabetic patients treated with metformin alone (1500-2500 mg/die). Two groups were matched for age, sex, BMI, and duration of disease.

In both groups a significant reduction of body weight was observed: -6.76 Kg ±4.46 (P<0.001) in the liraglutide-treated group, and -1.64 Kg ±3.21 (P<0.001) in the metformin-treated group. Weight loss was significantly greater in the former group than in the latter (P<0.01). Similarly, percentage of subjects reaching a weight loss ≥3% was significantly higher among liraglutide-treated patients (59.4%) with respect to metfromin-treated patients (12.7%) (P<0.001). Weight loss in the liraglutide group was independent from basal value of HbA1c. Both therapies were safe and well tolerated with no major treatment-related adverse events.

Overall, our data provide evidence that liraglutide plus metformin significantly reduced body weight compared with metformin monotherapy. Thus, they suggest that liraglutide **can be** beneficial to control weight loss in non diabetic obese individuals.

PP039 - FUNCTIONAL REGULATION OF INSULIN GENE BY THE HIGH-MOBILITY GROUP A1 (HMGA1) PROTEIN

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The High-Mobility Group AT-hook 1 (HMGA1) protein is a nuclear architectural element that regulates gene expression by controlling the formation of "enhanceosomes" complexes on the AT-rich regions of HMGA1-target genes. Previously, we reported that defects in HMGA1 caused decreased expression of the insulin receptor and increased susceptibility to type 2 diabetes mellitus in humans and mice. Furthermore, we found that Hmga1-knockout mice had smaller pancreatic islets of Langerhans and decreased insulin content, suggesting that HMGA1 may play a pivotal role in the regulation of the insulin gene, thus in insulin production.

Here, we investigated the regulatory roles of HMGA1 in insulin gene transcription. First, we provide evidence that HMGA1 physically interacts with PDX-1 and MafA, two critical transcription factors for beta-cell function and insulin gene expression. Then, we show that whereas overexpression of HMGA1 considerably improves the transactivating activity of PDX-1 and MafA on both human and mouse insulin promoters, knockdown of endogenous HMGA1 adversely affects this activity. Also, we show that HMGA1's activity to the endogenous insulin gene promoter is upregulated by glucose, suggesting that HMGA1 may act as glucose-sensor that contributes to the activation of the insulin gene, thus facilitating insulin release.

In summary, our findings consistently support the central role of HMGA1 in pancreatic beta-cell function and insulin production.

PP040 - BETTER OUTCOMES AFTER INSULIN PUMP IMPLANTATION IN A TYPE 1 DIABETIC PATIENT WITH CHARCOT NEUROARTHROPATHY AND RELAPSING OSTEOMYELITIS: A CASE REPORT.

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On December 2009 a 57-year-old Caucasian woman was referred to the outpatient Diabetology Unit of our University Hospital since she has a poor control of her diabetes. She had received diagnosis of type 1 diabetes at the age of 22, and developed over time diabetic nephropathy, proliferative diabetic retinopathy and Charcot neuroarthropathy, and she was moderately overweight. The patient exhibited a self-monitoring glycemic diary reporting an extreme glycemic variability with a number of episodes of hyperglycemia (up to 400 mg/dl) along with unaware hypoglycemia (up to 38 mg/dl). $HbA1_c$ level was 11.4% (101 mmol/mol). She was receiving nearly 40 UI of insulin per day according to a multi-injection regimen including two daily administration of NPH insulin. The insulin regimen was initially modified in order to reduce hypoglycemias, by introducing insulin analogues (both ultra-rapid and long-acting), instead of regular and NPH insulin. In addition, the patient exhibited an ulcer at the fourth metatarsal head of the left foot, deep to the bone surface with an associated osteomyelitis, which was diagnosed 1 year before and not still resolved by periodical and accurate surgical debridements, antibiotic therapy and orthotic management. Owing to the profound fluctuations in the glycemic profile provided by the patient, continuous subcutaneous insulin infusion (CSII) with continuous glucose monitoring system (CGMS) was implanted on June 2010. After six months, $HbA1_c$ decreased to 7.2% (55 mmol/mol) as well as Kovatchev indexes remarkably improved compared with relative baseline values (HBGI 14 vs 3; LBGI 6 vs 0.8). An appropriate glycometabolic control was maintained during the following four years, with $HbA1_c$ persistently below 7.5% and minimal rates of hypoglycemias. From December 2010 to October 2014 the patient had no recurrence of the osteomyelitic process and no further ulcers of the lower limbs occurred. To date, there is no evidence of relationship between appropriate glycometabolic control with low glycemic variability and tissue repair processes in diabetic patients. Our report could encourage further clinical research to verify this hypothesis and possibly support the use of insulin pump technique to improve wound healing processes in diabetic foot.

PP041 - THE POLYMORPHISM RS9677 OF VASOACTIVE INTESTINAL PEPTIDE RECEPTOR 1 IS ASSOCIATED WITH GLYCOLIPID CONTROL AND HEART FUNCTION IN FEMALES WITH TYPE 2 DIABETES: A FOLLOW-UP STUDY

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OBJECTIVE In a previous study, it was shown that the single nucleotide polymorphisms (SNP) rs9677 mapping in the 3'-UTR of Vasoactive Intestinal Peptide receptor 1 (VPAC1R) gene associated with type 2 diabetes (T2D) in females. In addition, the CC genotype correlated with a worse glycolipid profile. The aim of this study was to confirm the association of this polymorphism with glycolipid control and to search for the presence of coronary artery disease (CAD) in the same population, after a follow-up of 4.6 yrs.

PATIENTS AND METHODS 143 females with T2D, 53 carrying the CC genotype (age 71.7 ± 7.4 yrs, diabetes duration 17.2 ± 9.9 yrs) and 90 carrying the CT+TT genotypes (age 69.4 ± 8.8 yrs, diabetes duration 14.3 ± 8.2 yrs), were followed for 4.6 ± 1.8 yrs. At follow-up, clinical and hematochemical parameters were analysed. Twelve-lead electrocardiography (ECG) and Doppler echocardiography (echo) were obtained and the percentage of patients with acute myocardial infarction (AMI) or subjected to percutaneous transluminal coronary angioplasty (PTCA) and coronary artery bypass graft surgery (CABG) was evaluated.

RESULTS At follow-up there was no statistically significant difference in terms of clinical and hematochemical parameters between the two groups. However, despite a significant increase of statin therapy, in females with the CC genotype there wasn't a significant improving of LDL cholesterol levels unlike females with the CT+TT genotypes (P = 0.02). Moreover, females carrying the CC genotype presented a significantly higher percentage of echocardiographic abnormalities (P = 0.035), especially left ventricular diastolic dysfunction (P = 0.04).

CONCLUSIONS The rs9677 CC genotype could be correlated with a reduced response to statin therapy and also it seems to be involved in the development of diabetes cardiomyopathy in females with T2D.

PP042 - MACROVASCULAR COMPLICATIONS IN CALABRIAN POPULATION WITH TYPE 2 DIABETES MELLITUS

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Macrovascular complications (MCs), including cardiovascular disease, stroke, and peripheral vascular disease, represent the main cause of death in patients with diabetes. We aimed to evaluate the prevalence and risk factors of the macrovascular disease in a diabetic population from Calabria, the Italian region with the highest diabetic prevalence (8.5%).

We examined 1294 consecutive individuals with type 2 diabetes (T2D), who were seen at the Unità Operativa di Endocrinologia, University of Catanzaro, and at the Struttura Operativa Complessa Endocrinologia-Diabetologia, Ospedale Pugliese-Ciaccio, Catanzaro, during the period May 2010-December 2014. T2D was diagnosed according with the American Diabetes Association's criteria, and patients were clinically and instrumentally evaluated for MCs. All determinants of MC have been tested by regression analyses, employing the SPSS 20.0 statistical software.

The examined population included 685 female and 609 males with a median age of 63 years (interquartile range 55-71), BMI 27.5 Kg/m2 (25.3-31), and a duration of T2D of 7 years (3-12). 972 were treated with hypoglycemic oral agents, 429 with insulin, 865 with antihypertensive agents, and 461 with hypolipidemic drugs. 22.7% of subjetcs (20.1% females and 25.6% males) were affected by MCs, whose appearance correlated with age ($P<0.001$) and duration of T2D ($P<0.001$), and was associated with male gender ($P=0.016$), smoking ($P=0.026$), reduced HDL cholesterol($P<0.001$), and with insulin treatment ($P<0.001$), this latter independently from age and from duration of T2D.

In Calabrian population the prevalence rates of MCs are consistent with the prevalence rates for MC reported in the Italian general population, even with respect to gender. The association of MC with insulin treatment supports a pathogenetic role of insulin in MC.

PP043 - LEUKOCYTE TELOMERE LENGTH IS INDEPENDENTLY ASSOCIATED WITH INTIMA MEDIA THICKNESS IN ELDERLY PATIENTS WITH TYPE 2 DIABETES AND ATHEROGENIC DYSLIPIDEMIA

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Background and aim: Leukocyte telomere length (LTL), an emerging cardiovascular (CV) risk biomarker, is reduced in diabetic subjects compared to non-diabetics. Our aim is to assess whether LTL might be a surrogate marker of cardiovascular disease (CVD) also in patients with type 2 diabetes and atherogenic dyslipidemia (AD).

Methods: In a cohort of 114 type 2 diabetic patients (68M/46F, 65±9yrs, 31±4 kg/m^2) with atherogenic AD (triglycerides>150 mg/dl, HDL-cholesterol <40mg/dl) and LDL-cholesterol near to target (110±39mg/dl), we assessed anthropometric, hemodynamic and metabolic parameters, total cholesterol and triglyceride content in VLDL, HDL, LDL (ultracentrifugation), cholesterol content in HDL, IDL and LDL subfractions (Lipoprint©), oxidized LDL (oxLDL) and susceptibility of LDL to oxidation. Carotid intima-media thickness (IMT) was measured by a single operator and expressed as the maximum IMT (IMT max). LTL was assessed by a specific real-time PCR reaction.

Results: As age was the major determinant of IMT max in the whole population, and LTL is closely dependent on age, we performed analyses by dividing the population in two subgroups based on median cohort age (65 yrs). In a multivariate model, after controlling for traditional CV risk factors, smoking habit (pack/year, <0.0001) and previous CVD ($p<0.05$) were independently significantly associated to IMT max in patients with age<65 years ($R^2=0.27$). In elderly subjects (age >65yrs), LTL ($p<0,005$), previous CVD ($p<0.01$) and small dense LDL ($p<0.01$) were the only independent predictors of IMT max ($R^2=0.29$).

Conclusions: LTL is a promising sensitive biomarker of CVD also in patients with diabetes and AD, although this finding is limited to elderly patients.

PP044 - IS EARLY MEASUREMENT OF A1C USEFUL FOR THE PREDICTION OF TREATMENT RESPONSE IN TYPE 2 DIABETES?

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A1c, which is correlated with 3-month mean glycemia, is usually measured every 3-6 months. Effects of variations of treatment are routinely assessed through A1c not earlier than 3 months. Glycated albumin (GA) has been proposed as an indicator of shorter-term (2-week) glucose control Aim of the present pilot study was to explore the possibility of predicting 3-month A1c by measuring A1c or GA at 15-30 days.

Twenty-seven metformin-treated patients with type 2 diabetes initiating a pharmacological treatment other than insulin were enrolled after written informed consent. The patients (16M:11F, aged 64.7±10.1 years) had a duration of diabetes of 8.6±8.5 years with baseline A1c 59.0±12.0 mmol/mol (7.5±1.1). The prescribed treatment was maintained throughout the 3-month follow-up. A1c and GA were measured at baseline, 15, and 30 days, and A1c only at 90 (±3) days.

A1c at 90 days (50.0±7.2mmol/mol) was significantly ($p<0.001$) reduced from baseline (59.0±12.0 mmol/mol). A significant reduction was already present at 15 days (56±7.8 mmol/mol, $p<0.01$) and confirmed at 30 days (53.0±7.0 mmol/mol, $P<0.001$). A similar pattern was found for GA, which was significantly lower at 15 (28.8±10.8%, $p=0.03$) and 30 (27.0±9.8%, $p<0.001$) days than at baseline (31.9±11.3%). Variations of both A1c and GA at 15 days showed a significant correlation with 90-day variation of A1c: $R=0.895$, $P<0.001$ and $R=0.418$, $P=0.030$, respectively. Correlation of A1c improved at 30 days $R=0.908$, $P<0.001$, but GA lost significance, suggesting that A1c may represent a better short-time predictor of patient's response.

The early identification of patients not adequately responding to the prescription of a new drug could be very useful for clinicians, allowing a greater timeliness in further modifications of therapy. This pilot study suggests that a measurement of A1c as early as 15 days from the start of treatment can accurately predict three-months results being of help for the assessment of treatment response.

PP045 - GESTATIONAL DIABETES AND MATERNAL-NEONATAL OUTCOMES: IMPORTANCE OF DIFFERENT OGTT ALTERATIONS

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Introduction: According to the criteria described in the HAPO study (Hyperglycemia and Adverse Pregnancy Outcome), a single altered value at the glucose tolerance test (OGTT) is sufficient for Gestational Diabetes (GDM) diagnosis. The presence of multiple OGTT altered values does suggest a more serious metabolic condition compared to a single OGTT altered value. However, no data have been reported so far to support this concept. **Aim:** To evaluate the clinical course, maternal and neonatal complications, and glucose tolerance after delivery in GDM women, identifying subgroups with higher risk, on the basis of different OGTT alterations. **Patients-Methods:** 315 GDM patients were divided into 4 groups based on diagnostic OGTT: group 1 (n=106; 33.6%): impaired fasting glucose alone; group 2 (n=106; 33.6%): alteration of a single point different from baseline (1h or 2h after glucose load); group 3 (n=68; 21.6%): 2 abnormal points; group 4 (n=35; 11.1%): all 3 points altered. Data are presented as mean ± SD or as % of the total group. Logistic regression was used for statistical analysis. **Results:** Glycosylated hemoglobin (HbA1c), both at diagnosis and at last control before delivery, was not different among the four groups. Similarly, maternal and neonatal complications were not different among the four groups. It was necessary to add insulin therapy to almost all of patients with all OGTT points altered (group 4) (91.4%, $p<0.05$ vs. other 3 groups). Half of patients in group 4 showed an early postpartum alteration of glucose tolerance ($p<0.05$ vs. other 3 groups). Interestingly, group 1 represented a worse condition than group 2. In fact, the need for insulin therapy was 55.7% in group 1 vs. 41.5% in group 2 ($p<0.05$), and the need for only basal insulin therapy was 22.0% in group 1 vs. 4.5% in group 2 ($p<0.05$). In addition, group 1 had a higher rate of impaired glucose tolerance after delivery than group 2 (16.7% vs. 6.5%, respectively). Patients of group 1 were also younger ($p<0.05$ vs. other groups), and had a pregestational BMI similar to group 4 (28.8±8.2 and 29.4±6.7 kg/m^2, respectively), significantly higher than group 2 (26.1±5.6 kg/m^2, $p<0.05$). Logistic regression analysis indicated that the results were independent of pregestational BMI. **Conclusions:** Groups 3 and 4 were composed of patients with more severe clinical features**.** However even a single altered value at OGTT (groups 1 and 2) requires insulin therapy in a high number of cases and carries a risk of early postpartum glucose tolerance alterations. Group 1 is at higher risk for both the need of insulin therapy during pregnancy and early postpartum glucose metabolism disorders compared to group 2. Finally, the absence of differences between the 4 groups in terms of HbA1c, and maternal and neonatal complications may be explained by the fact that all patients in all groups were under strict glycaemic

control, and were immediately placed on insulin therapy when necessary.

PP046 - CORRELATION BETWEEN TESTOSTERONE AND ENDOTHELIAL PROGENITOR CELLS IN TYPE 1 DIABETIC PATIENTS

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Circulating endothelial progenitor cells (EPCs) are bone marrow-derived stem cells able to migrate to sites of damaged endothelium and differentiate into endothelial cells, there by contributing to vascular repair. Recent studies demonstrated a reduction of EPCs in patients with diabetes mellitus or erectile dysfunction (ED). The aim of this study was to evaluate the circulating levels of different EPCs phenotypes and their relation with testosterone levels in young type 1 diabetic patients with ED.

We studied 118 consecutively type 1 diabetic patients and 60 age-matched healthy controls. Erectile function was assessed by completing the International Index of Erectile Function (IIEF-5), and EPCs levels by flow-cytometry. Testosterone concentrations were evaluated in all the study population.

Thirty-eight diabetic patients had ED (Group 1). CD34+KDR+CD133+ cells, were significantly lower in diabetic patients with ED as compared with those without ED (Group 2) [median and interquartile range, $n/10^6$ events, 12(6,16) vs 18 (13,22), $P<0.001$)]. In all participants in the study, there was a significant correlation between circulating CD34+KDR+CD133+ cells and testosterone levels ($r = 0.410$, $P <0.001$), which was highest in Group 1, intermediate in Group 2, and lowest in Group 3 (controls). Finally, there was a significant correlation between IIEF-5 score and both CD34+KDR+ ($r = 0.459$, $P = 0.003$) and CD34+KDR+CD133+ ($r = 0.316$, $P = 0.050$) cells among patients of Group 1, as well as between testosterone levels and most of the EPCs phenotypes.

In conclusion, type 1 diabetic patients show reduced levels of CD34+KDR+CD133+ cells, whose number correlate with IIEF and testosterone levels.

PP047 - GLUCOSE VARIABILITY AND ENDOTHELIAL PROGENITOR CELLS IN TYPE 1 DIABETIC PATIENTS

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Glucose variability may contribute to the development of chronic vascular complications of diabetes. Intermittent exposure to high glucose concentrations may lead to vascular injury through increase of endothelial dysfunction and oxidative stress. Circulating endothelial progenitor cells (EPCs) are involved in the repairing mechanisms of vascular damage.

We aimed at evaluating whether glucose variability may affect circulating levels of EPCs in type 1 diabetic patients. Forty type 1 diabetic patients and 22 sex- and age-matched control subjects were included in this study. Seven subpopulations of EPCs were determined by flow cytometry on the basis of the surface expression of CD34, CD133 and KDR antigens. Assessment of glucose variability was obtained was obtained from 72 h-blinded continuous glucose monitoring (CGM) data by calculating the mean amplitude of glycemic excursions (MAGE) and the blood glucose standard deviation (BGSD).

Compared with control subjects, diabetic patients showed a significantly decreased number of most EPCs phenotypes. A significant inverse correlation between MAGE and both CD34+ ($r = -0.339$, $P=0.034$) and CD34+KDR+CD133+ cells ($r = -0.429$, $P=0.006$) was found in diabetic patients; BGSD and HbA1c did not correlate with EPCs phenotypes.

In young type 1 diabetic patients, glucose variability inversely correlates with EPCs phenotypes that predict vascular damage.

PP048 - EFFECTS OF SIX-MONTH TREATMENT WITH LIRAGLUTIDE ON DIASTOLIC DYSFUNCTION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS.

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It is known that liraglutide, a GLP-1 receptor (GLP-1R) agonist, is effective in reducing HbA1c and body weight in type 2 diabetes (T2D) patients. Since GLP-1R are present on the heart, liraglutide could also favorably affect diabetic cardiomyopathy (DCM). Diastolic dysfunction (DD) represents the earliest marker of the DCM development. Aim of this study is to investigate whether liraglutide can improve diastolic function in T2D subjects by using a specific echocardiographic evaluation performed with the pulsed-wave tissue Doppler imaging technique (TDI). Methods: T2D patients with diagnosis of DD who started Liraglutide (LG) in our Diabetes Center since January 2013 were enrolled in an observational prospective study. 32 subjects (16 F, 16 M) on various hypoglycaemic, anti-hypertensive and lipid-lowering therapies were analyzed. Six of them drop out in the first days because of gastrointestinal side effects and were used as a control group (CG). They continued with other hypoglycemic therapies which did not include GLP-1 analogues. Clinical and echocardiographic data were investigated in all the patients at baseline and after six months. Results: In LG HbA1c decreased from 9 ± 1.6 to 7.5 ± 1.3 % ($p < 0.01$) and fasting blood glucose from 190.8 ± 56.2 to 151.9 ± 43.2 mg/dL ($p < 0.01$); body weight (BW) reduced from 91 ± 15.4 to 87.3 ± 15.3 Kg ($p < 0.01$); a significant improvement of waist circumference (WC) and BMI was also observed. In CG a significative improvement of HbA1c (from 9.0 ± 1.8 to 7.8 ± 1.4 %, $p < 0.03$) and fasting blood glucose was seen. BW, WC and BMI didn't change. In LG TDI and Doppler of pulmonary venous flow techniques showed a significant improvement in diastolic function. e' lat.-wave velocity increased from 9.2 ± 3.4 to 11.6 ± 4.7 ($p < 0.01$); e' med.-wave velocity increased from 6.9 ± 1.7 to 8.4 ± 2.6 ($p < 0.01$); e'/a' lat. ratio increased from 0.7 ± 0.3 to 0.9 ± 0.4 ($p < 0.01$); e'/a' med. ratio increased from 0.5 ± 0.1 to 0.6 ± 0.1 ($p < 0.02$). The reduction of the E/e' ratio was notable (from 10.7 ± 4.3 to 8.5 ± 2.5, $p < 0.01$). We also found the reduction of peak Ar velocity ($p \leq 0.02$), Ar-wave duration ($p \leq 0.01$) and S/D ratio ($p \leq 0.03$). No gender differences was observed. A significant relationship between HbA1c reduction and increase of e' lat.-wave was found ($R^2 = 0.203$, $p < 0.01$). On the contrary, CG didn't show any improvement of diastolic function. Conclusions: A six-month treatment with Liraglutide produced weight loss and was associated with a significant improvement of diastolic function that could be protective from DCM in T2D patients.

PP049 - GENDER-RELATIONSHIP BETWEEN POST TRAUMATIC STRESS DISORDER AND TYPE 2 DIABETES

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Aim. Many psychopathological diseases, such as anxiety and depression, occur more often in diabetic women than diabetic men. The relationship between Posttraumatic Stress Disorder (PTSD) and Type 2 Diabetes has been partially investigated in literature, and in this study, we investigated gender differences in PTSD and in the use of coping strategies in type-2 diabetic patients.

Methods. In the local unit of diabetology and metabolic diseases, we sequentially enrolled 54 men and 46 women with type-2 diabetes. These two groups of males and females were matched according to age and education and we excluded patients with severe mental disorder and cardiovascular disease or neuropathy. A psychometric protocol composed of the DTS (Davidson Trauma Scale) to assess PTSD and its cause, and the Brief-COPE for coping strategies was administered.

Results. A significant difference between males and females was found in the prevalence of PTSD (men=29.6%; women=58.7%; p=0.0001). A remarkable difference was also observed in the severity of DTS score (men=31.65±23.06, women=53.5±27; p<0.0001) and in the three subdomains indicating the PTSD symptomatology (intrusion: women= 17.17 ± 9.33 vs. men= 9.70 ± 8.23; p<0.0001), (avoidance/numbing: women=17.79±12.09 vs. men=10.26±9.70; p=0.0008), (hyperarousal: women=18.94±11.12 vs. men=11.68±8.92; p=0.0005). Moreover, direct correlations between maladaptive coping strategies and PTSD were found only in the female group.

Conclusions. To conclude, we demonstrated, for the first time, clear gender differences in PTSD in the diabetic population. Hence, it is fundamental to consider that diabetic women are more vulnerable to developing PTSD than men. Therefore, in diabetic women major clinical cares are necessary to improve their coping skills in response to PTSD and also for a major adherence for diabetic therapy. Finally, it seems more and more necessary to also offer psychological counselling for diabetic patients paying particular attention to gender.

PP050 - EXTRAHEPATIC CHOLESTASIS IS ASSOCIATED WITH REDUCED BETA-CELL FUNCTION IN NON-DIABETIC SUBJECTS

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Several studies have shown that cholestasis is associated with altered glucose tolerance. However, the relationship between cholestasis and β-cell function has not been fully evaluated in the clinical setting. To investigate whether cholestasis, as evidenced by hyperbilirubinemia, affects β-cell function and insulin secretory response, we performed OGTT and hyperglycemic clamps (HC) followed by arginine stimulation in 44 patients (27 F/17 M, 51±15 yrs) scheduled for pancreatoduodenectomy for periampullary diseases, all without known history of type 2 diabetes (T2D). Based on bilirubin levels (BL), subjects were divided into 2 groups: with resolved cholestasis and/or normal bilirubin levels (NChol, n=21, BL: 0.29 ±0.03 mg/dl) and with active extrahepatic cholestasis (Chol, n=23, BL: 4.30±0.69 mg/dl). Amylasemia was similar between the 2 groups (76.6 ± 15.8 vs. 86.9 ± 10.3UI/L). To evaluate β-cell function, β-cell glucose sensitivity during HC was calculated as the ratio of insulin secretion (IS) and glucose increments. Chol group displayed a significantly lower Insulinogenic index (0.46±0.06 vs. 0.76±0.11, p=0.01), while no differences were detected in the Matsuda index (5.89±0.88 vs. 4.66±0.67, p=NS). The incremental 1st phase (p=0.01), 2nd phase IS (p=0.02) and β-cell glucose sensitivity(57.5±10.5 vs. 92.3±11.3 pmol•min-1•m-2•mM-1, p=0.03) were significantly lower in Chol group. Analysis of the entire group revealed an inverse correlation between bilirubin level and Insulinogenic index (r= -0.40; p<0.05), and Arginine-stimulated IS (r=-0.51; p<0.01). Our data indicate that cholestasis associates with impaired β-cell function and possibly reduced mass (as estimated by Arginine-stimulated IS). We speculate that, in subjects with extrahepatic cholestasis, impaired β-cell glucose sensitivity could represent a major determinant of the observed insulin secretory defect in response to both glucose and arginine stimulus. Further investigation of this mechanism might improve understanding of the pathogenic events leading to altered insulin secretion in T2D.

PP051 - DO STATINS PROMOTE THE ONSET OF TYPE 2 DIABETES MELLITUS? A RETROSPECTIVE, OBSERVATIONAL SINGLE CENTER STUDY IN A COHORT OF DYSLIPIDEMIC PATIENTS.

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Aim: The introduction of statins into clinical practice represented a breakthrough in the treatment of patients with increased cardiovascular risk but, in the last years, many studies highlighted a possible correlation between statins therapy and onset of type 2 Diabetes Mellitus (DM2). The objective of our study was to evaluate the effects of statin therapy on glycemic metabolism in an heterogeneous group of dyslipidemic patients referring to our Lipidologic out patients clinic in Novara.

Methods: A retrospective, longitudinal, case-control study was performed. We consecutively recruited dyslipidemic patients referring to our center. Subject were divided in two groups: group A, including patients on statin therapy and group B, including patients only on diet. A 5 years follow-up was performed, with baseline and annual evaluation of anthropometric parameters, lipid profile, fasting plasma glucose, A_1c and ongoing medical therapy. Data on family history of DM2 and dyslipidemia were collected at baseline.

Results: We recruited 308 patients, divided in Group A (n=265) and Group B (n=43). The percentage of new cases of DM2 were 12.98 % in the whole population, 13.96% in Group A and 9.5% in Group B. The analysis of the anthropometric and metabolic parameters showed that patients who developed DM2 presented an insulin resistance phenotype at baseline. In particular, patients from Group A who developed DM2 had, at baseline, central obesity and hypertension. Considering the whole population, the factors that were found to correlate with the onset of DM2 in euglycemic patients were: familiarity for DM2 ($p<0,03$), use of thiazide diuretic ($p<0,05$) and BMI ($p<0,01$). Conversely, when considering the onset of DM2 in subjects who had already glycemic alterations at baseline, our study pointed out the influence of statins ($p<0,0001$), metabolic risk factors (including triglycerides > 150 mg/dl, arterial hypertension and BMI > 30 Kg/m^2) ($p<0,03$) and arterial hypertension ($p<0,01$). Similar correlations were found by considering only patients from Group A with the addition of alcohol consume, which was found to be a risk factor for developing altered fasting plasma glucose ($p<0,02$).

Conclusions: Our study confirmed that the onset of DM2 and impaired fasting glucose was mainly due to initial patient's clinical conditions and anthropometric parameters rather than to statin therapy, which seemed to play a role only in patients with insulin resistance phenotype at baseline. Because of side effects of statine therapy on developing glycemic alterations were found to be lower than benefits, this kind of therapy should be considered in all patients at risk of any type of major vascular event.

PP052 - SEVERE HYPERTRIGLYCERIDEMIA IN A 7 YEAR OLD GIRL WITH ONSET OF DIABETES MELLITUS IN KETOACIDOSIS (DKA)

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PRESENTATION OF THE CASE

A 7 year old girl, 18 kg, Caucasian, came to observation after appearance of several symptoms for about three months: asthenia, polyuria, polydipsia, visual disturbances and weight loss (about 4 kg); no others remarkable deseases.

The child looks alert, pale, dehydrated.

Urgent blood tests: blood glucose 400 mg/dl, serum potassium 2.9 mEq/L, serum sodium 128 mEq/L. Arterial blood gas: uncompensated metabolic acidosis with increased anion GAP (7.18 pH, PCO2 28 mmHg, PO2 98 mmHg, HCO3 13 mmol/L). Ketone capillary 2 mmol/L.

Urinalysis: glycosuria and ketonuria. Therapy: infusion with saline solution (0.9%, then glucose for 24 hours), insulin (0.1 units/kg/h), potassium phosphate.

Further Blood tests detect: hypertriglyceridemia (2831 mg/dl), high cholesterol (602 mg/dl, HDL 22 mg/dl), lipase 72 IU/L (reference range <76), GOT, GPT and pancreatic amylase not be determined for serum strongly lipemic.

Abdominal ultrasound: pancreas moderately hyperechoic, without dilation of the pancreatic ducts. Hepatic, renal and thyroid functionality appear normal, celiac absent. Antibodies associated with type 1 diabetes: (Anti GAD> 100 IU / ml; IA2 46 IU/ml).

Second day: venous pH 7.28. It was necessary to increase insulin dose at 0.2 units/kg /h, with progressive resolution of DKA and transition to subcutaneous insulin therapy (insulin requirements: 1.5 units/kg/day) with refeeding. Triglyceride levels, lipase and amylase gradually reduced. Genetic testing and lipidologic the child and the family: negative.

After two months of diagnosis: 7% glycated hemoglobin, normal values of fasting triglycerides (53 mg/dl) with normal total cholesterol and HDL. Insulin requirement: 0.87 units/kg/day

CONCLUSIONS

At the DKA may be associated severe hypertriglyceridemia, even in the absence of genetic syndrom. This case demonstrates that high levels of triglycerides, besides being a risk factor for acute pancreatitis, may prolong the course of the DKA. The resolution of DKA and the next good glucose control, lead to the normalization of dyslipidemia, without the use of other therapies. The presence of high levels of triglycerides requires insulin dosages higher than those indicated in the Guidelines.

PP053 - THE IMPROVEMENT OF B CELL PERFORMANCE AFTER INTENSE AEROBIC EXERCISE IS DIRECTLY RELATED TO THE INCREASE IN THE LEVELS OF VITAMIN D

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Low concentrations of 25-hydroxy-vitamin D [25(OH)D], the best indicator of vitamin D status, have been associated with alterations in glucose tolerance, insulin sensitivity and β cell function. Few data are available on the effects of a single session of aerobic exercise on the levels of 25(OH)D and the possible correlation with insulin secretion and sensitivity.

We therefore recruited 20 young (25-31 years-old), healthy, normal weight volunteers, with a sedentary/moderate active lifestyle. The oral glucose tolerance (OGTT) was performed before and on the day-after a bout of aerobic exercise (a single workout of jogging or running for 30-40 minutes, or until exhaustion). Insulin secretion and sensitivity were estimated using OGTT-derived indices. During exercise, all subjects wore a metabolic holter to assess energy expenditure.

Depending on the energy expenditure during work-out , the subjects were divided (cut-off 7 METs , arbitrary value based on the median value in the cohort) in two groups: non-intense (<7) or intense group (>7). In both groups, a significant improvement in insulin sensitivity ($p < 0.04$ Quicki) has been observed.

After exercise, concentrations of 25(OH)D appeared increased in the intense group and reduced in the other one (p=NS for both). Furthermore, the levels of 25(OH)D were significantly greater in the intense group after physical activity, as well as the disposition index (respectively, $p<0.02$ and $p <0.03$, non-intense vs. intense group), with no significant change in insulin secretion. The improvement in the disposition index is closely related with the change from baseline in 25(OH)D in the intense group (p:<0.05, r:0.4).

The data demonstrate that a single session of aerobic exercise improves beta cell secretory performance and this improvement is closely related with the increase in the levels of 25(OH)D levels after strenuous physical activity

PP054 - METABOLIC EFFECTS OF INTERVAL TRAINING IN PATIENTS WITH TYPE 2 DIABETES

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Background and aims: major guidelines on the management of type 2 diabetes mellitus (T2DM) recommend performing at least 150 min/week of moderate-intensity aerobic physical activity. Nevertheless, the most effective type of exercise for glycaemic control has not been established. The aim of the present study was to compare the effects of interval training exercise, which pairs high-intensity exercise phases with low-intensity exercise recovery phases, with those of a conventional, constant-load aerobic exercise on glycaemic control (HbA1c), lipid profile and body composition in patients with T2DM. **Materials and methods**: Thirty sedentary patients (17/13 M/F; age [mean±SD] 63.0±8.0 yrs; BMI 29.0±3.9 kg/m^2; HbA1c% [median (1°-3° quartile)] 6.6 (6.5-7.1) with a diagnosis of T2DM ≥5 years and treated with oral hypoglycaemic agents and/or basal insulin were randomized to 3 treatment arms: constant-load aerobic exercise (CL), interval training aerobic exercise (IT) for 10 weeks (a 2-week conditioning phase + 8-week exercise programme, 3 times/week) or no activity (SED) for the same time period. Fasting plasma glucose, insulin, HbA1c, triglycerides (TG), total cholesterol, HDL and LDL cholesterol and body composition (DXA) were measured at baseline and at study end. **Results**: basal characteristics of the 3 groups were comparable. After 10 weeks, there were no significant differences from baseline in HbA1c% [pre *vs.* post: 6.7 (6.5-7.1) *vs.* 6.6 (6.5-6.9); 6.6 (6.5-7.0) *vs.* 6.5 (6.5-6.6); 6.7 (6.1-7.5) *vs.* 6.6 (6.4-7.2) in the CL, IT and SED groups, respectively; p for time and interaction=ns) or cholesterol levels. Significant improvements in fasting plasma glucose (p=0.03), TG (p=0.02) and insulin resistance estimated with the HOMA-IR index (p=0.04) were observed in the IT group only, despite similar and significant changes in total body composition in both exercise groups (fat mass: -4.3% and -4.9%, fat free mass +1.5% and +1.7% in the CL and IT groups, respectively; p<0.05 *vs*. baseline; CL *vs*. IT: p=ns). **Conclusion**: Interval training exercise might have specific effects on segmental body composition and/or lead to different adaptive changes in skeletal muscle as compared with constant load aerobic exercise. Such changes might be responsible for the observed benefits on fasting plasma glucose, TG and HOMA-IR. The lack of significant effects on HbA1c might be due to the good glycaemic control at baseline.

PP055 - COGNITIVE PERFORMANCE AND DIETARY HABITS OF SUBJECTS AT RISK FOR TYPE 2 DIABETES

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The incidence of glycaemic metabolic disorders is increasing at alarming rates, largely due to poor diet and lifestyle habits; epidemiological and clinical observations have accumulated showing that diabetic patients are significantly more likely to develop cognitive impairment and exhibit increased susceptibility to dementia.

To test whether diet features are associated to metabolic disorders and, possibly, to the resulting cognitive impairment, sixtyfour subjects at risk for T2D undergoing OGTT as screening test were consecutively enrolled. The nutritional habits evaluation has been carried out using the Italian version of the EPIC (European Prospective Investigation on Cancer and Nutrition) and the cognitive performance was assessed through validated neuropsychological tests to explore memory, attention and executive functions.

Based on OGTT, none of subjects was classified as IGT nor diagnosed as diabetic. Then, the cohort was divided in quartiles based on insulin sensitivity estimated on Matsuda (<3,9; 4-7.9; 8-11.9; >12). As expected, BMI and insulin sensitivity were inversely correlated through all the quartiles (Q). Unexpectedly, the worsening of insulin sensitivity corresponded to a higher neurological performance, as demonstrated by the significant and gradual deterioration of the executive tests along the quartiles of insulin sensitivity (trail making test, Q1 60.4±14, Q2 61.8±22, Q3 78.1±24, Q4 93.8±28 seconds, ANOVA $p<0.008$). The higher insulin sensitivity was significantly associated to increase intake of nutswallnuts (ANOVA $p=0.04$) and whole grains (ANOVA $p=0.01$).

After linear multiple regression, only wholegrain intake remained significantly associated to insulin sensitivity ($p<0,05$).

In subjects at risk for T2D, the increased intake of whole grains seems to be associated to better insulin sensitivity. Cognitive performance, unexpectedly, seems to be inversely related to the worsening of insulin sensitivity.

PP056 - CARDIOVASCULAR OUTCOMES AND CONVENTIONAL RISK FACTORS IN NON-DIABETIC ADULT PATIENTS WITH GH DEFICIENCY

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Background: Adult-onset growth hormone deficiency (GHD) has been reported to lead to a higher cardiovascular (CV) risk, but only scant literature is available about the role for conventional risk factors in this clinical setting. This study aimed to assess the long-term outcome rates and their possible relationship with conventional risk factors like systemic hypertension (SH), hypercholesterolemia and age in adult-onset GHD patients scheduled for GH replacement therapy (GHRT).

Methods: fifty-three non-diabetic GHD patients (22M-31F), aged 45.4±14.3 years, were divided into 2 groups based on the presence (*group-A*) or absence (*group-B*) of SH before starting GHRT. Clinical examination, laboratory sampling, ECG and echocardiography were performed. Composite (fatal and non-fatal) CV events in both groups and with respect to age and LDL-cholesterol were recognized. Relative risk (RR), standardized mortality rate (SMR), discrepancies between unfavourable and favourable outcome patients were performed.

Results: 17 patients (32%) entered the *group-A* and 36 (68%) the *group-B*. Mean follow-up length was 133±42 months. CV event rate was 22.6% [47.1% in *group-A* and 11% in *group-B* (p=0.01)]. Fatal outcomes occurred in 3 patients (5.7%), 2 (11.8%) *in group-A* vs 1 (2.8%) in *group-B*, with SMR=1.46, 3.01 vs 0.72 (p=0.06), respectively. SH and age>55 years resulted to be major determinants of CV events, with a RR=4.2 (p=0.007) and 3.9 (p=0.02), respectively. Unfavourable outcome patients showed greater left ventricular mass and atrial chamber size.

Conclusions: This study strongly suggests that CV events are chiefly related to SH and age in replaced non-diabetic GHD patients.

PP057 - MICROALBUMINURIA IS ASSOCIATED WITH AN INCREASED RISK OF CARDIOVASCULAR DISEASE IN SUBJECTS WITH TYPE 2 DIABETES AND HYPERTENSION

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Background and aim: microalbuminuria is an independent risk factor for cardiovascular morbidity and mortality. The aim of our study was to evaluate if the presence of microalbuminuria in patients with type 2 diabetes mellitus and hypertension was associated with an increased risk of cardiovascular disease.

Materials and Methods: We enrolled 104 patients (54.9% male; age: 65.6 ± 8.4 years; BMI: 28.7 ± 5.8 kg/m2; HbA1c: 7.1 ± 0.6 percent) with hypertension and type 2 diabetes, subsequently divided into 2 groups according to the presence (Group A) or absence (Group B) of microalbuminuria (> 20 mcg/min). The studied subjects underwent to metabolic evaluation and ultrasound Doppler of carotid vessels in order to assess the carotid intima media thickness.

Results: HbA1C (7.1 ± 1.1 vs. 6.7 ± 0.7%; p = NS), total cholesterol (176.8 ± vs 169.1 32.5 ± 50.3 mg/dl; p = 0.08), LDL (88.4 ± 16.1 vs 85.8 ± 44.4 mg/dl; p = 0.09) and HDL (48.6 49.2 ± ± 7.6 mg/dl; p = 0.07) levels did not differ between group A and Group B. The mean values of the carotid intima media thickness were significantly higher in Group A than in Group B both at the right (1.24 ± 0.15 ± 0.04 mm vs 1.02; p = 0.007) and the left (1.23 ± 0.15 vs 1.05 ± 0.05 mm; p = 0.03) carotid.

Conclusions: The presence of microalbuminuria has an additive effect on cardiovascular risk in patients with type 2 diabetes mellitus and hypertension. Screening for microalbuminuria might have a role to estimate cardiovascular risk in diabetic hypertensive subjects.

PP058 - EFFECT OF METFORMIN ON THYROID STIMULATING HORMONE IN TYPE 1 DIABETIC PATIENTS, IN INTENSIVE LONG-TERM INSULIN THERAPY

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Introduction: A possible influence of metformin on some parameters of thyroid function, in particular on levels of TSH, in patients with subclinical and clinical hypothyroidism has been reported several studies. This role was also highlighted in patients with insulin resistance, mild increase in TSH levels and absence of thyroid antibodies.

Materials and Methods: We followed-up 80 patients (45 F and 35 F, age range 16-30 years) with type 1 diabetes mellitus (DM1) in intensive long-term insulin therapy (at least 12 years).

The patients were divided into two groups: Group A consisting of 24 patients with associated Hashimoto's thyroiditis, Group B represented by 56 patients without chronic autoimmune thyroiditis.

In both groups TSH levels ranged between 4.5 and 10 MCU / ml.

Group A patients were positive for TPO and TG antibodies showing a US thyroid inhomogeneous pattern; Group B patients were negative for thyroid antibodies and a US pattern slightly inhomogeneous.

In both groups in addition to insulin therapy metformin (2000-2500 mg/die) was also associated; none of them underwent L-thyroxine replacement therapy.

Results: After 12 months, all patients showed a significant reduction ($p < 0.01$) of TSH values (range 2.5-4.5mcU / ml) with respect to baseline levels.

Conclusions: Our study confirms the role of metformin on reducing TSH levels in DM1 patients becoming insulin resistant with subclinical hypothyroidism.

PP059 - ERECTILE DYSFUNCTION AND CARDIAC AUTONOMIC NEUROPATHY: A STUDY OF PREVALENCE IN PATIENTS WITH DIABETES

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Background and aim

Cardiac autonomic neuropathy (CAN) is a complication of diabetes mellitus and can lead to severe morbidity and mortality. The prevalence of CAN is 1-90% in patients with type 1 diabetes (T1D) and 20-73% in patients with type 2 diabetes (T2D). CAN is also one of the cofactors related to erectile dysfunction (ED) in diabetes and few data are available in the Italian population about this association. The aim of this study was evaluate the prevalence of CAN in patients with diabetes affected by ED.

Materials and methods

We studied 26 patients with T1D (age: 42.33 ± 13.28 years) and 23 patients with T2D (age: 62.58 ± 6.8 years) affected by ED. We evaluated CAN using Neurotester Meteda® elaborating three simple noninvasive tests on the heart rate (HR) response to deep breathing, to standing, and to the Valsalva manoeuvre.

Results

Among 26 T1D patients (HbA1c: 7.8% ± 0.96; disease duration: 23.7 ±12.1 years; BMI: 27.4 ± 3.1) with ED, 6 subjects (23.1%) presented CAN, 5 of which at initial stage (early CAN) and one at advanced stage (definite CAN).

In the group of 23 T2D patients (HbA1c: 7.5 ± 1.1%; disease duration: 11.5 ± 7.1 years; BMI: 31.8 ± 4.8) with ED, 14 subjects (60.8%) showed CAN: 10 subjects with "early CAN" and 4 with "definite CAN". A multiple regression analysis was carried out to predict CAN from age, HbA1c and disease duration. Only age statistically significantly predicted CAN (beta=0,674, $p < 0.0001$).

Conclusions

These data underline even more the association between ED and CAN in a population affected by T1D and T2D diabetes. Larger and prospective studies are needed to evaluate the link between these two diabetes complications.

PP060 - LOCALIZED FACIAL HIRSUTISM: AN UNUSUAL CASE OF BECKER NEVUS

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Hirsutism is a frequent endocrinological complaint. Polycystic ovary syndrome (PCOS), idiopathic hirsutism (HI), congenital adrenal hyperplasia (CAH) and chronic hypercortisolism represent the most common causes. We report the case of a 18-year-old female patient, who presented an excessive hair growth localized on the right side of her face, particularly in the right submandibular region, developed during peripubertal period. General physical examination was unremarkable. Laboratory tests, including serum LH, FSH, prolactin, 17-OH-progesterone, free-testosterone, androstenedione, DHEAS and SHBG levels, as well as pelvic ultrasonography, were within normal limits. Based on the clinical findings, a diagnosis of Becker nevus was suspected, despite the absence of hyperpigmentation and the unusual face localization. Skin biopsy confirmed the diagnosis of "amelanotic Becker nevus".

Becker nevus is an acquired disorder that usually manifests in late childhood or adolescence and is characterized by a patchy hyperpigmentation with irregular outline and hypertrichosis. It is more common in men and the lesions are most frequently localized on the upper extremities and trunk. Female sex, face involvement and absence of hyperpigmentation are unusual features and made diagnosis challenging.

PP061 - SEVERE INSULIN RESISTANCE IN TWO HYPERANDROGENIC YOUNG GIRLS

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Background - Insulin resistance (IR) is defined as the reduced responsiveness of the body to the glucose-lowering activity of insulin and even in lean individuals very severe degrees of IR can be observed. Many such patients harbor pathogenic single gene mutations, affecting insulin signaling or adipocyte development and/or function. We report two different cases of severe IR, referred to our Clinic.

Cases - 1) 17-year-old girl had severe hirsutism (first noticed at 14 ys and subsequently worsened) and primary amenorrhoea. The resection of a large serous cystadenoma (with Sertoli-Leydig-like cells) in left ovary was performed a year before. The examination showed: BMI 22 kg/mq, absence of acanthosis nigricans (AN), modified Ferriman-Gallwey score (mFGs) 40, pelvic ultrasonography (US) with enlarged ovaries and multiple peripheral cysts (right ovary diameter 50 mm); testosterone (T) 2.5 ng/ml (nv <0.57), androstenedione (A) 7 ng/ml (nv <3.8) (pre-operative serum androgen levels were unknown), LH 6.2 mUI/ml, fasting insulin (FI) 150 mcU/ml (nv <10) with glucose (FG) 73 mg/dl, normal lipid profile and no biochemical or radiological evidence of fatty liver.

2) 20 year-old girl had hirsutism, acne and oligomenorrhoea since the puberal age. The examination showed: BMI 29 kg/mq, AN in the axillae and nape of the neck, mFGs 29, polycystic ovary at pelvic US, T 1.95 ng/ml, A 10.3 ng/ml, normal serum androgens and 24-hour urinary free cortisol, FI 115 mcU/ml with FG 66 mg/dl, hepatic steatosis, with normal hepatic function and lipid profile.

In both cases, the oral glucose load test showed a normal glucose tolerance with an insulin peak of 1655 mcU/ml and 949 mcU/ml, respectively. Low glycemic index diet and metformin until 2.5 gr/die were started in both patients. In case 2 this therapy resulted in normalization of menstrual cycles and reduction of androgens and insulin levels. Case 1 had menarche some months later but subsequent biochemical evaluation is not available.

Conclusion - Hyperandrogenism with menstrual irregolarity can be the early clinical signs of congenital IR, particularly severe in the second decade, when pubertal IR interacts with the underlying genetic defect, with AN and hyperglycaemia often only being noticed on subsequent evaluation. Virilising tumours have been described in the context of congenital severe IR. The management of severe IR is not easy. Life style change (diet, physical activity), insulin sensitization (metformin and thiazolidinediones) and acarbose, are the mainstay of early therapy, sometimes only partially effective. Management of these patients remains largely empirical. New molecular studies and genetic analyses might aid to improve knowledge of this

syndrome.

PP062 - FAILURE OF SELECTIVE OVARIAN VENOUS SAMPLING IN DETECTING THE ORIGIN OF SEVERE HYPERANDROGENISM: A CASE REPORT.

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BACKGROUND – Detecting androgen producing tumors can be a challenge, because they are often very small. Abdomen ultrasonography (US), CT or MRI scans are generally performed for diagnostic assessment. When these traditional radiological exams are negative, it has been suggested utilizing F18-FDG-PET, also with preoperative provocative hormonal testing (hCG and leuprolide), to identify an androgen producing ovarian tumor. It has also been suggested selective venous sampling.

CASE - A 43-year-old woman was referred to our Clinic for virilization. Since one year she experienced amenorrhea, diffuse hirsutism, temporal balding and deepening of the voice. Previously regular menses and fertility were reported. BMI was 29,8 kg/mq, modified Ferriman-Gallwey score was 35 and clitoromegaly was found. Biochemical tests revealed: total testosterone (T) 9 ng/ml (nv <0.57), FSH 1,18 IU/L, LH 0,32 IU/L, Estradiol 47 pg/ml; other androgens and 24-hour urinary free cortisol were normal; DEX-suppression test (0.5 mg qid for 7 days) excluded a functional form of hyperandrogenism. Pelvic US and abdomen CT scans showed only a functional cyst in the right ovary (diameter 4 cm). Since the conventional radiological exams were negative, in order to detect the origin of the severe hyperandrogenism, a selective venous sampling was performed: T levels at superior vena cava and bilateral renal veins sampling were similar to the periphery; instead a gradient greater than 1,9:1 was found between inferior vena cava/right iliac vein (15,8 ng/ml) and left ovarian/iliac veins (8,3 ng/ml), suggesting the right ovary as source of the hyperandrogenism. Therefore, right ovariectomy was performed, but a simple cyst in normal ovarian tissue was histologically confirmed. Moreover, high T levels (9-10 ng/ml) was found a week and a month later.

CONCLUSION - In our case, the selective venous sampling failed in detecting the site of androgen production. Levens et al. reviewed 132 cases: this procedure localized only 66% of androgen-producing tumors. Left sided lesions may occasionally be misdiagnosed, maybe because of differences in catheterization technique and placement; additionally, it has been reported that the left ovarian vein may have reflux and drain in some women into the right ovarian vein. In conclusion, even an advanced and invasive procedure may incorrectly affect the surgical approach. In this case, it could be advisable to remove the controlateral ovary, to correcting the severe syntomatic hyperandrogenism.

PP063 - GONADOTROPIN-RELEASING HORMONE AGONISTS IN POSTMENOPAUSAL OVARIAN HYPERANDROGENISM

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Background - The most frequent cause of virilization in postmenopausal women is an excessive ovarian androgen production, generally by benign tumors or hyperthecosis. This one predominantly occurred in premenopausal women, but symptomatic hyperthecosis has been reported in postmenopausal women, in whom active steroidogenesis takes place in ovarian stromal cells. In these cases, bilateral oophorectomy is usually performed. GnRH-agonists are generally administered for a diagnostic purpose. Few cases of long-term GnRH-agonists for hyperthecosis have been reported.

Cases - We present three women in post-menopause, without any history of infertility or PCOS, who were referred to our Clinic for severe hirsutism and androgenetic alopecia, caused by a functional ovarian hyperandrogenism. Modified Ferriman-Gallwey score was more of 20 (normal <10). In all patients abdomen ultrasonography and CT scans were negative. The patients showed metabolic syndrome with hypertension and dyslipidemia and patient 3 also diabetes mellitus type 2. The altered hormonal profiles are the following: case 1 (61-yr-old woman): total testosterone (T) 3.7 ng/ml (<1), androstenedione (A) 10.8 ng/ml (<2.67), LH 41.8 mU/ml; case 2 (71-yr-old woman): T 3.2 ng/ml (<1), LH 23.6 mU/ml; case 3 (62-yr-old woman): T 2.6 ng/ml (<0.57), LH 30 mU/ml.

After the basal hormonal evaluation, GnRH-agonist (leuprorelin 3.75 mg depot 1 fl. i.m.) was administered in all patients. LH decrease (8.6, 8,0 and 3,0 mU/ml, respectively) and T normalization (0.6, 0,8 and 0,2 ng/ml respectively) were detected in all patients already within 2 weeks, confirming the ovarian source and the functional form of the hyperandrogenism. Moreover, GnRH-agonist soppressive effect remained up to one year, suggesting the effectiveness of this treatment.

All patients received careful follow-up, including periodic testing of androgen levels and ovarian imaging, to exclude progression or undiagnosed ovarian malignancy.

Conclusion – GnRH agonist is an accetable choice for the treatment of post-menopausal hyperandrogenism in patients in whom the functional ovarian androgen excess is ascertained, also because of its possible long-term effect as previously described. This medical choice is especially indicated in patients with high surgical risk due to comorbidies or who are unwilling to undergo bilateral oophorectomy.

PP064 - MAZZANTI SYNDROME: CASE REPORT

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A female child of 7.6 years was referred to our Clinic because of delayed statural growth, since the age of six months. She was born at term (3.60 kg) from not consanguineous parents. The physical examination revealed: height 108.5 cm (<3° in Tanner scale, 2 SDS below the standards, predicted final stature 150 cm, 2 SDS below the genetic target of 168 cm), statural age 5.2 years, weight 17.7 kg, delayed bone age by 3 years; absence of secondary sexual characteristics; pterigium colli, left blepharoptosis, arched palate, valgus elbow, pectus excavatum, angioma in the left hand, darkly pigmented skin with eczema, hypernasal voice; easily pluckable, sparse, thin, slow growing hair, features reminescent of Noonan Syndrome (NS). Routinary biochemical and hormonal exams performed to investigate the cause underlying short stature were normal. Evaluation of GH-IGF1 axis revealed low serum IGF1 levels (59.4 ng/ml, nv 76-499) and normal GH peak after arginine stimulation (14.9 ng/ml), thus excluding a GH deficiency (GHD) and suggesting a mild GH insensitivity (GHI). Caryotype was normal (46,XX). Moreover, the patient has been screened for mutations of the SHOX gene, Array-CGH and NS gene (PTPN11), without revealing any causative mutation. In the following two years, because of the persistence of delayed growth, other genetic analyses were performed and showed a rare invariant c.4A>G missense change in SHOC2 (encoding a scaffold protein that positively modulates RAS-MAPK signal flow), with consequential diagnosis of Noonan-Like-Syndrome with loose anagen hair (NS-LAH OMIM60772). This syndrome was first described in 2003 and it was also known as Mazzanti Syndrome. In our patient, after genetic diagnosis, GH-therapy was started, resulting in improving of statural growth within 6 months.

It is well known that the RAS-MAPK transduction pathway plays a key role in GH signaling. However, the exact mechanism of impaired GH-IGF1-axis in patients with NS-LAH is not yet clear. An altered response of GH to stimulation tests has been reported only in some children, whereas other authors have reported normal GH secretion with low IGF1 levels suggesting a peripheral GHI. Since only a few cases of NS/LAH associated to SHOC2 mutations have been so far described, the complex phenotype of the syndrome and the exact mechanism impairing GH/IGFI axis still remain to be elucidated as the GH therapy effectiveness and studies on larger cohort of subjects are needed to better delineate this syndrome.

PP065 - THE INFLUENCE OF BREAST CANCER TREATMENT ON HORMONAL STATUS AND SEXUAL FUNCTION

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Background: Breast cancer patients are at increased risk of sexual dysfunction. Despite this, both patients and practitioners are reluctant to initiate a conversation about sexuality. We evaluated levels of total testosterone, estradiol, and gonadotropins and sexual dysfunction in 83 women in treatment for breast cancer.

Methods: Between March 2013 and May 2014, 108 patients entered the study and 83 completed questionnaires. The analysis included 9 women with advanced breast cancer treated with neoadjuvant therapy (NAT), 39 treated with adjuvant therapy (AT), 22 taking hormonal therapy (HT) and 13 with metastatic cancer on first line chemioterapic treatment (IIT). Sexual functioning (FS) was evaluated with the female sexual function index (FSFI) while sexual distress was assessed with the female sexual distress scale (FSDS-R). Demographic data, laboratory values, and LH, FSH, total testosterone (T) and estradiol (E2) were obtained. The patients with FSFI score <23.55 and FSDS-R < 15 were accepted as experiencing sexual dysfunction and sexual distress respectively.

Results: In breast cancer patients the serum level of estradiol (28.1±7 pg/ml) was lower than normal values, total testosterone (13.7±1 ng/dl) was at the lower level of the normal values and both LH and FSH were significantly elevated. We found that the total FSFI score was 17.1 and FSDS-R was 8.2, both significantly lower than normal values with a prevalence of female sexual dysfunction (FSD) of 69%. The prevalence of FSD was 78 % in NAT, 77% in IIT, 68% in HT and 65% in AT patients. A significant difference was found between NAT and HT in lubrication, pain domains and total FSFI score ($p<0.02$, $p<0.01$, $p<0.04$ respectively), between AT and HT in pain domain ($p<0.03$) and between AT and NAT in lubrication domain ($p<0.04$). Total FSFI score was positively correlated with T levels ($p<0.005$) in 83 breast cancer patients, while no significant correlation was found between estradiol and FSFI.

Conclusions: Low levels of sex steroids reflected the medication-induced postmenopausal status independent of the type of treatment, nevertheless, when FSFI scores are separately displayed for women with and without a history of chemotherapy, the scores of patients without chemotherapy reflect a better preservation of sexual function despite antihormonal treatment. Breast cancer women are also at high risk to developing FSD both for hormonal status and psychological implication of this condition. The significative correlation between T levels and FSFI opens new therapeutics strategies in this patients.

PP066 - GROWTH HORMONE EXCESS PROMOTES HUMAN ENDOMETRIAL CANCER CHEMORESISTANCE

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The growth hormone (GH)/insulin-like growth factor I (IGFI) axis may influence neoplastic development of endometrial epithelium. Human GH is produced by endometrial epithelial cells of normal and neoplastic tissues. Moreover, patients with endometrial adenocarcinoma (EA) display GH levels in the upper limit of the normal range. GH is indeed produced by EA, where it stimulates growth, suggesting that GH may play an important role in promoting EA aggressiveness. Chemoresistance often develops in advanced metastatic EA, the most frequent malignancy of the female genital tract, the fifth leading cause of cancer-related death in women. We previously demonstrated that GH protects breast cancer cells towards the cytotoxic effects of doxorubicin. Therefore, we investigated whether this holds true also in HEC-1A endometrial adenocarcinoma cell line.

Our results show that GH significantly reduces doxorubicin antiproliferative and proapoptotic effects. In addition, GH reduced protein kinase C delta (PRKCD) expression, a protein involved in chemotherapic-dependent apoptosis. Furthermore, we explored whether the antiproliferative effects of GH are mediated by extracellular signal-regulated kinases (ERK1/2) activation, which contributes to drug resistance development. First, we verified that doxorubicin reduces the expression of phosphorylated ERK1/2. Later, we demonstrated that an ERK1/2 inhibitor significantly reduces cell viability and blocks GH-induced cell viability.

These data altogether indicate that GH promotes resistance to apoptosis induced by chemotherapeutic drugs likely via ERK1/2 phosphorylation and PRKCD expression reduction. These findings support the hypothesis that blocking GH receptor may be viewed as a potential new therapeutic approach to overcome chemoresistance in endometrial cancer.

PP067 - SALIVARY CORTISOL RESPONSE TO PSYCHOLOGICAL STRESS IN LATE ADOLESCENT AND YOUNG WOMEN: IMPACT OF MENSTRUAL IRREGULARITY, HIRSUTISM AND HYPERANDROGENEMIA.

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Hyperandrogenic disorders cause psychological implication in young girls, limiting the quality of life. Salivary cortisol (SalF) testing was proved to be useful in the evaluations of acute stress responses. Aim of this study was to investigate SalF responses to a stressor event in late adolescent females.

We selected 165 drug-free females aged 16–19y from a cross-sectional epidemiological study. Saliva was collected in the morning before and after a stressor event consisting in a physical examination by a trained physician for anthropometric data collection and hirsutism scoring and in a structured interview about familiar and menstrual history. Blood was collected for biochemical and hormonal evaluation. SalF and serum total testosterone (TT) were assessed by liquid chromatography-mass spectrometry. Subjects were subdivided in: menstrual irregularities group (MI, ≤10 bleeding/year; n=27), isolated hirsutism group (IH, modified Ferriman-Gallwey score≥8; n=37), isolated hyperandrogenemia group (IHA, TT>age/menstrual phase-specific cut-off; n=11), and normal controls (NC; n=90).

Glucose, insulin and lipid profile were normal and non different among groups. Compared to NC (21.2±0.3kg/m2), IH (22.4±0.52kg/m2, p=0.0169) and IHA (23.5±0.77kg/m2, p=0.004) displayed higher body mass index (BMI); IHA also displayed higher waist circumference (75.4±0.71 *vs* 81.0±2.3cm, respectively, p=0.015). Compared to NC, IH had lower SHBG (48.11±1.75 vs 42.7±2.96 nmol/L, respectively, p=0.039). Basal SalF was not different among groups (p=0.977); a significant SalF increase after the stressor event was observed only in ICH (1.21±0.15 vs 1.67±0.23ng/ml, p=0.029). SalF relative increase (dSalF%) was significantly different among groups (p=0.015); in particular, dSalF% was significantly higher in ICH compared to NC (56.1±18.2 *vs* 3.4±5.1%, respectively, p=0.010), and this data was confirmed after adjustment for BMI, SHBG and waist circumferences (p=0.0051).

We conclude that hirsutism, major feature of clinical hyperandrogenism but not mestrual irregularities nor hyperandrogenemia, plays a major role in the responsiveness to stress as measured by SalF in young girls.

PP068 - OVARIAN LEYDIG-SERTOLI CELLS TUMOR IN AN ADOLESCENT WITH VIRILIZATION

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Introduction: Sertoli-Leydig cell tumors (SLCTs) are rare neoplasms, accounting for 1% of all sex cord-stromal tumors and for 0.1%–0.5% of all primary ovarian neoplasms.

Case report: A 15-years-old female was referred to our outpatient clinic for rapidly progressive clinical manifestations (which have started 3 months before) of androgen-excess such as hirsutism, alopecia, deepening of the voice, secondary amenorrhea and clitoromegaly. Laboratory tests showed increased serum levels of total testosterone (11,96 nmol/L; normal range: 0.4-2.60 nmol/L) and of delta-4-androstenedione (6,78 ng/mL; normal range: 0.5–3.3 ng/mL). Abdominal ultrasound examination revealed a semisolid mass suggestive of ovarian tumor. Magnetic resonance imaging confirmed a solid mass of 66x52x60mm in the left ovary.

The patient underwent laparoscopic left salpingo-oophorectomy plus standard staging surgery (omentectomy, appendectomy, and pelvic lymphadenectomy). Histopathological and immunocytochemical analyses revealed a moderately-differentiated Sertoli-Leydig cell tumor of the right ovary (FIGO IA).

Surgical resection of the tumor led to normalization of androgen levels and gradual disappearance of all related clinical manifestations. Treatment with adjuvant chemotherapy or radiation was not performed, and the patient underwent follow-up visits every four months.

Twelve months postoperatively the patient was in good health, alopecia and hirsutism had substantially improved, and serum total testosterone levels (0,5 nmol/L) and delta-4-androstenedione (0,9 ng/mL) remained within the normal range. MRI did not show recurrences.

Conclusion: SLCTs typically occur in the second and third decades of life.

55% percent of the patients presents with clinical evidence of a hormonally active tumor, and 38% show frank virilisation. The prognosis depends on tumor grading and staging; moderately and poorly differentiated SLCTs are associated with 11% and 59% malignant potential respectively in terms of recurrence rate and 5 year survival of 50% in the poorly-differentiated forms.

Due to the severe prognosis, and the young age in which SLCTs occur, it is of paramount importance to consider SLTCTs in the differential diagnosis of hyperandrogenemia occurring in women in the reproductive age, also if adolescents.

PP069 - PCOS AND MUSCLE STRENGTH: PRELIMINARY RESULTS IN NORMAL WEIGHT WOMEN

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Polycystic ovary syndrome (PCOS) is a common and heterogeneous endocrine disorder, often associated with body fat excess and insulin resistance, factors that may limit physical performance. However, androgen excess could be an advantage in these women, in terms of increased muscle strength. Till now, only a few studies in overweight/obese women have addressed the possibility that PCOS may be associated with changes in muscle strength, with controversial results. The aim of this study was to assess muscle strength in a sample of normal weight PCOS women, compared with BMI-matched sedentary controls, to avoid the confounder effect of excess body fat. In this preliminary report, 8 PCOS women and 10 healthy controls (mean age±SD: 22.2±1.1 vs 27.4±1.5 years; BMI 21.0±0.7 vs 20.7±0.6 kg/m2) were studied. In all subjects isokinetic quadriceps strength was assessed by isokinetic dynamometry at two different rates of execution (30°/second and 120°/second) in concentric and eccentric phase, whereas muscle architectural characteristics (thickness, fascicle length and pennation angle) were analyzed by ultrasound scan of the vastus lateralis muscle. Anthropometric and metabolic features and serum total and free testosterone levels (as measured by LC-MS/MS and equilibrium dialysis) were also assessed. As expected, testosterone levels were higher in PCOS women than in controls, while no significant differences in body composition and metabolic features were observed. The PCOS group showed greater isokinetic muscle strength in concentric phase at slow rate of execution (30°/s) (difference between groups 17%, p=0.04), whereas borderline differences were observed at higher rates of execution. No differences in muscle architectural characteristics were found. In conclusion, this preliminary study suggests that women with PCOS may have increased muscle strength. We hypothesize that this phenomenon may be related to the effects of the increased androgens levels on muscle fibers expression. However, it is necessary to increase the sample size and to perform muscle biopsy to confirm this hypothesis.

PP070 - PHOSPHODIESTERASE TYPE 5 TRANSCRIPTIONAL REGULATION IN HUMAN MYOMETRIAL CELLS

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~~Phosphodiesterase type 5 (PDE5) is a cGMP-specific hydrolytic enzyme that has been the subject of many clinical studies because it was shown to be the target of sildenafil, the active ingredient of the widely prescribed impotence drug Viagra. PDE5 is expressed in several tissues, especially in smooth muscle tissues. Its activity in smooth muscle cells (SMCs) is regulated by Nitric Oxide (NO) that is produced by endothelial cells under the control of nitric oxide synthase (NOS)1. Although PDE5 enzymatic activity has been extensively studied, little is known on how PDE5 gene is transcriptionally regulated. A putative androgen responsive element (ARE)2 as well as cAMP and cGMP responsive elements have been identified in the PDE5 promoter, which are upregulated by increasing concentrations of either cAMP or cGMP3. To investigate if cGMP, cAMP, NO and Testosterone can modulate PDE5 at the transcriptional level in smooth muscle cells we adopted as experimental model myometrial primary cultures. We independently treated confluent uterine cell cultures stimulated with 1mM 8BrcGMP, 1mM 8BrcAMP, 100M GSNO or 100nM di-hydrotestosterone (DHT) for 24h and then analyzed PDE5 mRNA and protein levels. We found that each of these factors upregulated PDE5 mRNA and protein levels. We cloned the PDE5 genomic region containing the two putative promoters in a luciferase reporter vector, which was then transfected in myometrial cells. Transfected cells were then stimulated with 8BrcGMP, 8BrcAMP, GSNO or DHT and analyzed for luciferase expression. None of the factors induced an increase of luciferase expression, as revealed by luciferase assay, suggesting that their respective responsive elements are not present in the genomic region spanning from -10000 to +200 of PDE5 locus. We then focused on Notch3, a single-pass transmembrane receptor that is a positive regulator of smooth muscle differentiation4 and NO/sGC signaling. We performed overexpression experiments in myometrial cells using a retroviral vector for Notch3 intracellular domains (N3ICD) along with EGFP as reporter of infection. Pde5 protein levels were strongly up-regulated in Notch3 transduced cells and a faster migrating band immunoreactive to the anti-PDE5 antibody was also induced. PDE5 up-regulation was not was mediated at the transcriptional level as resulted from semiquantitative RT-PCR analysis and luciferase assays, suggesting that Notch3 post-transcriptionally regulates PDE5 protein levels. 1 Omori and Kotera. Circulation Research 2006. 2 Lin CS et al. Int.Journal Impotence Research 2013. 3 C-S Lin et al. Human PDE5A gene encodes three isoforms from two alternate promoters Int.Journal Impotence Research 2002. 4 Guruharsha et al. Nature 2012.

PP071 - LOW LEVELS OF VITAMIN D ARE ASSOCIATED WITH CHRONIC INFLAMMATION MARKERS IN WOMEN WITH POLYCYSTIC OVARY SYNDROME (PCOS)

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Background and Aim: Epidemiological studies have demonstrated the inverse association between hypovitaminosis D and inflammatory disease. The aim of this study was to investigate the association between vitamin D levels and inflammation markers (C-reactive protein (PCR), leucocyte count and fibrinogen) in women with PCOS.

Materials and Methods: We assessed 25-hydroxyvitamin D (25(OH)D), PCR and fibrinogen levels in 50 women with PCOS (age 26.8±8.4 years; BMI: 27.5±8.3 kg/m^2).

Results: The prevalence of vitamin D deficiency (< 20 ng/ml) was 64% in women with PCOS. PCR (0.5±0.2 vs 0.3±0.1 mg/dl; p=0.03) and fibrinogen (254.8± 60.5 vs 217.8±77.8 mg %; p=0.03) levels were significantly higher in women with PCOS and vitamin D deficiency compared to women with PCOS without vitamin D deficiency. No significant difference were found in leucocyte count between the 2 groups (6.0±1.9 x 10^3/mcl vs 6.4±2.4 10^3/mcl; p=0.7). Multivariate analysis (p<0.01) was performed using PCR, leucocyte count and fibrinogen as independent variables and 25(OH) D as dependent variable. Fibrinogen levels was found to be the most powerful predictor of 25(OH)D concentration (p< 0.03).

Conclusions: Low levels of vitamin D are associated with chronic inflammation markers and in particular with fibrinogen levels in women with PCOS. However, supplementation studies are needed to establish whether vitamin D treatment may be able to reduce the degree of chronic inflammation, and thus the related cardiovascular risk, in women with PCOS.

PP072 - LOW LEVELS OF VITAMIN D ARE ASSOCIATED WITH THE INCREASED RISK OF CHRONIC AUTOIMMUNE THYROIDITIS IN WOMEN WITH POLYCYSTIC OVARY SYNDROME

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Background and aim: Several in vivo and in vitro studies demonstrated the association between low levels of vitamin D and autoimmune diseases such as thyroid, rheumatologic and neurological disease. The aim of our study was to evaluate the association between low levels of vitamin D and chronic autoimmune thyroiditis (TCA) in women with Polycystic Ovary Syndrome (PCOS).

Materials and Methods: Serum 25-hydroxy-vitamin D (25 (OH) D, FT3, FT4, TSH, anti-thyroglobulin and antithyroperoxidase Antibodies have been assessed in 50 women with PCOS (age: 26.8 ± 8.4 years; BMI: 27.5 ± 8.3 kg/m2).

Results: 25 (OH) D levels were significantly lower in women with PCOS with TCA (19.9 ± 8.0 ng/ml) than women with PCOS without TCA (32.0 ± 22.6 ng/ml) (p = 0.03) although vitamin D levels did not correlate with serum anti-thyroglobulin (p = 0.07) and anti-thyroperoxidase antibodies (p = 0.08). TSH levels were significantly higher in women with PCOS with TCA (2.7 ± 1.1 mcUI/ml) compared to women with PCOS without TCA (1.9 ± 0.8 mcUI/ml) (p = 0.02) while FT3 (3.3 ± 0.3 vs. 3.4 ± 0.4 pg/ml; p = 0.09) and FT4 (1.1 ± 0.1 vs. 1.2 ± 0.1 ng/dl; p = 0.3) levels were similar between the 2 groups.

Conclusions: 25(OH)D levels were significantly lower in women with PCOS with TCA. These data allow to hypothesize that vitamin D may play a role in the immunomodulation of thyroid function and that vitamin D supplementation may have a beneficial effect in thyroid autoimmune disease.

PP073 - VITAMIN D IS INVOLVED IN REGULATORY MECHANISMS OF PLASMA AMH LEVELS IN YOUNG SUBJECTS AFFECTED BY POLYCYSTIC OVARY SYNDROME.

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Role of Vitamin D (VitD) in ovarian physiology and its implication in reproduction is supported. VitD deficiency is related to a poor ovarian response in women following In Vitro Fertilization(IVF) and VitD supplementation (VitDsup) seems to improve IFV outcomes. Recent studies suggested that VitD and AMH are involved in follicular development, ovarian hormonal secretion and reserve. Because, in PCOS subjects, lower VitD and higher AMH levels can be linked in the ovulatory dysfunction, **aim of the Study** was to evaluate the role of VitDsup on AMH levels in a group of PCOS patients.**Material and Methods:** Eighteen subjects (aged 18-32 yrs) affected by PCOS were studied before and 1 month after oral VitDsup (3x 10^6 UI/ml). LH,FSH, PRL, ovarian steroids, AMH and 25-OHVitD3 were measured in all subjects in winter season and at the same menstrual cycle period (3-7 day). We further subdivided our PCOS patients in: **-lean**(BMI<24.9; n:12) and **-obese**(BMI>30;n.6). A group of subjects affected by hypothalamic amenorrhea (**HA**;n.8), premature ovary failure (**POF;**n.4) and healthy subjects (Con;n 8) were also evaluated.All give their informed consent.**Results:**Main hormonal data were summarised:

	VitDpre	**VitD**post	**AMH**pre	**AMH**post	**PTH**pre	**PTH**post
PCOS tot	31.6±10^{a,d}	53.3±14	11.8±4^{b,d}	7.1±3	37.1±8^{c,d}	28.6±4
Lean	42.4±10^{a}	59.9±12	14.2±3^{b,d}	9.5±1	33.0±3^{c,d}	27.0±3
Obese	18.0+4^{a,d}	40.2±6	7.9±.6^{d}	5.3±1	45.0±9^{c,d}	31.8±3
CONTR	49.9±3^{a}	69.8±16	5.0±1	3.7±.8	26.0+2	23.0±3
HA	36.7±9^{a}	65.6±10	14.1±5^{b,d}	9.8±3	36.4±9^{c,d}	27.3±5
POF	37.2±12^{a}	64.4±16	<0.1^{d}	<0.1	31.2±3^{c,d}	26.0±2
P : a <0.005 vs Post; b <0.01 vs Post; c :<0.02 vs Post; d <0.002 vs Contr						

Conclusions: In our PCOS patients,VitD and AMH levels were respectively lower and higher than Controls. All subjects showed VitD deficiency, that improved after VitDsup. VitDsup decreased AMH levels in all subjects, but reaching a significant effects only in PCOS and HA patients.Finally,the evidence that in obese PCOS patients VitD seems to induce a lower effect on AMH levels,suggests an involvement of more complex mechanisms

PP074 - INVOLVEMENT OF MTOR PATHWAY IN REGULATING RAT GH SECRETING PITUITARY ADENOMA CELL LINES

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Background: Acromegaly is mainly due to a G-secreting pituitary adenoma. Surgery is the first therapeutic option, but medical therapy is frequently employed, being represented mostly by somatostatin analogues (SSA). However ~10% of patients is resistant to SSA. PI3K/Akt/mTOR pathway, activated by IGF-1, is important in regulating many cellular processes and it is deregulated in neoplasms.

Aim: to understand how PI3K/Akt/mTOR pathway can influence viability and GH secretion in pituitary adenomas, we tested two mTOR inhibitors (Everolimus and NVP-BEZ235) in two rat GH-secreting pituitary adenoma cell lines (GH3 and GH4C1)

Material and methods: we assessed cell viability by ATPlite, GH secretion by ELISA, GH expression by RT-PCR and Akt phosphorylation by AlphaScreen assay.

Results: Everolimus and NVP-BEZ235 caused a significant reduction in cell viability up to 60% in the two cell lines. On the other hand, IGF-1 induced cell viability by 40% in GH3 cells and by 30% in GH4C1 cells. This effect was counteracted in both cell lines by Everolimus and NVP-BEZ235, indicating that these compounds could act, at least in part, on IGF-1 activated pathways. GH secretion was reduced by IGF-1 in GH3 cells; this effect was not enhanced by Everolimus, but was blockaded by NVP-BEZ235 in GH3 cells. IGF-1 reduced GH expression in GH3 cells, suggesting that IGF-1 acts at transcriptional level. On the contrary, Akt phosphorylation was not affected by treatment with IGF-1 or Everolimus, while NVP-BEZ235 reduced the Akt phophorylation.

Conclusions: our results suggest that Everolimus and NVP-BEZ235 interfere with IGF-1 signaling in rat GH-secreting cell lines, that could be used as a model to identify alternative pharmacological targets for GH-secreting pituitary adenomas resistant to SSA therapy.

PP075 - PI3K/AKT/MTOR SIGNALING PATHWAY MAY LEADS TO DIFFERENT RESPONSES TO EVEROLIMUS IN PNETS

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Introduction: Neuroendocrine tumors (NETs) are rare neoplasms arising from neuroendocrine cells of the respiratory and gastro-entero-pancreatic systems. The mammalian target of rapamycin (mTOR) pathway regulates cell growth, metabolism, and apoptosis representing a novel molecular target. This pathway is constitutively activated in pancreatic NETs (pNETs) promoting the development of specific mTOR inhibitors as new therapeutic tools.

Aim: To evaluate the cell viability and apoptosis activation in response to Everolimus, a m-TOR inhibitor, in pNETs and to identify new putative markers that may be involved in the response to Everolimus.

Methods: 20 pNET primary cultures were treated with IGF1 and/or Everolimus. Cell viability and caspase activity were evaluated. Alpha Screen Assays for IGF1R, AKT, mTOR and 4EBP1 phosphorylation were performed.

Results: in 6 pNETs Everolimus caused a reduction in cell viability and an increase in apoptotic rate up to 30% (Responders). In addition, the proliferative and antiapoptotic effects mediated by IGF-1 were blocked by Everolimus. 14 pNETs were resistant to Everolimus and IGF-1 treatments (Non Responders). Furthermore, the expression of phosphorylated IGF1R, AKT, mTOR and 4EBP1 was significantly ($p<0,001$) higher in the Responder group than in Non Responder group.

Conclusion: Our data suggest that PI3K/AKT/mTOR protein pattern may predict the response to Everolimus in pNETs.

PP076 - CXCR4/CXCL12/CXCR7 AXIS IS FUNCTIONAL IN NEUROENDOCRINE TUMORS AND SIGNALS ON THE MTOR PATHWAY

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Background Neuroendocrine tumors (NETs) are rare neoplasms with variable biological behavior. mTOR inhibitor, Everolimus (Eve), was approved in the subset of progressive pancreatic NETs (pNETs). The chemokine receptor CXCR4 interacts with CXCL12 to exert proliferative effects. CXCR4 activates mTOR through phosphorylation of its two effectors: 4EBP1and S6K. **Aim** To evaluate the role of the axis CXCR4-CXCL12-CXCR7 and its cross-talk with mTor pathway in NET. **Methods** 61 human NET were included into the study:40(GEP), 21(MTC). The mRNA was extracted from fresh/paraffin tissue and CXCR4, CXCL12 and CXCR7 expression was determined by qRT-PCR. CXCR4 axis and mTOR pathway was evaluated by immunohistochemistry (IHC).The expression of CXCR4/CXCR7 was also evaluated in NET cell lines (NH727 and BON) by qRT-PCR. The CXCR4-dependent mTOR induction in human NET cell line, BON, was evaluated by Western-blotting and the effects of Eve and AMD3100 (CXCR4-antagonist) on NET cellular growth, were evaluate by MTT assay. **Results** CXCR4-CXCL12-CXCR7 mRNA was significantly overexpressed in tumour as compared to normal tissue. The IHC score of CXCR4, mTOR, p-mTOR, p-4EBP1, p-S6K1 were significantly higher in G1/G2 tumors. CXCR4,CXCR7,mTOR,p-mTOR and p-4EBP1 significantly correlated with poor prognosis, while CXCL12 with favorable prognosis. CXCR4/CXCR7 expression levels in NET cells are comparable to positive controls. In BON cells, CXCL12 increased pP70S6K and p4EBP1, instead AMD3100 inhibited this induction. Eve blocked the mTOR targets. Moreover, CXCL12 induced the phosphorylation of ERK1/2 and P38 while AMD3100 inhibited CXCL12-induced pERK1/2 and p38. Eve modestly impaired CXCL12-mediated pERK1/2, p38 and pAkt induction. In NH727 and BON cells, the AMD3100 and Eve showed inhibitory effects on proliferation: the combined treatment reduced cell growth and the anti-proliferative effect was improved respect to single treatment. **Conclusions** CXCR4/CXCL12/CXCR7 and mTOR pathways are expressed in NETs, might represent a prognostic factor in these tumors and their concomitant inhibition might represent a new effective therapeutic approach.

PP077 - NEUROENDOCRINE ASPECTS OF CUTANEOUS MELANOMA: CHARACTERIZATION AND SUBCELLULAR LOCALIZATION OF SOMATOSTATIN RECEPTORS AND IN VITRO EFFECT OF PASIREOTIDE ON CELL VIABILITY, PROLIFERATION AND CELL CYCLE IN HUMAN CUTANEOUS MELANOMA CELL LINES

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Cutaneous malignant melanoma (CMM) is a highly aggressive skin cancer, whose incidence is increasing in the last years. Melanocytes originate from the neural crest and messenger expression of somatostatin receptors (SSTRs) has been demonstrated in CMM, thus suggesting the presence of neuroendocrine features in this tumor. The aim of the study is twofold: 1) to characterize the pattern of expression of SSTRs and the subcellular localization and 2) to evaluate the *in vitro* effect of the somatostatin (SST) analogue pasireotide on cell viability, proliferation and cell cycle in four human CMM cell lines. The CMM cell lines considered were: A375, HMCB, COLO38 and M14. SSTRs messenger expression was evaluated by RT-qPCR and protein expression was confirmed by immunocytochemistry (ICC). The subcellular localization of SSTRs was investigated by western blotting (WB) on membrane and cytoplasmic protein extracts in basal conditions and after treatment with pasireotide. Cells were treated daily with serial doses of pasireotide for 72 hours. Cell viability and proliferation were assessed by MTT and DNA assay, respectively. CMM cell lines expressed SSTRs messenger and protein. SSTR2 mRNA is the most expressed, followed by SSTR5, SSTR3 and SSTR1 in A375 cells, by SSTR3, SSTR5 and SSTR1 in COLO38 and M14 cells, and by SSTR1, SSTR3 and SSTR5 in HMCB cells. ICC confirmed the expression of the evaluated receptors in the four CMM cell lines. WB showed a predominant cytoplasmic pattern of expression for SSTR1 and SSTR2; mild plasma membrane localization for SSTR3 and only membrane localization for SSTR5. Pasireotide significantly inhibited cell viability (maximal effects at the dose of 10^{-7}M: 41% $p<0.01$ and 44% $p<0.001$, *vs* control, respectively) and proliferation (maximal effects at the dose of 10^{-7}M: 20% $p<0.01$and 23% $p<0.05$ *vs* control, respectively) in A375 and M14 cells. Flow cytometry analysis revealed that pasireotide did not induce significant change in the cell cycle. SSTR3 and 5 receptor did not internalized after pasireotide treatment. In conclusion, this is the first study describing the protein expression of SSTRs in CMM cell lines and the effect of pasireotide on cell viability and proliferation n some cell lines. These data encourage further studies to better define the role of SST pathway in the diagnosis and prognosis of CMM and to determine whether SST analogues, in combination with other drugs, could have a role in the treatment of CMM.

PP078 - INFLUENCE OF THE HYPOTHALAMIC–PITUITARY–ADRENAL AXIS DYSREGULATION ON THE METABOLIC PROFILE OF PATIENTS AFFECTED BY DIABETES MELLITUS-ASSOCIATED LATE ONSET HYPOGONADISM.

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Background and aims: Functional hypercortisolism (FH) is a harmful systemic condition generated by clinical states able to chronically activate hypothalamic-pituitary-adrenal (HPA) axis, such as major depression, diabetes mellitus (DM), simple obesity, etc. However, no study has evaluated FH influence in worsening the metabolic profile of male patients affected by DM-associated hypogonadism. In this report, we assess the possible association between HPA axis-dysregulation and cardiovascular risk factors in men simultaneously affected by DM and late-onset hypogonadism (LOH). Methods and results. Fourteen DM and LOH subjects affected by FH (Hypercort-DM-LOH) and fourteen DM and LOH subjects who were not suffering from FH (Normocort-DM-LOH) were retrospectively considered. Clinical, hormonal [testosterone, sex hormone-binding globulin, 24-h urinary free cortisol, oral dexamethasone suppression test and corticotropin releasing hormone (CRH) test] and metabolic parameters [glycemia, glycated haemoglobin, total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, low density lipoprotein cholesterol and blood pressure] were considered. All metabolic parameters, except for systolic blood pressure, were significantly worse in Hypercort-DM-LOH than in Normocort-DM-LOH. In Normocort-DM-LOH, no significant correlation between general/hormonal parameters and metabolic variables was present. In Hypercort-DM-LOH, positive and significant correlations of cortisol area under the curve (AUC) after CRH with glycemia, triglycerides and blood pressure were evident; on the other hand, negative and significant correlation was present between cortisol AUC and HDL cholesterol. The associations of AUC cortisol with glycemia, HDL cholesterol and DBP were further confirmed at quantile regression. Conclusions: FH may determine a worsening of the metabolic profile in DM-associated hypogonadism.

PP079 - GENE EXPRESSION PROFILING IN HUMAN ACTH-SECRETING PITUITARY TUMORS

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Introduction. ACTH-secreting pituitary adenomas present considerable variability both in terms of clinical presentation, responses to endocrine testing, surgical outcomes and efficacy of medical therapy. Heterogeneity is evident also at molecular level with variable responses to known modulators of ACTH secretion *in vitro* (Pecori Giraldi *et al* J. Neuroendocr. 2011) as well as diversity in receptor, transcription factor and miRNA expression. Microarray analysis provides a powerful means to evaluate the expression pattern of thousands of genes and has yielded interesting results in several endocrine tumors, such adrenal carcinoma and thyroid neoplasia. **Aim** of the study was to evaluate transcriptome expression pattern in human ACTH-secreting adenomas and verify whether gene profiles are associated with clinical variables.

Methods. Forty-six human ACTH-secreting pituitary adenoma formalin-fixed paraffin-embedded specimens were cut into 20 μm thick sections and RNA extracted using Recover All Total Nucleic Acid Isolation Kit (Invitrogen, Carlsbad CA). One normal pituitary tissue block was used as control. Real-Time PCR with house-keeping *RPL13A* gene attested to the efficacy of RNA recovery. RNA (300 ng) was hybridized to Human HT-12V4 expression bead Chip (approx 29,000 transcripts) and analyzed with the WG-DASL-HT assay (Illumina, San Diego CA). Results were processed with Genome Studio software.

Results. Hybridization yielded informative data in 41 pituitary adenoma specimens. *POMC* was overexpressed in all corticotrope adenomas attesting to validity of microarray analysis. Unsupervised clustering analysis revealed several different clusters, all clearly distinct from the normal pituitary gene expression profile. Clustering according to surgical outcome revealed distinct expression profiles.

Conclusion. Microarray analysis on archival pathology specimens proved a valid and informative technique for the study of Cushing's disease molecular features. Human ACTH-secreting adenomas appear to present several, distinct gene expression profiles.

PP080 - THE ROLE OF EGFR AND IGF1R IN HUMAN BRONCHOPULMONARY NET TARGET THERAPY

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The main treatment for bronchopulmonary NET (BP-NET) is surgery, not feasible for infiltrating and metastatic disease. In those cases, medical therapy is often tried. It is important to identify new therapeutic targets to provide adequate medical treatment for patients with BP-NET. Sunitinib is a multi-targeted receptor tyrosine kinase inhibitor (TKI), mainly described to inhibit VEGFR.

The aim of our study is to verify whether IGF1R and EGFR could be involved in Sunitinib mechanism of action in BP-NET cells.

For this purpose human BP-NET cell lines and human BP-NET primary cultures were treated with Sunitinib and/or EGF, IGF1, or VEGF. Cell viability and caspase 3/7 activation were measured after 48 h of treatment. Receptor expression was detected by Western blot in both cell lines and primary cultures. Phosphorylation of IGF1R and EGFR was analysed by Alphascreen sure fire assay.

Our results show that Sunitinib is capable of inhibiting cell viability of BP-NET cell lines and primary cultures, and activates caspase 3/7. Both events are counteracted by EGF and IGF-1, but not by VEGF. Moreover, both cell lines and tissues express IGF1R and EGFR, while VEGFR receptors are not expressed in all samples. Treatment with Sunitinib decreased the phosphorylation of both EGFR and IGFR1, in both cell lines and primary cultures.

In conclusion, these data indicate that the expression of EGFR and IGF1R are important for Sunitinib activity in BP-NET. The effects of Sunitinib on BP-NET cell viability could be due to a double inhibition of EGFR and IGF1R, opening new possible therapeutic opportunities.

PP081 - HIGH-DOSE TREATMENT WITH SOMATOSTATIN ANALOGS IN NEUROENDOCRINE TUMORS

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Somatostatin analogs (SSA) effectively control symptoms in neuroendocrine tumours (NET), besides showing antiproliferative activity. In progressive or metastatic NET, increasing SSA dose or shortening the dosing interval are common clinical practice, though empirical.

Aim of this study is to evaluate efficacy and safety of high-dose SSA treatment in patients with progressive disease under standard SSA dose.

Twenty-one patients (median age 56.8 yrs) with NET of different origin were retrospectively identified among 118 patients under SSA therapy. All 21 patients were treated with SSA high dose schedule treatment, after disease progression under standard dose.

The median follow-up was 22.3 months (range 4-76). High dose schedule included octreotide LAR in 15 patients (73%) and lanreotide Autogel in 6 (27%).

Progression free survival was significantly higher with high-dose treatment compared with standard dose (32 vs 8 months, $p<0.05$). Partial objective tumor response was recorded in 1 patient (5%), stabilization in 10 (47.5%) and progression in 10 (47.5%). Among 16 patients who were symptomatic under standard dose, complete clinical response was obtained in 1 (6%), partial response in 9 (57%). Side effects were abdominal discomfort (5%), asymptomatic gallstones (5%) and type 2 diabetes mellitus (5%).

High-dose SSA treatment in progressive NET is still effective in patients refractory to standard SSA doses. No additional toxicity is observed compared with standard dose.

PP082 - AN UNUSUAL CASE OF ECTOPIC PITUITARY GLAND

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INTRODUCTION

Cushing's syndrome can be ACTH-dependent (pituitary adenoma/ectopic production) or ACTH- independent (adrenal adenoma/carcinoma).

CASE DESCRIPTION

A 47 years old man, with a history of high blood pressure, depression, osteoporotic vertebral fracture and diabetes mellitus, underwent a visit, in another Italian endocrinology centre, for the onset of nervousness, widespread bruising and cushingoid face. He was diagnosed with a ACTH-dependent Cushing disease of pituitary origin (urinary free cortisol was 2760 mcg/24h; failure overnight suppression test with dexamethasone 1 mg and with dexamethasone 8 mg). Even though pituitary MRI documented a normal gland, the patient was referred for a transsphenoidal surgical exploration, that was interrupted for early bleeding. After the surgical attempt, he was referred to our Endocrinology Department and started on ketoconazole; due to the onset of important side effects the patient was switched to pasireotide, but he didn't show any response to the drug. Because of a bronchopneumonia, a chest CT was performed detecting a 13 mm nodule in the left lung. Suspecting an ectopic Cushing disease, a FDG-PET and a 68Ga-PET were performed, both with negative results. Finally a PET/CT with fluoride-choline (FCH) showed an increase uptake of FCH in a 11 mm polypoid nodule of the left ethmoid sinus. This lesion was excised and the histology revealed an "ectopic pituitary corticotrophic adenoma". After four months a repeated FCH-PET/CT gave negative results. After one year FCH-PET/CT was still negative. The ACTH and cortisol levels were also found to be normal as well as blood pressure and glucose levels and the patient showed a complete resolution of the clinical signs.

CONCLUSIONS

We believe that the present case has 4 points of interest: 1) unusual localization of the pituitary adenoma (ethmoid sinus); 2) inability of conventional imaging methods in localizing the disease; 3) unusual FCH uptake by the ectopic pituitary adenoma; 4) unresponsiveness to pasireotide. To our knowledge only 5 cases of ectopic ACTH-secreting adenomas are described in the literature and all of them were in the paranasal sinuses, none in the ethmoid ones. Moreover, although FCH can be absorbed by normal pituitary tissue, this is the first description of an FCH uptake by an ectopic pituitary tumor.

PP083 - COMPLETE REMISSION OF HEPATIC METASTASIS AFTER TOTAL GASTRECTOMY FOR A GASTRIC CARCINOID TUMOR TYPE 1: A CASE REPORT

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Gastric carcinoids secondary to autoimmune atrophic gastritis (GC type 1) are usually well differentiated neoplasia, with an indolent course and an excellent overall prognosis. However, a subset of these tumors (< 5%) may develop advanced disease, with lymph node and/or hepatic metastasis. The pathogenesis of these carcinoids is related to chronic trophic stimuli to enterochromaffin-like (ECL) cells due to chronic hypergastrinemia.

Treatments directed to remove the source of hypergastrinemia (such as antrectomy and SSAs) have been used with good results in localized tumors. These approaches had been used in localized disease, while its effectiveness had never been demonstrated in metastatic cases.

In this report, we describe the case of a woman with type I gastric carcinoid with liver metastasis documented by abdominal CT and 68Ga-PET DOTATOC. The patient underwent total gastrectomy with lymph node dissection; during surgery an hepatic US was performed, showing 7 subcentimeter metastases. Histological examination revealed a neuroendocrine neoplasm G2 (WHO 2010) of 30 mm, Ki67 20%, 15 mitoses/10 HPF; chronic gastritis and micronodular hyperplasia of endocrine cells was associated. During the early months of follow up a gradual reduction in size of liver metastases, until the complete disappearance of them was observed.

This is to our knowledge the first case ever described in literature of complete remission of liver metastatic type 1 GC after removal of the source of excessive gastrin, showing a possible preservation of responsiveness of metastases to gastrinemic stimuli. This could lead to a possible change in therapeutic approach in these neoplasm.

PP084 - SEVERE HYPONATRIEMIA AT THE CLINICAL ONSET OF SHEEHAN'S SYNDROME IN A WOMAN WITH MULTIPLE SCLEROSIS AND MALNUTRITION: A CASE REPORT

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Sheehan's syndrome occurs as a result of pituitary ischemic damage after postpartum hemorrhage and hypotension. Clinical features, the pattern of hormonal deficiencies, and time delay from partum to onset of disease may vary greatly. We present a case of 41-year-old woman admitted to the Emergency Department for the development of subacute severe psychomotor retardation, associated with deterioration of general conditions, loss of appetite, progressively marked asthenia, which had started several weeks earlier and worsened after a upper respiratory tract infection and vomiting. The patient had been affected by relapsing remitting multiple sclerosis for 10 years. Clinical history indicated the development of apparent depression with impairment of psychological state after childbirth, 9 months earlier, with the gradual onset of arthalgia, asthenia, and loss of well-being, which had been treated with alternative medicine including a low-calorie vegetarian diet leading to significant weight loss (10Kg). An episode of massive macrohematuria with acute anaemia (haemoglobin fall from 10.5 to 5.5g/dl) and arterial hypotension had occurred 12 days after a caesarean section, and required hospitalization and transfusion of 4 units of blood. The patient breastfed the baby just for 12 days, without lactation onset. The menstrual cycles became irregular after partum with oligomenorrhea. Laboratory tests at the Emergency Department documented severe hyponatremia (113mEq/L), hypochloremia (80mEq/L), hypoglycaemia (49mg/dl), hypothyroidism (TSH11.8 µUI/ml, FT3 1.81pg/ml, FT4 0.5ng/dl), and normal kalemia (3.7mEq/L). After admission, hypoglycemia was treated with intravenous glucose; oral supplementation with levothyroxine and infusional therapy with hypertonic saline solution were started but without a significant increase in natremia after 48 hours, with stable values around 112mEq/L. In differential diagnosis of severe hyponatremia, we found plasma osmolarity of 234mOsm/kg that confirmed true hyponatremia, and urinary osmolarity of 308mOsm/kg, as an indication of reduced free-water urinary excretion. We excluded both hyper and hypovolemia, and suggested a possible hormonal cause of hyponatremia. Thus, we found subnormal levels of ACTH (<5pg/ml), cortisol (0.2µg/dl), IGF-1 (<25ng/ml), GH (2.26ng/ml), prolactin (<0.3ng/ml), LH (0.91mUI/ml, follicular phase), FSH (4.58mUI/ml, follicular phase), estradiol (20.3pg/ml), progesterone (<0.15ng/ml), total testosterone (<0.10ng/ml), DHEA-S (<0.15µg/dl), Δ4androstenedione (<0.3ng/ml), the presence of TPOAb (124IU/ml), and of nutritional indexes indicative of moderate-severe malnutrition (weight: 39.5kg; BMI: 15.6Kg/m2; phosphatemia: 2.5mg/dl; serum albumin: 3.2g/dl; lymphocyte: 2100/microL; transferrin: 154mg/dl; prealbumin: 5.37mg/dl). Following indication of secondary adrenal insufficiency, intravenous

hydrocortisone replacement was started with immediate and gradual recovery of natremia in 48 hours (until 134mEq/L) and a concomitant relevant water diuresis and amelioration of general conditions. Pituitary magnetic resonance imaging showed a reduction in pituitary gland size (maximum height 3.5mm), with a morphological aspect of the sellar region compatible with a partial empty sella. In conclusion, for this woman with acute emergengy presentation, clinical history, laboratory and radiological findings and the prompt response of hyponatremia to hydrocortisone, suggested the diagnosis of Sheehan's syndrome, associated with multiple sclerosis, autoimmune thyroiditis, and malnutrition. Cortisol deficit might have exacerbated autoimmune comorbidities, whereas all the comorbidities presumably contributed to subacute clinical presentation.

PP085 - BONE QUALITY IN ACROMEGALY

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Introduction: Acromegaly is characterized by chronic exposure to high GH and IGF-I levels that leads to increased bone turnover. Regardless of BMD value, acromegalic patients seem to have an increased vertebral fracture risk probably due to a reduction of bone quality. Trabecular bone score (TBS) is a new index used for assessing bone microarchitecture. In this study TBS was used for the first time to analyze bone quality in acromegaly. **Methods:** 16 new acromegalic patients (12F, age 56.3±13.8, BMI 28.1±6.3) and 16 controls matched for age, gender, menopausal state and BMI, were enrolled. Patients with MEN1, ectopic GHRH secretion and history of secondary osteoporosis were excluded. All participants underwent lumbar radiograph and dualenergy X-ray absorptiometry scan on lumbar spine (LS) and femur (total:FT, neck:FN). TBS was assessed in the region of LS-BMD. **Results:** 56% of patients had a macroadenoma, 18% had hypopituitarism (all hypoadrenalism) and nobody had cosecretion. BMDs were not different between the two groups (acromegalic patients vs controls: LS T-score -0.5±1.3vs-0.7±1.0 *P*=0.7, LS Z-score 0.5±1.3vs0.5±1.5 *P*=0.7; FN T-score -0.6±0.9vs-0.7±1.2 *P*=0.8; FN Z-score 0.3±0.7vs0.2±0.8 *P*=0.4; FT T-score 0.02±1.01vs-0.5±0.99 *P*=0.2; FT Z-score 0.64±0.84vs0.4±0.8 *P*=0.4) while acromegalic patients had lower TBS than controls (TBS Z-score -2.27±2.05vs-1.00±0.9 *P*=0.04, TBS value 1.195±0.14vs0.8±0.6 *P*=0.01). Two patients and one control had vertebral fractures (*P*=1.0). In acromegalic patients, at bivariate analysis TBS was associated with age at diagnosis (r^2-0.25, *P*=0.04), GH serum levels ($r^2$0.36, *P*=0.01), FN T-score ($r^2$0.35, *P*=0.02) and Z-score ($r^2$0.81, *P*=0.01). Vertebral fractures were associated with age at diagnosis ($r^2$0.36, *P*=0.02). LS-BMD and FT-BMD were related to alteration of glucose metabolism ($r^2$0.25, *P*=0.04 and $r^2$0.49, *P*=0.002 respectively). **Conclusions:** Acromegalic patients had impaired bone quality despite normal bone density. Further larger studies are needed to define TBS role in fracture risk in acromegaly.

PP086 - CRITICAL HYPONATREMIA FROM WATER INTOXICATION IN A PATIENT WITH BIPOLAR AFFECTIVE DISORDER.

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Introduction. Psychogenic polydipsia (PP) is often seen in patients with psychiatric disorders (5-15%), especially schizophrenia. This disorder is rare in patients with mood disorders. We report a case of life-treatening severe hyponatremia due to ingestion a very large volume of water in a patient with a diagnosis of bipolar disorder.

Case report. A 41-year-old woman affected by bipolar affective disorder was admitted with the diagnosis polydispia and polyuria (12-14 L/day). S-Na was 129 mEq/L, glycemia 88 mg/dL, U-osm 210 mosm/L. No history of smoking and alcohol abuse and no drugs known to induce hyponatremia. In the preceding two months she experienced two episodes of water intoxication (25-30 L in 24 hrs) leading to hyponatremic encephalopathy (S-Na 110-117 mEq/L) manifesting as a worsening of psychiatric symptoms, nausea, vomiting, delirium, ataxia, seizures and stupor. A U/P osm ratio <1 suggested a water intake exceeding the maximal dilution capacity of the kidneys (around 20 L). Hyponatraemia secondary to antipsychotic medication was also considered, but the patient had been taking the same antipsychotic dosages for years. The management of this condition in patient included fluid restriction, hypertonic saline and behavioral treatment.

Conclusions. We describe a rare case of water intoxication with critical hyponatremia in a patient with mood disorder. Most case of PP complicated by water intoxication are observed in schizofrenic patients when both polydipsia and impaired water excretion are present. However, it is not uncommon that psychiatric settings minimize overlooking this association of psychosis and disturbed water balance. This case emphasizes the need for greater awareness of the development of this serious and potentially fatal complication in psychiatric patients, including those with mood disorders. Water intoxication in these cases is difficult to manage and may become a recurring problem. S-Na should be measured periodically in such patients with polydipsia for early detection of potential life-treatening hyponatremia.

PP087 - PRESURGICAL TREATMENT WITH PASIREOTIDE IN CRITICAL PATIENTS WITH CUSHING'S DISEASE DUE TO ACTH-SECRETING PITUITARY MACROADENOMAS

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Pasireotide is indicated for the treatment of patients with Cushing's disease (CD) for whom pituitary surgery is not an option or has not been curative. Few data are available about the use of this drug as first line or presurgical treatment. We report the effects of presurgical pasireotide treatment in two patients with ACTH-secreting pituitary macroadenoma in whom hypercortisolism caused dramatic alkalosis and cardio-respiratory complications precluding surgical approach. In both cases pasireotide administration (60 µg s.c. bid) induced a rapid, partial decrease of plasma ACTH, serum cortisol and UFC levels and normalization of both serum potassium concentration and arterial blood gases parameters during the first 21 days of treatment. MRI evaluation performed before surgery did not show pituitary tumor shrinkage. Surgical cure of CD was obtained in the first patient, while debulking allowed the pharmacological control of hypercortisolism in the second case. In conclusion, pasireotide can induce a rapid improvement of clinical and metabolic conditions in critical patients with CD, in whom transsphenoidal approach is considered hazardous and should be delayed.

PP088 - HIGH DOSES LANREOTIDE AUTOGEL AND CABERGOLINE AS FIRST-LINE TREATMENT: MEDICAL DEBULKING OF A GIANT INVASIVE GH-SECRETING PITUITARY MASS

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Objective: In the last years primary medical therapy of acromegaly with somatostatin analogs has been suggested for patients with larger tumors when a surgical cure is unlikely. We report efficacy and safety of the association of high dose somatostatin analogs with cabergoline as a first-line treatment in a giant invasive GH secreting pituitary adenoma

Report: a 24 years old man with giant invasive pituitary adenoma presented with classical features of acromegaly, GH and IGF-1 markedly elevated (GH 21 ng/ml and IGF-1 >1000 ng/ml). Nuclear Magnetic Resonance (NMR) imaging showed a pituitary mass extending into suprasella, obliterating the cavernous sinus and invading third and lateral ventricle with a maximum diameter of 65 mm. Due to several less favorable predictors of surgical outcome, according to neurosugeon we started medical treatment with lanreotide autogel 120 mg one injection every 21 days. After three months cabergoline 0,5 mg a week was added. After two months of therapy a decrease in GH and IGF-1 levels (7.6 ng/ml and 725 ng/ml respectively) was observed. NMR showed a significant and progressive shrinkage of the lesion, reached 25 mm reduction in maximum diameter at sixteenth months.This shrinkage was associated with a persistent reduction of GH and IGF-1 levels (2.7 ng/ml and 560 ng/ml respectively), a restoration of a normal visual field pattern but not a normal gonadic function The patients displayed amelioration of acromegaly-related symptoms and a decrease in body weight and fat mass.

CONCLUSION: we demonstred a clinically significant 40% tumor volume reduction of a giant GH secreting adenoma with medical first-line therapy combining high doses lanreotide autogel and cabergoline. Pharmacological approach can be used in those patients poor surgical candidates and in those in whom there is a low probability of a surgical care.

PP089 - PERIPHERAL RAPID CALCIUM INFUSION TEST IN ADULT HYPOGLYCEMIC DISORDERS

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Introduction

The most frequent causes of endogenous hyperinsulinemic hypoglycemia are insulinomas and beta-cell hyperplasia (nesidioblastosis). In addition to clinical and biochemical findings (fasting plasma glucose, insulin and C-peptide), diagnostic work-up of hypoglycemic syndromes includes functional test (prolonged fasting test) and exams for tumor localization (abdominal US, CT, MRI, Octreoscan, PET-CT, selective pancreatic arterial calcium injection). Rapid Calcium Infusion (RCI) test is a functional test which has been proposed in order to investigate beta cells secretion after Calcium gluconate infusion in a peripheral vein. We examined RCI test as diagnostic tool in comparison with prolonged fasting test.

Materials

13 patients (all females, mean age 54) consecutively referred for hypoglycemic symptoms underwent prolonged fasting test. RCI test was performed in the same subjects in a separate day and it was repeated after surgery in 3 patients. Plasma glucose, insulin and C-peptide were measured before and after 2'-5'-10'-15'-30' calcium gluconate infusion (2 mg/kg in 90''). RCI test was previously performed in 6 healthy subjects as controls and none of them reported adverse events.

Results

Prolonged fasting test resulted diagnostic for endogenous hyperinsulinism in 7 patients, confirmed at surgery (5 insulinomas, 2 beta-cell hyperplasia). In all these patients preoperative RCI test showed a transient insulin elevation (at 2' and 5') without hypoglycemia. At variance, insulin levels did not change in patients with normal prolonged fasting test and in controls. Insulin did not increase during RCI test performed in 3 patients postoperatively. No relevant side effects were reported during RCI test.

Conclusions

In our experience RCI test and prolonged fasting test were comparable in diagnostic performance of endogenous hyperinsulinemia. Prolonged fasting test requires hospitalization, with the potential risk of symptomatic hypoglicemia. RCI test is minimally invasive and it seems to be safe. RCI test could be suggested as a screening test in the diagnostic work-up of hypoglycemic disorders but validation and standardization on a larger population are needed.

PP090 - IMPAIRED VITAMIN D CONCENTRATIONS: A LINK BETWEEN PITUITARY AUTOIMMUNITY AND HYPOGONADISM IN TYPE 2 DIABETES MELLITUS

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Hypogonadotropic hypogonadism (HH) frequently occurs in type 2 diabetes mellitus (T2DM), sometimes associated to pituitary autoimmunity. Impaired concentrations of vitamin D have been reported in hypogonadism, in T2DM and in autoimmune diseases. Aim of this study was to investigate whether vitamin D insufficiency or deficiency may be considered a link between pituitary autoimmunity and hypogonadism in patients with T2DM.

To this purpose we enrolled 95 males with T2DM (aged 35-70) and 100 age-matched helthy controls. Vitamin D by chemiluminescent assay, glycemic pattern, pituitary, gonadal, adrenal, thyroid and parathyroid hormones and pituitary antibodies (APA) by indirect immunofluorescence, were investigated in all patients and controls on morning samples drawn in the same month of the year.

Results. HH was diagnosed in 37/95 patients with T2DM (38%) on the basis of testosterone values ≤ 12 nmol/L in presence of normal/low gonadotropin levels. A higher prevalence of APA was observed in T2DM patients (26/95, 27%) vs controls (5/100, 5%; P< 0.001). In particular, APA was found in 15/37 patients with HH (40%) and in 11/58 without HH (18%; P=0.002). T2DM patients with HH showed vitamin D concentrations significantly lower with respect to those without HH and to controls (19.4±7.06 ng/ml vs 24±5.6 ng/ml and vs 34.3±7.2 ng/ml, respectively, P < 0.02 and < 0.001, respectively). A higher prevalence of vitamin D deficiency was found in T2DM with HH, positive for APA.

Conclusions. Taking into accont the well-known involvement of vitamin D in autoimmunity and its promoting role on the testicular steroidogenesis (Hofer et al JCEM,2014), the results of our study seem to indicate that an impaired concentration of vitamin D in T2DM patients may favour the occurrence of HH promoting pituitary autoimmunity but may also act negatively on the testicular synthesis, inducing a combined primary/secondary hypogonadism.

PP091 - A RARE CASE OF MASS-LIKE THICKENING OF THE PITUITARY STALK

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BACKGROUND: Ectopic posterior pituitary (EPP) is a rare morphological alteration of the hypothalamic-pituitary region described as part of the pituitary stalk interruption syndrome (PSIS). Until now, a thousand cases of PSIS have been reported. EPP appears on MRI as an high-signal round nodule in the floor of the third ventricle or along the infundibulum. Since the portal circulation carrying hypothalamic-releasing hormones to the adenohypophysis is disrupted, EPP can be associated with isolated GH deficiency or combined pituitary hormones deficiency. Nevertheless, some cases in otherwise normal individuals have been reported. CASE REPORT: A 45-year-old man was referred to our Endocrine Unit because of headache and decrease in libido. Born after an uneventful pregnancy and suffering from allergic bronchial asthma from childhood (treated with inhaled corticosteroids), our patient experienced a mild traumatic brain injury when he was 8 year-old. General laboratory data were normal. Plasma cortisol showed low response to h-CRH 100 mcg i.v. The plasma GH level was low, but its response to GHRH + arginine i.v. was preserved. Beyond this, hormonal secretion was preserved. On MRI, T1 weighed images revealed the absence of the high intensity signal of the posterior pituitary within the sella, while a focal rounded area (5x7 mm) was located at the median eminence, suggesting the presence of an EPP. During a 5 year follow-up, neuroradiological findings were unchanged. Three years after the diagnosis of secondary adrenal failure, glucocorticoid treatment was stopped, because plasma cortisol levels showed a normal response to low dose ACTH stimulation. After a significant weight loss, we noticed an improvement of both symptoms and GH level peak after GHRH + arginine i.v. provocative testing. CONCLUSIONS: The diagnosis of EPP was based on MRI findings. We observed only a transient adrenal insufficiency, which seems to be indipendent of pituitary stalk lesion and probably due to chronic corticosteroid therapy. Moreover, we noted an improvement of GH response to GHRH + arginine i.v. after weight loss. We can hypothesize that anatomical integrity of the adenohypophysis has allowed a normal secretory function. This case underscores the importance of a proper evaluation of the patient with signs and symptoms suggestive of hypopituitarism. All patients with pituitary stalk lesions should have a careful clinical and biochemical assessment for both anterior and posterior pituitary hormones deficiencies, as well as long-term follow-up.

PP092 - PSYCHOLOGICAL AND NEUROCOGNITIVE EVALUATION IN PATIENTS WITH PITUITARY ADENOMA.

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Reduced health-related quality of life (HRQoL) and impairment in neurocognitive functions are a possible complaint in patients with pituitary adenoma. Psychiatric comorbidities in Cushing's disease are well known. However recent studies showed HRQoL reduction and psychiatric symptoms in patients with pituitary disease with or without hormonal excess. Aim of this study is to analyze HRQoL, psychiatric symptoms and neurocognitive functions in patients with pituitary adenomas, with either Cushing's disease or non-functioning pituitary adenoma (NPFA) before and after surgery. Through three validated questionnaires (SF-36, BDI-II, MMPI-II) and an interview with a psychologist, we assessed HRQoL, psychiatric symptoms and neurocognitive functions in 20 adult patients (age 49.6±11.4, M/F=12/8) harboring pituitary tumors (10 Cushing's disease and 10 NPFA, macro/micro=12/8). We conducted a baseline measurement before transphenoidal surgery as well as after 12 months. All Cushing's patients were in remission at the follow-up visit and pituitary deficiencies, when present, were adequately substituted. 20 healthy subjects, age- and sex-matched, were analyzed as controls.Regarding HRQoL, patients with NFPA did not show significant differences compared to controls. On the contrary, patients with Cushing's disease had significantly lower HRQoL than NFPA and controls in all scales of SF-36 questionary, both at baseline and at follow-up. At follow-up NFPA patients showed an improvement in all scales while patients with Cushing improved only in role-physical and general health ones. Furthermore, BDI-II and MPI-II scales showed a significant increase of depression (p=0,045) and social inversion (p=0,031) in the Cushing's group. Lastly, both groups of patients, without any difference, showed a significative impairment in all neurocognitive functions tests at baseline compared to controls. At follow up, though, the difference had disappeared. According to the literature, this study confirms that Cushing's disease leads to a much larger impact on HRQoL and psychiatric comorbidities, with significant improvement after treatment but without a complete remission, probably due to irreversible changes in neural function. Interestingly, however, neurocognitive impairment is present and appears in all patients with pituitary tumors, independently of hormone secretion.

PP093 - GENDER DIFFERENCE IN CLINICAL PRESENTATION AND CO-MORBIDITIES OF NON-FUNCTIONING PITUITARY TUMORS: A PROSPECTIVE SURVEY OF A NATIONAL REFERRAL CENTER

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Background: Several studies investigated the hormonal differences in the male and female gender in determining development of several diseases, but only limited data are available for pituitary diseases.

Objective: To evaluate the presence of gender differences of patients newly diagnosed with non functioning pituitary tumors on a three years period in a national referral center in Italy.

Subjects: 91 patients with non functioning tumor (39 men, mean age 51.0 ± 16.7 years; 52 women, mean age 40.9 ± 17.1 years) were included in this study.

Methods: Presenting clinical symptoms, clinical, biochemical and endocrine evaluation, and tumore size at magnetic resonance were measured in all subjects.

Results Rathke's pouch cysts were 4.3 % and were exclusively female. Regarding to other pituitary lesions no statistically significant gender differences were found. Pituitary lesions (>1 cm) were significantly more frequent in male than in female ($p = 0.0001$). The female gender presented a higher prevalence of pituitary microadenomas (<1cm) ($p = 0.013$). The size of the pituitary lesions was significantly higher in males than in females ($p= 0.044$). There was no significant difference in males and females as to clinical and biochemical evaluation. Hypopituitarism was more frequent in the male than female gender ($p = 0.008$). There was no gender difference regarding the presence of GH and gonadotroph deficiency ($p = 0209$); while hypothyroidism and hypocortisolism were more frequent in male ($p<0.05$). No differences were found about dose of L-tiroxine and acetate cortisone between male and female patients ($p=0.169$).

Conclusion: No significant gender differences was observed in the prevalence of different histotypes of pituitary non functioning lesions except for Rathke's pouch cysts . Pituitary macroadenomas were significantly more frequent in the male than in the female gender, while female showed a higher prevalence of microadenomas. Severe hypopituitarism, was more frequent in the male gender. No gender differences were found in the hormonal values at baseline and in doses of replacement therapy.

PP094 - PREGNANCY MAY FAVOUR THE DEVELOPMENT OF SEVERE AUTOIMMUNE CENTRAL DIABETES INSIPIDUS IN WOMEN WITH VASOPRESSIN-CELL ANTIBODIES: DESCRIPTION OF TWO CASES

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Recently, an increased incidence of central diabetes insipidus in pregnancy, and less frequently in the post-partum period, has been reported, most likely favoured by some conditions occurring in pregnancy. This study was aimed at investigating the influence of pregnancy on a pre-existing potential/subclinical hypothalamic autoimmunity.

We studied the longitudinal behaviour of vasopressin-cell antibodies (AVPcAb) and post-pituitary function in two young women with a positive history of autoimmune disease and presence of AVPcAb, but without clinical central diabetes insipidus (CDI), and who became pregnant 5 and 7 months after our first observation.

The behaviour of post-pituitary function and AVPcAb (by immunofluorescence) was evaluated at baseline, during pregnancy and for 2 years after delivery.

AVPcAb, present at low/middle titres at baseline in both patients, showed a titre increase during pregnancy in one and after delivery in the other, with development of clinically overt CDI. Therapy with 1-deamino-8-D-arginine-vasopressin (DDAVP) caused a prompt clinical remission. After a first unsuccessful attempt of withdrawal, the therapy was definitively stopped at the 6th and the 7th month of post-partum period, respectively, when AVPcAb disappeared, accompanied by post-pituitary function recovery, persisting until the end of the follow-up.

The determination of AVPcAb is advisable in patients with autoimmune diseases planning their pregnancy, because they could be considered good predictive markers of gestational or post-partum autoimmune CDI. The monitoring of AVPcAb titres and post-pituitary function during pregnancy in these patients may allow an early diagnosis and an early replacement therapy, which could induce the disappearance of these antibodies with consequent complete remission of CDI.

PP095 - LATE PRIMARY AUTOIMMUNE HYPOTHYROIDISM IN A PATIENT WITH POST-DELIVERY LACTATION FAILURE AND AUTOIMMUNE HYPOPITUITARISM IN THE PRESENCE OF ANTIBODIES TO GROWTH HORMONE- AND PROLACTIN-SECRETING CELLS

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Pituitary and thyroid autoimmunity can be triggered by pregnancy. We report the first association of combined growth hormone (GH) and prolactin secretion deficiency due to autoimmune damage to GH- and prolactin- secreting cells in a patient with post delivery lactational failure, presenting subsequently with primary autoimmune hypothyroidism.

A 34 year old woman presented with failure of lactation following delivery of her first child. She had a family history of hypothyroidism without a history of pituitary dysfunction.

Physical examination did not show any abnormal findings. Laboratory investigations showed normal gonadotropin levels after the restoration of normal menstrual cycles following pregnancy, normal basal and stimulated cortisol but low prolactin concentrations and low serum insulin-like growth factor-1 with impaired GH response to insulin-induced hypoglycaemia. Thyroid function was normal when initially investigated 3 months after delivery, but 5 months later marked primary hypothyroidism (TSH levels >100 mIU/L) occurred. Immunological investigation revealed the presence of antipituitary antibodies, identified by double immunofluorescence as targeting GH- and prolactin- secreting cells. Anti thyroid antibodies, in the normal range 3 months post-partum, were significantly elevated when the hypothyroidism appeared.

Autoimmune hypophysitis is responsible for selective or multiple pituitary- hormone deficiencies, sometimes involving TSH secretion causing secondary hypothyroidism but usually associated with hyperprolactinemia. To our knowledge this is the first observation of autoimmune hypopituitarism involving deficient growth hormone and prolactin secretion in a patient with lactation failure after delivery subsequently followed by severe primary autoimmune hypothyroidism, thus falling into an unusual autoimmune polyendocrine syndrome type 3.

Considering the well known relationship between pregnancy and autoimmunity, an early post delivery immunological and functional investigation in women presenting with disorders of lactation may be useful to detect potential pituitary and thyroid dysfunction even in a subclinical stage.

PP096 - HORMONAL AND CLINICAL CHANGES DURING THE PUBERTAL PERIOD: OUR EXPERIENCE

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Pubertal onset requires activation of hypothalamic neurons to increase pulsatile GnRH secretion. Throughout childhood, GnRH secretion undergoes small but progressive increases until the onset of puberty. The reactivation of the axis results in increasing circulating levels of FSH and LH that stimulate both the growth of the gonads' size and their production of gametes and hormones. This process is defined gonadarche. The development of secondary sexual features is due to the rise of adrenal androgens (DHEA, DHEA-S and Δ4) and is called adrenarche. It takes place approximately 2 years before gonadarche but the trigger remains unknown.

Aim of this study was to evaluate hormonal and clinical changes that happen during pubertal development. 94 healthy subjects were evaluated: 69 males (aged between 8 – 18 years) and 25 females (aged between 7.8 – 15.7 yrs.) were sorted by age. They all underwent clinical and hormonal evaluation every 6 months. The transition between Tanner stage G1 to G2 for boys and T1 to T2 for girls was assumed as semester 0. Blood samples were analyzed to detect serum concentrations of LH, FSH, Te, E2, DEAS, Δ-4, SHBG. For statistical analysis Excel Microsoft Office and SPSS were used.

We found that puberty begins at median age of 12.2 years for male and 10.9 years for female. During the semester before puberty onset (S -1), testis volume increased sooner than testosterone's rise. FSH increased between 4° (S -4) and 3° (S -3) semester before puberty onset, whereas LH increased just before 1 year. DEAS increased about 2 years before S 0, and its level was constant until G2. After that time, DEAS gradually increased. One year after the beginning of puberty, 62% of boys were in G3 phase, to demonstrate that 2 semesters are necessary for transition from G2 to G3 and G3 to G4. 68.7% of boys reached G5 after 3 years (S6). Complete sexual maturation of all boys was gained at S7. Females' FSH levels, unlike males, increased around the second semester before puberty (S -2). In girls FSH and estradiol levels raised before LH (whose increase started at S-1). Female LH values were lower than male at S0. 50% of girls reached T3 stage at S1, the remaining 50% at S2. At S5 all girls were in T5 and menarche was reached. The median age of menarche was 13.5 years. In conclusion our data showed that 3-3.5 years are necessary to complete boys' development, whereas girls reach menarche in 2.5 years. The proper knowledge of pubertal timing is helpful for physicians to reach proper diagnosis of puberty development diseases.

PP097 - TWO SIBLINGS WITH CEREBELLAR INVOLVEMENT, PROMINENT MAXILLA/INCISORS, GROWTH DELAY AND RETARDATION: A NEW SYNDROME?

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We report on two siblings (female and boy) from a non-consanguineous Italian family, who presented overlapping clinical features. They were born at 39 weeks by spontaneous vaginal delivery. At birth, growth parameters were below 3rd percentile and were evident dysmorphic features, short neck and limbs, convergent strabismus, oedema of feet and cryptorchidism in male. They underwent clinical evaluation at the age of 12 (girl) and 9 (boy): pubertal stage was T2P2 and G1P1 respectively. Clinical examination revealed: short stature and low weight, microcephaly, craniofacial dimorphisms with sparse hair and eyebrows, wave shaped palpebral fissures, hypotelorism, low set ears, prominent teeth and alveolar bone with malocclusion. The boy presented bilateral single palmar crease and cutaneous syndactyly; genitalia evaluation disclosed testis of 1.5 ml of volume, localized above the external inguinal ring. US revealed the presence of bilateral cryptorchidism, thus hCG was given for two months obtaining testis descent. Cardiac evaluation demonstrated in girl mitral valve prolapsed, in boy mild dilatation of the ascending aorta. Skeletal survey showed sloping ribs, dysmorphic vertebral bodies with lordosis in girl and right convex dorsal-lumbar scoliosis in boy. In girl slender and mild bowed long bones was evident, associated with bilateral clinodactyly of digit V. Both of them had a femoral head crushed with irregular mineralization (right for boy with the outcome of Legg-Calvé-Perthes disease) and marked delayed bone age. Cognitive functioning was mildly delayed. Cranial MRI revealed enlarged cisterna magna (Dandy-Walker variant) with cerebellar vermis hypoplasia and enlargement of the IV ventricle. Sinuses/Face CT scan showed thickening of cranial bones, mandibular hypoplasia with anterior dislocation of TMJ articular disc only in girl. Karyotype analysis and metabolic investigations were normal. Clonidine stimulation test for GH was performed in both and an impaired pick level was observed in girl. Therefore she underwent a second test (ITT), with a peak level of 3.38 ng/mL, consequently rec-GH therapy was given. Hormonal evaluation showed most values in the normal range according to age, but in the boy LH, Te, FTe and DHEA-S were below the lower limit. The girl pelvic US demonstrated normal uterus and ovaries; menarche appeared at 15y4m.

PP098 - BIOCHEMICAL CONTROL AND CLINICAL IMPROVEMENT IS INDUCED BY LONG-TERM PASIREOTIDE ADMINISTRATION IN THE MAJORITY OF PATIENTS WITH CUSHING'S DISEASE PERSISTENT AFTER PITUITARY SURGERY

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Pasireotide (SOM230) is a multireceptor ligand somatostatin analog with high binding affinity to somatostatin receptor subtype 5, which is predominantly expressed in ACTH-secreting pituitary adenomas. It is indicated for the treatment of adult patients with Cushing's disease (CD) for whom pituitary surgery is not an option or has not been curative. Our study evaluated the effects of pasireotide, administered for 12 months at a dose of 600-900 mcg/daily, in five CD patients with persistent/recurrent disease after pituitary surgery. 24-hour urinary free cortisol (UFC) levels, weight, body mass index, waist circumference, blood pressure and glucose and lipid metabolism parameters were evaluated in all patients. After six months of therapy 4/5 patients (80%) had normal UFC levels, confirmed at 12 months. UFC normalization was associated with a slight improvement in anthropometric values and amelioration of lipid profile. In the remaining 20% of patients (1/5), normalization of UFC levels after 1 month of therapy was followed by escape from response. No patient experienced adrenal insufficiency. Hyperglycemia due to pasireotide therapy occurred in 2 patients with normal glucose metabolism prior to treatment. These findings suggest that Pasireotide is an effective treatment for most of the patients with persistent/recurrent CD after surgery.

PP099 - OCCURRENCE OF LIVER FAILURE IN POST-SURGERY HYPOPITUITARIC PATIENTS

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Multiple pituitary hormone deficit and hypothalamic obesity are common complications after brain surgery for childhood tumors. Despite adequate replacement hormone therapy, obesity and metabolic syndrome develop equally, but liver failure is not usually described. In this study, four subjects who had undergone surgery for brain tumors involving the peri-hypothalamic area when they were nursing or as youths (9 months, 6 years, 9 years and 20 years). They were referred to our Unit when they were 19, 23, 23 and 31 years old, respectively. One of this patients had a pilocytic astrocytoma, two of these patients had a craniopharyngioma, and the last a third ventricle germinoma. One patient underwent radiotherapy and chemotherapy after surgery, and another one received four transphenoidal and transcranial surgical operations. All patients developed panhypopituitarism, but only two had taken in childhood and adolescent age adequate hormone replacement therapies since brain damage. Since the other two patients came to our observation, adequate hormonal replacement therapy was proposed. All patients developed obesity (BMI 39.2-46.6 Kg/m^2) or overweight (BMI: 27.1). The two patients adequately treated for hypopituitarism after surgery developed a fatty liver, when they were 16 and 21 years old respectively. In both cases, steatosis progressively evolved into cirrhosis after two years. One of these patients also developed a hepatic-pulmonary syndrome and underwent liver transplant at the age of 25 years. In the two patients with inadequate or absent hormone replacement therapy, a diagnosis of cirrhosis was concomitant with the first appropriate endocrinological care during hospitalization for liver failure.

The analysis of these four cases shows that pediatric peri-hypothalamic surgery may be associated with very severe hepatic clinical features, induced by mechanisms not yet known, regardless of hormone replacement therapy. It is therefore very important to start a careful follow-up of these patients from childhood for early detection of possible liver failure.

PP100 - CLINICAL, BIOCHEMICAL, IMAGING CHARACTERIZATION AND TREATMENT OUTCOME OF PATIENTS WITH CLINICALLY SILENT ACROMEGALY

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Clinically silent acromegaly is a condition characterized by biochemical and immunohistochemical evidence of GH excess in the absence of typical clinical manifestations of acromegaly.

We identified 113 consecutive acromegalic patients referred to our Endocrinology Unit from 1st January 1995 to 31st December 2014. Eleven out of 113 had no clinical features of acromegaly but elevated basal serum GH concentrations that did not suppress after OGTT and/or elevated IGF1 concentrations (clinically silent acromegalic patients, CSAP).

Overt acromegalic patients (OAP) group and CSAP group showed similar age at disease's diagnosis (46.53±SE 1.22 *vs* 48.82±SE 4.77), M/F ratio (0.52 *vs* 0.57), Macro/microadenoma ratio (3.43 *vs* 2.67) and prevalence of AIP mutations (0,03% *vs* 0,1%). Despite similar IGF1 values at diagnosis (IGF1 ULN 1.75±0.17 *vs* 2.9±0.18), we reported significantly increased GH values in OAP group compared with CSAP group (25.19±SE 4.88 *vs* 4.84±1.48 *p<0,001*). Finally, statistical analysis showed no significant difference between OAP group and CSAP group concerning prevalence of cardiovascular and metabolic complications (arterial hypertension, ventricular hypertrophy, impaired glucose tolerance, type 2 diabetes mellitus) surgery effectiveness and somatostatin analogues resistance.

PP101 - REVALUATION OF THE CLINICAL AND METABOLIC BEHAVIOR OF GHD CHILDREN DURING GH TREATMENT ACCORDING TO NEWLY PROPOSED NOTE 39 OF THE ITALIAN MEDICINES AGENCY (AIFA)

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Background The newly proposed note 39 of the Italian Medicines Agency (AIFA) revisited the diagnostic criteria of growth hormone deficiency (GHD) in children and, applying it, a percentage of children previously diagnosed as GHD may have received a wrong diagnosis and a unnecessary treatment, with potential clinical implications.

Aim To evaluate the clinical and metabolic behavior of GH-treated children according to the new criteria of GHD diagnosis.

Subjects and Methods: We retrospectively analyzed clinical and metabolic data of 310 prepubertal children (220 M, 90 F, age 10.8 ± 2.9 yrs) with short stature admitted to our section of Endocrinology during the years 2005-2014, having at least a 24 months follow-up. All children were divided, according to new AIFA note 39, into group A (n°181 with a peak of GH < 8 ng/dl after 2 tests), group B (n°103 with a peak of GH ≥ 8 and < 10 ng/dl) and group C (n°26 with a peak of GH > 10 ng/dl).

Results At baseline, group A showed higher waist circumference than B (p=0.031) and C (p=0.041), while no difference in metabolic parameters was found between the 3 groups. As expected, group C showed a better height (-1.70 ± 0.35 SD) than A (-2.04 ± 0.72; p=0.002) and B (-2.06 ± 0.86; p=0.010), associated with higher bone/chronological age ratio (p=0.044 and 0.00, respectively) and IGF-1 (p=0.013 and 0.015, respectively). After 12 and 24 months of treatment, group B showed lower height velocity (p<0.001 and 0.049) and QUICKI (both p<0.001) and higher fasting glucose (p=0.001 and 0.015), insulin (p=0.001 and 0.008), Homa-IR (p<0.001 and 0.001) than group A, and HbA1c levels higher at 12 months (p=0.017), although always within the normal range.

Conclusions Children considered as affected by GHD on the basis of previous, but not current, AIFA criteria showed a worse auxological and metabolic response, probably confirming that the previous cut-off of 10 ng/dl seems to be too high and those patients may not fully benefit from the GH treatment.

PP102 - USEFULNESS AND SAFETY OF PASIREOTIDE IN A CASE OF ACROMEGALY HIGHLY RESISTANT TO TREATMENT

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Background: Pasireotide is a novel somatostatin analogue with higher affinity to 4/5 known human somatostatin receptors subtypes. It could have utility in acromegalic patients not responding to available treatments, although a deterioration in glucose metabolism is described during its use.

Case: We describe our experience in the management of a case of a 41 years-old man affected by mixed GH-PRL secreting pituitary macroadenoma (32 mm) high resistant to different treatments. At baseline, the patient showed GH nadir after OGTT 26 ng/ml, IGF-1 1369 ng/dl, PRL 2386 ng/ml, normal glucose tolerance (NGT) with HbA1c 5.9%. Eight months of pre-operative treatment with monthly lanreotide LA 120 mg and weekly cabergoline 0,5-1 mg were ineffective in improving biochemistry and tumor mass (GH nadir 45 ng/dl, IGF-1 908 ng/ml, PRL 589 ng/ml; tumor mass 30 mm), but have slightly worsened the GT (IFG + IGT, with HbA1c 6,8%). After surgery, no significant improvement was achieved (GH nadir 12 ng/dl, IGF-1 952 ng/ml, HbA1c 6.1%, unchanged tumor mass). After 9 months of poor response to monthly octreotide LAR 30 mg and weekly cabergoline 1.5-2.0 mg (GH nadir 15 ng/dl, IGF-1 758 ng/ml, PRL 201 ng/ml; tumor mass 28 mm, IFG + IGT with HbA1c 6.5%) and 6 months of the daily dose of pegvisomant 10-15 mg (IGF-1 655 ng/ml, unchanged tumor mass, IFG with HbA1c 6.1%), pasireotide LAR was started 5 months ago at the monthly dose of 40 mg. To date, a slight not complete biochemical response was obtained (GH nadir 9 ng/dl, IGF-1 408 ng/ml, PRL 41 ng/ml, tumor mass 25 mm) but an unexpected improvement in GT was shown (NGT, with HbA1c 6.0%), likely related to the trend of reduction in GH levels.

Conclusion: Pasireotide could be a useful therapeutic option in acromegaly not necessarily associated with a worsening in glucose metabolism.

PP103 - METABOLIC EFFECTS OF GH THERAPY IN ADULTS WITH GH DEFICIENCY: A 2 YEAR PROSPECTIVE STUDY

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Background Growth hormone deficiency (GHD) in adults is associated with adverse metabolic profile, while data about the metabolic effects of GH treatment are controversial.

Aim To evaluate the metabolic influence of GH replacement in GHD.

Subjects and Methods Data of 65 patients (35 M, age 54 ± 18 yrs) were analyzed. Before GH therapy and yearly up to 2 years we measured BMI, WC, visceral adiposity index (VAI), IGF-1, lipide profile, HbA1c and basal insulin-secretion (Homa-β) and -sensitivity (Homa-IR) indexes.

Results No subject showed overt dysglycemia at baseline and during GH therapy. Hypertriglyceridemia was detected in 36 patients at baseline and in 31 and 20 respectively at 12 and 24 months, while low HDL cholesterol was detected in, respectively, 25, 17 and 8 patients during follow-up. Compared to baseline, WC and VAI significantly decreased after 12 ($p<0.001$) and 24 months ($p<0.001$), without changes in BMI. IGF-1 (both $p<0.001$), total-(both $p<0.001$), LDL-cholesterol (both $p<0.001$) and triglycerides ($p=0.84$,<0.001 and <0.001, respectively), significantly decreased from baseline to 1 or 2 years, with an increase in HDL (both $p<0.001$). Insulin, Hba1c, and Homa-IR increased (all $p<0.001$), while a slight increase in glucose was shown only at 24 months (89.1 ± 13.6 and 92.4 ± 13.9 vs.88.1 ± 14 mg/dl; $p=0.85$, 0.65 and <0.001, respectively). Homa-β did not significantly change. The favorable changes in lipid profile was more closely related to change of VAI than IGF-1 (data not shown).

Conclusions In adults GHD, a slight deterioration of insulin sensitivity, although without overt worsening in glucose metabolism, seems to occur during the first 2 years of GH treatment and it appears well balanced by a significant improvement in body composition (VAI and WC) and lipid profile.

PP104 - INSULIN SECRETION AND SENSITIVITY DURING PASIREOTIDE TREATMENT IN PATIENTS WITH RECURRENT CUSHING DISEASE: A CASE SERIES

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Background: Pasireotide is currently recommended in patients with persistent or recurrent Cushing disease (CD) after surgery and has demonstrated long-term effectiveness for the biochemical control and clinical improvement of patients with CD. However, it is associated to high frequency of hyperglycaemic adverse events.

Aim, materials and methods: In a prospective study, we evaluated the effects of pasireotide on glucose metabolism and insulin secretion and sensitivity in a group of five patients with CD recurrence. Anthropometric, metabolic and hormonal parameters were evaluated at baseline, after 6 and 12 months. We also studied M value during euglycemic hyperinsulinemic clamp, HbA1c and AUC2hc-peptide during meal mixed test.

Results: Significant differences were observed between HbA1c baseline and at 6 months [median (IQR): 6 (5.35-6.65) vs. 6.4 (5.7-7.05) %; p= 0.042], HbA1c baseline and at 12 months [6 (5.35-6.65) vs. 6.7 (5.75-7.15) %; p= 0.043]. A significant decrease was observed between baseline BMI and at 6 months [40.8 (28.6-51.7) vs. 35.4 (26.5-44.7) kg/m2; p=0.042], and baseline BMI and at 12 months [40.8 (28.6-51.7) vs. 31 (23.8-42.7) kg/m2; p=0.043]. A significant decrease of waist circumference (WC) was observed comparing baseline and 6 months [119 (101.5-131.5) vs. 114 (94.5-124) cm; p=0.042] and baseline and 12 months [119 (101.5-131.5) vs. 112 (93.5-121.5) cm; p=0.043]. No significant differences were observed about AUCcpeptide and M value. One out of five patients did not experience hyperglycaemia, while four out of five who were diabetic, experienced a worsening of glycaemic levels, managed by addition of GLP-1 analogues to the antidiabetic treatment.

Conclusions: Pasireotide treatment causes a mild increase of glycaemic levels, without an apparent impairment of insulin secretion and sensitivity. However, the sample of analysed patients is small and further studies on larger samples are required in order to confirm the observed results and eventually clarify the pathophysiological mechanisms of the hyperglycaemic effect.

PP105 - POTENTIAL ROLE OF RET MUTATIONS IN GLIOBLASTOMA: A CASE REPORT

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INTRODUCTION Glioblastoma multiforme (GBM) is a hide grade glioma, characterized by rapid and infiltrative growth and high level of cellular heterogeneity associated with therapeutic resistance. Missense mutations in the RET proto-oncogene are usually associated with MEN2A, MEN2B and FMTC. The association between GBM and RET mutations has never been described. CASE REPORT A 55-year-old man presented for the appearance of several episodes of headache. He had familiarity for Medullary Thyroid Cancer (mother, brother, cousin, 3 nephews) related to point mutation of proto-oncogene RET. Brain MRI showed encephalic lesion in fronto-mesial region. He underwent craniotomy. Histological examination revealed grade IV GBM. Promptly, radiotherapy (60 Gy) and chemotherapy has been started. Genetic assessments of the patients showed the presence of point mutation in codon 768 exon 13 of proto-oncogene RET. Thyroid ultrasonography showed no nodules, serum CT was 2.75 pg/ml (normal value < 11). DISCUSSION We suppose that RET constitutive activation can play a crucial role in the pathogenesis of GMN, causing uncontrolled downstream signaling. Our patient presented the mutation in the tyrosine kinase domain at codon 768 that activates RET in monomeric form. RET encodes for a transmembrane receptor for one glial derived neurotrophic factor family ligands (GFLs), GDNF, in conjunction with co-receptors, GFRα. It is well-known that RET regulates critical processes in cancerogenesis (cell proliferation, survival and growth) via activation of the MAPK and PI3K-AKT pathways. In vitro animal and human studies demonstrated that: (1) GDNF and its receptor promote the survival and differentiation of neuronal cell population and they are also involved in pancreatic cancer, biliary carcinoma and neuroblastoma. (2) GDNF may act as an autocrine or paracrine factor for gliomas and it is overespressed in glioblastoma. (3) Knockdown for GDNF and GFRα1 in rat C6 glioma cells significantly reduces proliferation of these cells. (4) Recent experiments demonstrate that glioma migration is dependent on GDNF levels and on RET-AKT activity and that tumor invasion in animal model is blocked by a RET inhibitor. We hypothesize that the constitutive activation of RET due to RET mutation of our patient activates the phosphorylation of multiple targets and signal cascade in glial cells and it is involved in GBM etiopathogenesis. Further analyses are needed to support our hypothesis.

PP106 - PITUITARY ADENOMAS: DATA FROM THE PITUITARY UNIT OF THE MAGGIORE DELLA CARITÀ UNIVERSITY HOSPITAL, IN NOVARA.

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Aim: The aim of our study was to describe clinical features of patients affected by pituitary adenoma (PA) referred to our pituitary unit.

Methods: retrospective study. We collected data from 181 consecutive patients affected by PA referring to our centre from 1994 to 2014, analyzing the clinical symptoms at diagnosis, tumour size (micro versus macroadenoma) and therapeutical approach.

Results: most of patients (n=113, 62.4%) had a macroadenoma, while 68 patients (37.6%) presented lesions < 10 mm of diameter. Regarding symptoms at diagnosis, 140 patients (77.3%) were symptomatic, in particular: 79 subjects (56.4%) showed hormonal hypersecretion symptoms, while 47 (33.6%) referred symptoms related to the compression of adjacent anatomical structures, including headache, visual or neurological alterations and 14 patients (10.0%) had both of them. On the other hand 41 patients (22.7%) had an incidentally discovered tumour diagnosed because of an imaging study performed for unrelated reasons. The distribution of each PA subtype was: PRL-oma 40.9%, non-functional pituitary adenoma (NFA) 39.2%, GH-secreting adenoma 18.3%, corticotroph adenoma 1,1% and both PRL- and GH-secreting adenoma 0.5% . Overall, we found that PA are similarly common in both sexes: 94 female (51.9%) and 87 male (48.1%), although in the GH-secreting adenoma subtype there was a male preponderance. Mean age at diagnosis was 41.0 ± 16.7 years. PRL-oma was the most frequent PA diagnosed before the age of 40 and GH-secreting adenoma after the age of 40 years, accordingly with the insidious onset of symptoms. Among acromegalic patients, 12 (36.5%) underwent primary neurosurgery, while 21 (63.5%) were treated with SSa before NRX. Both the ACTH-secreting adenomas underwent surgical treatment and radiotherapy. NFAs, 42 patients (59.2%) underwent surgery, while for 29 patients (40.8%) a conservative approach was preferred. We had 1 patient with a pituitary carcinoma (Ki-67=19%), who underwent two surgical approaches, radiotherapy and a palliative temptative with temozolomide, unsuccessfully. Considering only patients with pituitary incidentaloma (n=41), 15 (36.6%) had a macroadenoma and 11 (26.8%) underwent surgical therapy, the others were in follow-up.

Conclusions: Our data from a single Italian centre confirm the high clinical impact of pituitary adenomas in an endocrine clinic, even if real epidemiological studies are limited by the lack of population-specific registries. Interestingly, the high proportion of acromegaly in our geographical area, characterized by high petrol-chemical industrial density, could suggest a role of the environmental factors, as reported in previous series.

PP107 - EPIDEMIOLOGY OF ACROMEGALY IN EAST-SICILY AND DETECTION OF NEW CASES BY ICD-9 SYSTEM APPROACH IN THE GENERAL PRACTITIONERS' DATABASES

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Introduction: Epidemiology of acromegaly has been poorly investigated in Italy only by endocrine referral centers. We searched for acromegalic patients and potentially new cases in 27 Sicilian General Practitioners (GPs) databases by ICD-9 system.

Design and Methods: 27 out of 35 GPs from different areas of East-Sicily reviewed their patient-records by the ICD-9 system approach.

Results: 6 acromegalic patients (4M/2F, mean age 55.4±10.5 years) were identified out of 37348 individuals (prevalence: 160 cpm). Mean age at diagnosis was 45±8.7 years. All patients harbored GH secreting macroadenomas and underwent surgery, 3 cases were treated with somatostatin analogs and 1 also with pegvisomant. One case underwent radiosurgery. Diabetes mellitus occurred in 1 case, cardiomyopathy in 2, sleep apnea syndrome (OSAS) in 4, carpal tunnel syndrome (CTS) in 1 and multinodular goiter in 2. Fifteen out of 27 GPs searched for occurrence of prognatism (P), OSAS and/or CTS on the basis of ICD-9 system in 21919 individuals and found 12 patients with P, 143 with OSAS and 614 with CTS. One out 14 patients complaining of both OSAS and CTS showed increased GH and IGF-1 levels.

Conclusions: This study confirms that prevalence of acromegaly in Sicily is higher than that reported by literature and that other undiagnosed cases can be detected on the basis of ICD-9 system investigation.

PP108 - EFFECTS OF CYBERKNIFE RADIOTHERAPY TREATMENT OF PITUITARY ADENOMAS

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Introduction: CyberKnife (CK) is an emerging treatment for pituitary tumours (PT) resistant to other therapies.

Patients and methods: We report long-term CK effect on endocrine function and tumour volume in 20 PT patients (11M/10F, mean age 58.6±14.4yrs). Twelve patients harboured a non functioning adenoma, 2 an ACTH, 5 a GH (1 case of TSH co-secretion) and 2 a PRL-secreting PT. Before CK 9 patients had normal while 11 presented impaired pituitary function. CK was used as first line treatment in 3 cases. The mean follow-up period was 21.16± 16.35 months (range, 2-90 months).

Results: MRI demonstrated tumour shrinkage in 50% of patients. Tumor increase was evident only in 2 cass. Pituitary function impairment occurred in 3 of the 9 patients with previous normal pituitary function who developed isolated deficiency in 2 cases and multiple deficiencies in 1. Among 6 patients with previously multiple or isolated hypopituitarism, 2 became panhypopituitary and 1 developed a new deficit.

Conclusions: CK treatment for PT is safe and effective, ceasing tumour growth in 90%, and inducing tumour shrinkage in 50% of cases. Nevertheless, impairment of pituitary secretion was demonstrated in 30% of cases with previously intact pituitary function and in 50% of already hypopituitary patients.

PP109 - MANAGEMENT IN THE "REAL-LIFE" OF ACROMEGALY PATIENTS WITH "POOR RESPONSE" TO SOMATOSTATIN ANALOGS: AN ITALIAN SURVEY

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Somatostatin analogs (SSAs) are the milestone in the medical treatment of acromegaly, but data from the real-life suggest that a large part of acromegaly patients does not achieve full biochemical control of disease under SSAs. Indeed, the therapeutic decision-making in patients “poor responder” to SSAs is still a matter of clinical controversy. In 2013, a survey was addressed to 143 endocrinologists across Italy to disclose the management of “poor responder” acromegaly patients in the real-life and to determine whether peer-reviewed consensus statements have changed clinical practice in our Country. More than one half of endocrinologists (59,8%) defined “poor response” to SSAs by serum growth hormone (GH) higher than 1 µg/L and high insulin-like growth factor-1 (IGF-1) normalized per age, showing a good knowledge of diagnostic-therapeutic guidelines. Forty-one percent of interviewed clinicians considered resistant those patients treated with SSAs for at least 6 months with the highest conventional doses of SSAa. Interestingly, 24% of clinicians considered “poor response” to SSAs the lack of tumor mass control during treatment, consistently with the concept that in the real life, as well as in the trials and literature studies, shrinkage effect of SSAs is largely considered as a therapeutic end-point. Some heterogeneity appeared when the questions dealt with the therapeutic management of “poor responder” acromegaly patients. Only 19,9% of interviewed used pegvisomant alone, whereas 25,7% used the combination pegvisomant+SSAs and 11,8% cabergolina+SSAs. Twelve percent of interviewed used high dose of SSAs in patients “poor responders” to conventional doses. Indeed, 33,1% of interviewed had an experience with high doses of SSAs and 72,9% of them would use it in non-responding to standard dosages patients and 18,8% in patients with low surgical success, suggesting a clear role of high doses SSAs in the real-life with a good level of adoption between the interviewed clinicians. About 50% of these clinicians would use SSAs high dosages before pegvisomant treatment, and 27,4% in patients non tolerating pegvisomant. Furthermore, 72% of these clinicians believed a good or excellent safety profile of high dose SSA compared to conventional dosages. In conclusion, this survey suggests that the definition of biochemical resistance to SSAs is in line with the published consensus. However, the management of “poor responder patients” in the real-life is still a matter of controversy, often not supported by published guidelines. However, the positive opinion on high doses SSAs may be an interesting starting point for a deeper insight into the adoption of this therapeutic schedule in specific patients settings through a Delphi method, which is now ongoing.

With an unrestricted grant from Ipsen

PP110 - OUTCOME OF VERTEBRAL FRACTURE RISK IN ACROMEGALY PATIENTS UNDERGOING DIFFERENT GROWTH-HORMONE-LOWERING TREATMENTS

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Patients with acromegaly may develop skeletal fragility with high prevalence and incidence of vertebral fractures (VFs). In a recent prospective study we observed that patients with controlled/cured acromegaly had lower incidence of VFs as compared to patients with active disease. We also demonstrated that patients with controlled/cured disease maintained high risk of VFs as compared to subjects without acromegaly, in relationship with coexistent hypogonadism and prevalent VFs at the baseline. In this *post-hoc* analysis we aimed at investigating whether neurosurgery and medical therapies may have different effects on fracture risk in acromegaly patients, such as already demonstrated for other clinical outcomes. We enrolled 88 patients with acromegaly (33 females, 55 males; mean age 50, range 21-88); 10 patients were cured by neurosurgery alone, 38 had controlled disease by somatostatin analogs (SSAs) (15 naive and 23 already treated by neurosurgery) or pegvisomant while 40 patients had active acromegaly. Patients were evaluated for incidence of VFs using a quantitative morphometric approach on spine X-ray, which was performed at baseline and after 3 years of follow-up.

Patients with baseline cured/controlled acromegaly remained so for the whole study period. Among 40 patients with active acromegaly at the study entry, 26 patients had a controlled disease at the end of 3-year follow-up (19 patients treated with pegvisomant, 7 with SSAs), whereas 14 patients remained with active disease notwithstanding the treatments. After 3-year follow-up, 37 patients with acromegaly (42.0%) developed VFs. The incidence of VFs was significantly higher in patients with active disease as compared to those who had controlled/cured acromegaly at the study entry (62.5% vs. 25.0%; $p<0.001$). In controlled acromegaly, the incidence of VFs was significantly lower in patients treated with SSAs alone as first line therapy as compared to those already treated by neurosurgery (13% vs 43%; $p=0.03$), but such a correlation was lost after correction of analysis for untreated hypogonadism, which tended to be more frequent in patients treated with neurosurgery as compared to those treated with SSAs alone.

In conclusion, this *post-hoc* analysis suggests that first-line therapy of acromegaly with SSAs may provide some advantage in terms of decrease in fracture risk as compared to neurosurgery, mainly when this latter approach may cause post-surgical hypopituitarism contributing to maintain high fracture risk notwithstanding the cure of acromegaly.

PP111 - IMPACT OF PRE-TREATMENT WITH SOMATOSTATIN ANALOGS ON SURGICAL MANAGEMENT OF ACROMEGALIC PATIENTS REFERRED TO A SINGLE CENTER

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Background and Aim of the study: First-line treatment of patients with growth hormone (GH) secreting adenomas is surgical resection. Disease control can be obtained by surgery (one or multiple steps), in case followed by medical treatment, or adjuvant radiation therapy (radiosurgery or radiotherapy). The impact of pre-surgical treatment with somatostatin analogs (SSAs) on surgical outcome is still controversial.

Aim of this study is to retrospectively evaluate the impact of SSA pre-treatment on biochemical outcome and post-surgical hypopituitarism in a consecutive surgical series from a single referral center, with data covering 17 years' experience and to investigate the possible predictive value of early postoperative IGF-I on long-term biochemical control.

Patients, Methods and Results: Data from 68 acromegalic patients were revised. Endocrinological long-term follow-up (minimum 6 months) was available for 57 patients. Eighty-eight percent of patients received a single-step surgical treatment (single surgery, with or without adjuvant medical therapy). The remaining 12% underwent a multi-step strategy: redo-surgery (3 macroadenomas) and/or radiation (4 macro-and 2 microadenomas). Pre-surgical SSA treatment was performed in 77.9% and resulted in a significant lowering of basal IGF-I values (p=0.0001). Early postsurgical IGF-I was significantly lower in patients biochemically controlled with single surgery alone (p=0.016) and after overall treatment strategies (p=0.005). Normalization of GH and IGF-I was obtained in 56.1%, and normalization of either one of them in 27.8% of patients. No major surgery-related complications occurred. Post-treatment hypopituitarism occurred in 11.9% and was lower in SSA pre-treated patients.

Conclusions: Our results well compare with other recently published series. Very early post-surgical IGF-I improvement might be a useful predictor for biochemical disease control. Moreover, our results suggest that pre-surgical treatment with SSAs seems to prevent hypopituitarism.

PP112 - PANHYPOPITUITARISM OCCURENCE IN A 72-YEAR-OLD MALE PATIENT TREATED WITH IPILIMUMAB FOR METASTATIC MELANOMA: A CASE REPORT

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Ipilimumab is a monoclonal antibody currently licensed for the treatment of metastatic melanoma. It prevents the interaction between cytotoxic T-lymphocyte antigen 4 (CTLA-4) and its ligands, blocking the inhibitory signaling on cytotoxic T lymphocytes which thereby can proliferate and infiltrate tumors attacking the cancer cells. Although ipilimumab demonstrated an overall improvement in survival of patients with advanced melanoma, it can also result in immune-related adverse events such as enterocolitis, hepatitis, dermatitis and endocrine disorders. We present the case of a 72-year-old male patient with evidence of panhypopituitarism following his fourth dose of ipilimumab, administered once weekly after the radiological evidence of pulmonary and lymph nodes metastases from primitive melanoma. Basal endocrine blood tests performed at the Emergency Department of our University Hospital, where the patient came for intense fatigue and recurrent presyncopal episodes, documented undetectable TSH levels (<0.01 mU/l) associated with low values of FT4 (0.74 ng/dl), low levels of ACTH (<5.0 pg/ml) with reduced plasmatic cortisol (0.6 mg/dl), low levels of gonadotropins (LH 0.85 mU/ml and FSH 2.57 mU/ml) and total testosterone (0.28 ng/ml), decreased prolactin levels (<0.3 ng/ml) and low values of IGF-1 (69 ng/ml). Diabetes insipidus was excluded in light of the normal values of plasma (294 mOsm/l) and urine osmolality (422 mOsm/l). Neither electrolyte imbalance nor hypoglycemia were detected. Irrespective of fatigue and transient hypotension, the patient did not show any other relevant symptoms or signs of secondary adrenal insufficiency or hypothyroidism. In addition, no symptoms such as headache or visual disturbances, potentially related to the inflammatory enlargement of the pituitary gland, were detected. In contrast to what reported in most cases of auto-immune hypophysitis, magnetic resonance imaging (MRI) showed a reduction in size of the pituitary gland with anterior-posterior diameter of 8.2 mm and a maximum thickness at the pituitary stalk of 2.5 mm. For these reasons, we decided not to introduce high dose corticosteroid therapy, as current management guidelines recommend, in order to avoid potential side-effects of this regimen. Oral corticosteroids replacement was therefore initiated and adjusted according to clinical response and cortisol levels, followed by L-thyroxine replacement after few days, leading to a progressive clinical improvement of the patient. Androgen replacement therapy was introduced two weeks after the patient was discharged, whereas GH replacement was avoided because of the concomitant diagnosis of malignancy. In conclusion, ipilimumab-induced hypophysitis is a rare disease with various clinical presentation. High dose corticosteroid therapy could be restricted to patients with severe clinical presentation or substantial pituitary enlargement.

PP113 - GENDER AND METABOLIC DETERMINANTS OF FRACTURE RISK IN CUSHING'S SYNDROME

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In Cushing's syndrome (CS), the excess cortisol leads to a clear increase of fracture risk. There are also systemic complications: cardiovascular damage, dyslipidemia, diabetes mellitus and hypogonadism. Our purpose is to evaluate the prevalence of fragility vertebral fractures and their association with other metabolic effects of hypercorticism. In our study we considered 51 patients suffering from CS that in addition to the usual diagnostic tests, had performed a spine RX, an ultrasound carotid doppler and a metabolic evaluation (blood glucose, insulin, cholesterol, HDL cholesterol and LDL, triglycerides, uric acid). The fractures were evaluated by Genant method. The patients performed a DEXA of lumbar spine and femoral with an evaluation of bone metabolism: creatinine, serum calcium, phosphorus, bone alkaline phosphatase (BAP), PTH, serum cross laps, DPD, 24h urinary calcium . 57% of our patients presented fragility fractures: 14 males (82%) and 15 females (44%). The variables significantly related to fractures were: male gender ($p = 0.0057$), presence of calcified plaques ($p = 0.0260$), disease duration ($p = 0.020$), waist ($p = 0.040$) and the T-score lumbar spine ($p = 0.0482$). We found a positive correlation between 8 h plasma cortisol, triglycerides and Z-score lower back. This positive correlation between cortisol and Z-score, the higher incidence of fractures in men, whom present more altered metabolic indices, and the close association between bone damage and indices of atherosclerosis suggest that bone fragility in CS is more closely correlated with qualitative changes than quantitative.

PP114 - ALTERNATIVE TREATMENT OF CUSHING'S DISEASE: A PECULIAR CASE

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INTRODUCTION: management of Cushing's syndrome (both ACTH dependent and independent) continues to be challenging. The treatment of choice, specially when a pituitary microadenoma is detected, is surgery. Surgical approach is mainly via the transsphenoidal route. Rarely the presence of anatomical abnormalities could block the neurosurgical approach and pose the necessity of an alternative strategy

CASE: A 37 years old woman came to our attention for the evidence of high levels of plasmatic and urinary free-cortisol (CLU) detected for an important increase of weight gain (about 20 kg in a period of about 4 years) in a clinical picture of severe obesity (BMI 45 Kg/m^2). Patient complained of depressive syndrome with suicide attempt, amenorrhea and hypertension. Physical examination revealed moon face, central obesity with red strie and buffalo hump. Diagnostic evaluation comprised low and high dose dexamethasone test (with no suppression of cortisol levels) in association with high levels of ACTH (101 pg/ml). Pituitary MRI showed a microadenoma (diameter 8 mm) and the inferior sinus petrosal sinus sampling (IPSS) point out data compatible with a pituitary origin of ACTH hypersecretion.

The pre-surgical TC of maxilla-facial zona to evaluate and plan the neurosurgical approach showed, unexpectedly, a failure of pneumatization of the frontal and sphenoid sinus. The presence of these anatomical disorder was a condition at risk for the transphenoidal surgery. Patient began treatment with ketoconazole with little results.

We therefore decided to carry out a combined treatment with stereotactic radiotherapy and pasireotide, tapering and stopping ketoconazole. After one year we could reduce pasireotide reaching normal value of cortisol and CLU with a general improvement in well-being (specially mood), weight reduction, recovery of menses and a good improvement in metabolic profile. Moreover the pituitary MRI didn't show the focality previously described..

CONCLUSION: In Cushing disease the diagnostical work out should be take in account the possibility of anatomical variant that could be a high risk factor for the neurosurgical approach. In these rare cases a multimodality alternative approach could allow a similar good result.

PP115 - VERY EARLY RESPONSE TO SUNITINIB IN VHL PATIENT WITH PANCREATIC NEUROENDOCRINE TUMOR.

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Von Hippel Lindau (VHL) disease is an inherited, autosomal dominant syndrome caused by mutations in the tumor suppressor VHL gene. Patients can develop various benign and malignant lesions: central nervous system and retinal haemangioblastomas, renal cell carcinomas and cysts, pheochromocytoma, epididymal and broad ligaments cystoadenomas and pancreatic cyst or tumor.

Pancreatic neuroendocrine tumor (pNET) arise in 8-17 % of VHL patients and surgery plays a key role for resectable lesions. Alternative medical therapy can be used when surgery is unfeasible. Among targeting drugs, sunitinib, that inhibits several thyrosine kinase receptors, including VEGFR, has recently been approved for treatment of pNET.

Here we present the case of 32 years old female diagnosed with VHL during radiological investigations for secondary hypertension. CT scan displayed a major nodule in the pancreatic body (62x71x45 mm) and similar lesions in the head (max 27 mm) with vascular invasion. Moreover, bilateral renal cysts and solid lesions consistent with renal carcinoma were detected. The diagnosis of pNET was confirmed by endoscopic ultrasound fine needle aspiration (EUS-FNA). Brain MRI revealed multiple cerebellar and spinal hemangioblastomas. Disease staging was completed by ^{68}Gallium positron emission tomography (^{68}Ga-PET) that revealed only pancreatic uptake. Genetic testing showed a heterozygosis mutation (p.Asn131Thr). Pancreatic lesions were judged unresectable, then, considering the young age and the high tumor burden, medical therapy with sunitinib (37.5 mg/day, 4 weeks on + 2 weeks off) was started. After only 12 days of treatment, the patient was admitted to the emergency due to severe abdominal pain and nausea. Surveillance CT confirmed the same size of major pancreatic lesion, but displayed an increased hypodensity, suggesting a colliquative area. Tumor density, on the portal venous phase of CT, measured 85 HU compared to 133 HU in the prior treatment CT, with a density reduction of 36%. According to RECIST criteria this is considered as stable disease, while with CHOI criteria this is considered a partial response. Target therapy, as well as VEFGR inhibitors, may often modify tumor density and this is not adequately captured by RECIST criteria. Therefore, specific radiological criteria that can better evaluate the effects of target therapy in NETs are strongly needed. According to the literature, this is the first case of documented early (< 8 weeks) response to sunitinib treatment in VHL patient.

PP116 - EFFECTIVENESS OF A MULTIMODAL TREATMENT IN A CASE OF MALIGNANT INSULINOMA

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Malignant insulinoma (MI) is rare (0.4 cases per milion /year). Treatment is aimed to cure the neoplasm and control blood glucose levels (BG). Surgery, Nonsurgical liver-directed therapy [(NSLDT) -radiothermal ablation (RFA), Hepatic artery embolization (TACE)] and Cytotoxic Chemotherapy (CCT) are effective therapies, while SSRAs are less useful since they potential worsen hypoglycemia. Everolimus (EV) is particularly effective in MI due to its hyperglycemic action. Arterial radioembolization with Y-90 microspheres (TARE) has been anecdotally reported for the treatment of liver mts in MI. We report the case of a patient with a MI in whom a multimodal treatment leaded to long-term survival. In November 2011 SG (35 yrs) was admitted to our department for recurrent episodes of insulin mediated hypoglycemia (mean glucose =20-30 mg/dl, mean insulin 120 mU/ml). CT of the abdomen showed multiple liver mts and a pancreatic node of 6 cm. Citology on a liver mts diagnosed a NET. On Diazoxide (600 mg/die) and Octreotide (0.6 mg/die) BG were normal. He then underwent spleno-distal pancreasectomy and enucleresection of liver mts in January 2012. Istology confirmed the diagnosis of giant cell neuroendocrine carcinoma (Ki 67 =40%). After the evidence of a liver progression of disease (PD) at CT, in june 2012, EV 10 mg/die was started, leading immediately to achieve normal BG, that lasted until may 2013: serial CTs stable disease. In june 2013, hypoglycemic episodes reappeared with increased insulin (200 mU/l). CT confirmed liver PD. TACE of liver mts in July 2013 restored normal BG and was repeated in Nov 2013 and mach 2014.High insulin (500 mU/l) with normal BG were recorded on EV 10 mg/day. The worsening of hypoglycemic episodes and PD at CT in May 2014 lead to stop EV, and to start CCT (cisplatin + etoposide). After 3 cycles, CT displayed PD at and the patient was hospitalized for continuous iv dextrose. Neither another TACE nor CCT with streptozotocin (3 cycles) lead to clinical response. In October 2014. CT confirmed marked PD, then EV treatment was restored with the aims to increase insulin resistance and radiosensitivity. EV treatment induced a reduction in the need of dextrose (from >1000 to 100 g/d). TARE–SIRTEX was performed in November 2014 delivering 1.4 GBq dose to liver mts. Dextrose infusion was stopped within 48 hours and patient was discharged from hospital. 2 months after TARE SIRTEX while continuinig EV, BG were normal but insulin values high (900-1200 U/l). The CT of 20-01-2015 was stable. Oral cytotoxic treatment with capecitabin and temozolomide (CAPTEM) was started. The treatment of MI requires a Multimodal approach. Surgery, NSLDT and CCT are crucial, but EV seems to be effective also when its cytotoxic effect disappears in increasing insulin resistance and pheraphs radiosensitivity. TARE in liver mts from malignant insulinoma was feasible safe and effective.

PP117 - PREVALENCE OF HYPONATREMIA IN HOSPITAL SETTING: AN ITALIAN EXPERENCE.

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Hyponatremia is the most common electrolyte disturbance encountered in clinical practice. It is associated with poor clinical outcomes, increased mortality, morbidity and length of hospitalization. Its prevalence in hospitalized patients is highly variable in different series, up to 38 and 42% in the two largest ones collected in Toronto and Singapore respectively. Very few data are available for Italian hospital where a different situation may be present due to different health care sistem conditions. Aim of the present study is to verify the prevalence of hyponatremia, its treatment and outcome in a generalist universitary hospital in a large Italian town. METHODS: observational retrospective study. Data on all the sodium measuremts perfomed during a 3 months period in 2014 were obtained from the central lab of S. Giuseppe hospital, a 200-bed generalist urban university hospital in Milan. According to the more recent guidelines hyponatremia was defined as PNa<135 mmol/L, with moderate hyponatremia defined as PNa<130 and severe hyponatremia as PNa<125. The analysis is on going and only preliminary data will be here presented. RESULTS: Sodium values were tested in 1539 in-side hospital patients out of a total of 2499 cases (62%), and in 1280 emergency room patients out of a total of 6499 admissions (20%), of which 1033 yellow and red codes. Overall the prevalence of hyponatremia was 8.7% (2.5% moderate or severe) among in-patients and 9.7% (2.1% moderate or severe) among emergency room patients. Sodium was measured in almost all the patients admitted to the units of internal medicine, endocrinology, neurology, and pneumology, but in less than one third of most of the other units. Patients with hyponatremia belonged more often to the units of oncology (25 %), internal medicine (23%), epatology (19 %) general surgery (16%), endocrinology and diabetology (14%), cardiac rehabilitation (13%) and respiratory rehabilitation (11%). CONCLUSIONS: The prevalence of hyponatremia in our experience was lower than expected. However the small number of sodium tested in many units suggests that hyponatremia remains under recognized and under diagnosed. Ongoing analysis focused on clinical and treatment data of patients with low sodium levels will provide informations on morbidity (in particular cardiovascular) and outcomes associated to hyponatremia in an italian reality.

PP118 - SLEEP APNEA SYNDROME IN ACROMEGALIC PATIENTS: WHICH PREDICTORS?

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The sleep-disordered breathing affects the majority of acromegalic subjects. The sleep apnea syndrome (SAS), diagnosed by polysomnography, is described in about 70% of patients with active disease; in the majority of the cases, the cause is obstructive (OSA). The high levels of GH and IGF-1 cause hypertrophy of the soft tissues of the upper airways and it alters irreversibly the skeletal structures. We selected 21 acromegalic patients (10 males and 11 females), attending our Center, aged between 36 and 77 years (57 ± 8.8), on the basis of the daytime sleepiness medical history's findings and/or Epworth index ≥ 10. The duration of illness was respectively < 10 years in 4/21 subjects and > 10 years 17/21 subjects. 8/21 patients did not show a biochemical control of disease, 13/21 had normal levels of IGF-1 age-related. The mean BMI was 39.59 ± 3.6 kg/m². The enrolled patients were submitted to polysomnography and 24 hour ambulatory blood pressure monitoring. 13/21 showed 24 hours high blood pressure levels and 8/21 patients did not show the physiological nocturnal blood pressure decrease (non dipping pattern: diurnal/nocturnal means ratio < 1).

Results: 15/21 patients had an AHI > 5, of which 4 had an AHI > 30 (severe OSA), 4 between 15 and 30 (moderate OSA) and 7 between 5 and 15 (mild OSA). We did not observe a significant difference between the prevalence of OSA in patients with disease control and those without disease control, while we found a correlation between the presence of OSA and disease duration (in 12/17 with disease duration > 10 years, P = 0.0016), and a trend of significance between the prevalence of OSA and non-physiological decrease nocturnal blood pressure (6/8 "non-dippers"). **Conclusions:** The SAS is the main respiratory complication of acromegalic patients and it worsens the prognosis by mean of other complications (cardiovascular risk, diabetes). The 24hour blood pressure monitoring non-dipping pattern could also play a significant role as an additional prognostic predictor. The PSG is therefore the gold standard diagnosis of OSAS and should be part of the screening protocol for acromegalic patients, especially if presenting a blood pressure nocturnal "non-dipping" pattern.

PP119 - KI 67 LI AND PROGNOSTIC FEATURES IN 51 NON FUNCTIONING PITUITARY ADENOMA (NFPA): RETROSPECTIVE ANALYSIS

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Patients and Methods. 51 patients (34 M/17 F) aged from 17 to 78 years old (Mean 53.5 ±15.5) underwent to transsphenoidal adenectomy from 2001 to 2011.

Tumor size was between 18 and 32 mm in maximum diameter (Mean19.7±7mm). Adenomas were intrasellar in 9.8% of cases,suprasellar in 23.5%,parasellar in 17.6%,supra and parasellar in 49%;extension to the cavernous sinus was in 33 parasellar adenomas (64.7%),with Knosp index>3 in 13 cases (25.4%). All tissue specimens were examined for anterior pituitary hormones, p53,and Ki-67 MIB-1 monoclonal antibody. Atypical adenomas were excluded. Immunohistochemistry revealed in 62.7% non immunoreactive adenomas,in 15.6% expression of LH/FSH,in 5.8% plurihormonal expression,in 7.8 % PRL, in 3.9% GH,in 2.1% TSH,and in 2.1% ACTH. Mean KI67LI values were 2.04±1.37 SD. Patients were followed with postsurgical MR after 3,6,12 months and every year,and observed within 5 years. KI67LI was compared between the group of NFPA with progression and NFPA without progression. Other tumor characteristics (size, cavernous sinus invasion,suprasellar extension,extent of the removal) were also compared. **Results.** Radical excision was obtained in18 NFPA (35.2%):16 cases cured within 5 follow up years,1 NFPA relapsed after 24 months. Partial excision was obtained in 33 NFPA (64.8%):17 cases (33.3%) cured;16 cases (31.7%) in progression of disease within 41±11 months. Postsurgical progression rate was 33.3%. No statistical difference in KI67LI between 17 NFPA in progression and 34 NFPA not in progression was observed. No significant correlation between KI67LI and size of adenoma,parasellar and cavernous sinus involvement but significant correlation between KI67LI and suprasellar extension (P=0.016) was confirmed. Comparing NFPA in progression vs NFPA not in progression,was also observed a statistical correlation between prognosis and tumor size (P=0.002) and between prognosis and partial excision (P=0.025). There was no significant correlation between prognosis and extension and cavernous sinus involvement and invasion (Knosp index 3-4). **Conclusions**. The results demonstrate no statistical difference in KI 67 LI between recurrent and non recurrent NFPA:in this series it can't be established a threshold for prognostic purposes. Tumor size was the only feature related to prognosis. Radical excision remains the primary purpose,so because of postsurgical progression rate,a stereotactic radiotherapy of residual tissue could be individualized. We recommend a more closely follow up in case of "wait and see" approach. It is important to assess in this series the long-term follow-up.

PP120 - MEDICAL THERAPY IN A CASE OF SACCULAR ANEURYSM OF THE LEFT CAROTID SIPHON THAT COMPRESSES THE CAVERNOUS SINUS AND IS SURROUNDED BY A GIANT PROLACTINOMA

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Introduction: Characterized by large size (>40mm in diameter), high aggressiveness and massive extrasellar involvement, giant prolactinomas are a rare subset of macroadenoma usually associated with high serum PRL levels (>1000ng/ml).An intracranial aneurysm can be up to seven times more frequent in patients with pituitary adenomas than with any other type of brain tumor.However, an internal carotid artery aneurysm totally embedded by a macroprolactinoma is rare.3 similar cases are reported in literature.**Case Report:**A 73 years old woman was admitted to the Department of Neurosurgery of our Hospital for diplopia in the left eye and transient headaches.**Clinical history**:Amenorrhea since the age of 27, not investigated; arterial hypertension. **Angio-MR**:"Saccular aneurysm of 24x18x16mm at the origin of the left carotid siphon, flexed in the sphenoid sinus and the sella turcica.Presence of a large alteration of the signal, with irregular margins and expansive process of erosion, solid and with its epicenter at the level of the sphenoid".Patient underwent a biopsy of the lesion.**Histopathology:**Prolactinoma, intermingled with accumulation of amyloid-like amorphous material.Immunohistochemistry was positive for PRL and estrogenic receptor, with KI 67 L.I. of 2%.Surgical adenectomy was contraindicated so an observation of symptoms was proposed and, in case of worsening of the clinical status, a flow diversion with stent.Low-dose cabergoline, 0.5mg twice a week, was chosen.Patient was monitored with visual field examination.The lowest effective dose was administered to normalize serum PRL and avoid a rapid shrinkage of the lesion that could lead to aneurysm rupture.After 6 months of medical therapy, visual symptoms and cephalalgia did not worsen.Serum PRL was 45ng/ml and 1 year after was normalized: 16ng/ml.A **MR** showed a slight increase in size of the saccular aneurysm (27x24x20mm) while the size of the adenoma was unchanged so an endovascular flow diversion with stent was performed.**Discussion:**In giant prolactinomas, targets of medical therapy are shrinkage of the lesion and normalization of serum PRL.First-line therapy are dopamino agonists and cabergoline is the best choice.Some giant prolactinomas are responsive to low-dose cabergoline: in our case we had only normalization of serum PRL within 1 year.Usually, the drop in PRL from treatment's onset ranges from several months to more than 2 years.This variability is not related to tumor size, baseline PRL level or tumor shrinkage.We chose a cautious approach in titration of cabergoline to avoid aneurysm rupture in case of shrinkage.

PP121 - GIANT PROLACTINOMA IN A "OLD-OLD" PATIENT

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R.V. male 86 years (Pt) married without children, performs investigations to worsening of headache and asthenia. In history: hypertension, type II diabetes, hyperuricemia; severe chronic renal failure, aortic valve disease. Physical examination is negative. The MRI showed "dysmorphic clivus, replaced by iso-hypo-intense (T1) and hyperintense (T2) tissue with contrast-enhancement"; invasion of the sphenoid sinus and carotid siphons. Proliferative lesion is suspected and the Pt arrives in Oncology. Blood tests show: hyperglycemia, anemia and high level of prolactin (PRL) seriata: 2480 ng / ml (4.60-21.40); TSH = 6.37 UUI / ml (.25-4.20), ACTH, cortisol, GH, FSH and LH at lower limits; Testosterone level is reduced. The diagnosis is PRL-secreting pituitary macroadenoma (MA). Counseling excludes neurosurgical indications. It starts with cabergoline therapy: 0.5 mg / week for 15 days, and then 1 mg / week. After 30 days PRL: 1200 ng / m; 60 days: 376 ng / ml. The Pt refers progressive disappearance of headache and asthenia improvement. The dosage of cabergoline is reduced to 0.5 mg / week. PRL concentration is: 129 ng / ml at 4 months and 32 ng/ ml to 7 months. The TSh remains constant: 5.92 UUI / ml with negative AbTPO and AbHtg and normal thyroid ECO. The Pt performs a RM control that highlights "significant reduction in size of the lesion with increased signal in T2 and T1 following the increase in the fibro-fatty component".

Conclusion: PRL-secreting pituitary macroadenomas are very rare in elderly subjects; in this Pt is very likely that the adenoma is occurred many years before, probaably causing infertility and has shown symptoms when it reached the size of "giant adenoma" with very high PRL and exerting mass effect. In the literature it is described a similar case occurred in a 80 years old Patient. The PRL-secreting adenoma is mainly described in young adult women with amenorrhea but should also be suspected in males of all ages showing neurological symptoms such as headache, asthenia and reduction of the libido, due to possible hyperprolactinemia.

PP122 - PRELIMINARY RESULTS OF A PROSPECTIVE PHASE II TRIAL WITH HIGH DOSES LANREOTIDE IN PATIENTS WITH PROGRESSIVE NEUROENDOCRINE TUMORS

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CLARINET study has recently provided further evidence on the antiproliferative effect of somatostatin analogs (SSA) in neuroendocrine tumors (NET). In patients (pts) with progressive metastatic NET, dose escalation or reduction of the interval between injections is common in clinical practice. However, to date, no systematic prospective studies have been carried out to evaluate the safety and the efficacy of this treatment schedule in NET, while high doses of SSA have been already shown effective in resistant acromegaly.

Here we present the preliminary results of the first multicentre, prospective, open label, single arm phase II study, with high doses of lanreotide ATG (180 mg/28 days for 12 months) in pts with progressive (PD) NET under standard maximal doses of SSA. Primary endpoint was safety, while secondary was efficacy.

The planned recruitment of 35 pts was completed in November 2014. Mean age was 63±11 years, 16% thoracic and 84% gastroenteropancreatic NET were enrolled and -49% were G2. Pts entered the study with radiological PD in 94% of cases. To date, 6 pts completed the study and the trial is still ongoing. Eight serious adverse events (SAE) in 6 pts have been recorded so far, including 2 treatment-related (cholelithiasis and consequent cholecystitis, considered as 2 SAE, although occurred in the same pt and at the same time), with a SAE frequency rate of 23%. Statistical analysis for the verification of primary endpoint (safety), with the binomial test (null hypothesis value at 65%), has shown that treatment with high dose lanreotide is safe ($p < 0.0001$).

At moment, minor considerations can be made about the secondary endpoint (efficacy), since only 6 pts completed the trial. However, all these 6 pts were in stable disease at the end of study. Pts prematurely dropped out, have been analyzed for different factors, in order to find a potential correlation with the withdrawal from the study.

PP123 - KI-67 IS NOT A PREDICTOR OF RESPONSE TO PEGVISOMANT IN SSA-RESISTANT ACROMEGALY

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Previous studies showed that Ki-67 labeling may predict clinical outcome and somatostatin analogs (SSA) response in acromegaly. No data are available about pegvisomant (PEGV) treatment and proliferative markers. Therefore, we evaluate whether Ki-67 is a predictor of pegvisomant response in somatotropinomas resistant to SSA

We selected 27 consecutive acromegalic patients referred to our hospital during a 7-yr period. The Ki-67 index was determined by immunohistochemistry on tissue samples obtained from each adenoma after neurosurgery. All patients, who were not completely cured after surgery and after at least 1 year of SSA, began medical therapy with PEGV. Periodical pituitary magnetic resonance imaging and hormonal evaluation were performed during the follow-up.

No correlation was found between Ki-67 index and age, tumor size and extension, GH, or IGF-I plasma levels. There was no difference in Ki-67 levels between patients controlled and not controlled with PEGV, but in not controlled IGF-I levels before PEGV were higher than in controlled one. Final daily PEGV dose was significantly increased in not controlled patients.

In conclusion, in acromegalic patients resistent to SSA, Ki-67 not appear to be a predictor of response to Pegvisomant.

PP124 - THREE NEW CASES OF ADIPSIC HYPOTHALAMIC DIABETES INSIPIDUS AFTER CLIPPING OF ANTERIOR COMMUNICATING ARTERY ANEURISM: A COHORT STUDY.

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Introduction. In general, the estimated incidence of central diabetes insipidus after the rupture of cerebral artery aneurysm seems to be very low (0.04% in accordance with some previous studies). In addition, over the last 25 years some sporadic cases of adipsic hypothalamic diabetes insipidus (AHDI) after clipping of a ruptured aneurysm in an anterior communicating artery have been reported. This is a potentially life threatening condition, because severe hypernatraemia may develop in the absence of thirst and increased fluid intake. Accordingly, recent data show that adipsia increases the risk of death in patients with diabetes insipidus, independently from its cause. *Methods/design*. We retrospectively reviewed the records of 19 consecutive patients (11 females; male to female ratio: 0.727; median age, 60 yrs, range 36-79 yrs) who underwent clipping of an aneurism in an anterior communicating artery from January to December 2014. Of these, 12 (63.6%) had a ruptured aneurism, while the remaining 7 (36.4%) underwent clipping of an intact aneurysm. All patients were tested for anterior pituitary function. In addition, urine volume, fluid intake, fluid balance, urine osmolality, plasma osmolality, plasma sodium, and routine laboratory tests were carried out. In the patients who had no desire to drink despite a serum osmolality in excess of 300mOsmol/kg, hypertonic saline infusion test was performed. *Results*. No patients had anterior pituitary dysfunction. All the 3 patients with a confirmed diagnosis of AHDI were females. What's more, each of them was treated by clipping of an intact aneurysm of an anterior communicating artery in the absence of subarachnoid haemorrhage. *Conclusion*. Previous studies reported sporadic cases of AHDI after clipping of anterior communicating artery aneurisms. In these studies, subarachnoid haemorrhage was almost always present either before treatment or during the procedure. Our cohort study clearly shows that subarachnoid haemorrhage cannot be the only mechanism responsible for the development of AHDI after clipping of anterior communicating artery aneurysm. By contrast, it is the surgical procedure per se that can facilitate this event, which seems to be not so rare as previous studies had suggested. Actually, the vascular supply of the organum vasculosum laminae terminalis, the putative site of the osmoreceptors, derives from small perforating branches of anterior cerebral and anterior communicating arteries. As a consequence, it is not unlikely that clipping of the haemorrhaging anterior communicating artery aneurysm may compromise the blood supply to the osmoreceptors cells. What's new is that also clipping of an intact aneurysm of an anterior communicating artery may lead to the same result.

PP125 - THE ASSOCIATION BETWEEN SIADH AND DIGEORGE SYNDROME:

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INTRODUCTION: We report the clinical case of a patient affected by DiGeorge Syndrome and Syndrome of inappropriate antidiuretic hormone secretion (SIADH). The DiGeorge Syndrome (microdeletion of chromosome 22) is characterized by hypoplasia of the thymus and parathyroid glands, congenital heart disease and facial dysmorphisms. SIADH characteristics are hyponatremia, plasma hypoosmolarity and hypertonic urine.

CASE REPORT: A 19 years old patient came to our observation for follow-up of SIADH, diagnosed at the age of 17 months. At birth had cleft palate, polydactyly, interventricular difect and peculiar facial dysmorphisms (hypertelorism, elongated palpebral fissures and tubular nose). A molecular karyotype analysis was performed: "two partial duplication of the region 12q24.33, containing respectively 6 and 2 OMIM genes and a partial monosomy of 22q11.2 region containing 12 genes that partially covers the critical regions for the Cat-Eye Syndrome and DiGeorge". At the admission laboratory tests performed in fluid restriction (250-300 cc/day) showed serum sodium at the lowest concentrations (137 mEq/L) with urinary hyperosmolarity (1276 mOsm/kg). MRI of the pituitary gland showed "absence of physiological pituitary hyperintensity". A positive HCV-Ab (genotype 1b) with normal liver function was found. After gradual increase in fluid introit, a treatment with tolvaptan 15 mg, a selective antagonist of the vasopressin V2 receptor was started. In a few days a good fluid balance (1.5 L/day, in and out) and the stability of the electrolyte profile (Na^+ 140 mEq/L) was obtained.

DISCUSSION: The treatment with tolvaptan was well tolerated even in the presence of a HCV-related liver disease and, by avoiding the water restriction, it has greatly improved the quality of life of the patient. Being the absence of the pituitary hyperintensity most frequently associated with diabetes insipidus, genetic and molecular analyses are ongoing to clarify the underlying mechanism of SIADH in our patient. One hypothesis is that genetic mutations identified might involve crucial regions for an adequate biological action of vasopressin or for the proper functioning of aquaporins channels.

CONCLUSIONS: The tolvaptan resulted effective and safe in our patient with DiGeorge Syndrome, SIADH and liver disease. Additionally, by avoiding the fluid restriction and reducing the risk of hyponatremia, it has improved the clinical status and quality of life of the patient.

PP126 - EATING HABITS AND MEDITERRANEAN LIFESTYLE IN SANZA' S POPULATION(SALERNO, ITALY)

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After the studies of the eating behaviors of Sapri and Centola's students, we examined the community of Sanza, a small town in the valley of Bussento near of Mount Cervati (National park of Cilento and Vallo di Diano). We used to make these exams the questionnaire "the Evaluation of visceral adiposity index and adherence to the Mediterranean diet". This questionnaire was suggested by "Campus della salute onlus", Department of Endocrinology, Molecular and clinical Oncology of University Federico II of Naples and National Cancer Institute of Naples ,G.Pasquale Foundation Service of Epidemiology ".

In the early 80s of the last century, the clinical-epidemiological studies of "Naples Medical Clinic of New Hospital" noticed some problems in that population like glucose intolerance, diabetes and high levels of the blood pressure and also a modification in eating habits in a typical rural population that was changing.

The sample: 763 people, 460 women and 303 men - mean age: 51 years old.

The sample showed good adherence to the Mediterranean way of life by the evaluation of a final score obtained by the sum of the partial scores defined for each question.

The results of this interview was compared with a sample of Naples which examined during the American's Cup edition.

More than half of population eats pasta only 3 times a week while white bread was preferred to integral one and consumed daily. Most consume meat at least three times a week and prefer the fresh meat to the sausages.

The red meat is preferred by the men, while the white meat is preferred by the women. The people eat fish few times a week (< 3 times) and only the 22% eat vegetables every days and the women under 30 eats more then men. Half sample and more drinks wine every days, while the 38% of the sample doesn't answer to the question(3/4 the people who can't answer are women). It should be emphasized the almost exclusive use of olive oil as a sauce. The 72% spends sedentary life but the 81% have breakfast in the morning. The 95% eats fruit and season vegetables and the 87% knows the benefits of the Mediterranean diet. There is also a good control of the own weight. The 56% says to consume iodized salt. The adult people consume more iodized salt than the old people.

PP127 - EFFECTS OF FAT DISTRIBUTION ON HYPERTROPHIC CARDIOMYOPATHY PHENOTYPE

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Introduction: Hypertrophic cardiomyopathy (HCM) is the most common genetic heart disease, characterized by heterogeneous phenotypic expression with extreme diversity in the pattern and extent of left ventricular (LV) hypertrophy. Among extrinsic factors, body mass index (BMI) and male sex were found independently associated with LV mass increase and progression of heart failure symptoms in HCM. The aim of our study was to assess whether adipose tissue distribution may influence cardiac morphology and function in HCM patients with respect to gender.

Methods: We studied 24 overweight/obese subjects (age 59.4±12 yrs, 9F/14M, BMI 29.3±3.7 Kg/m^2) with echocardiography- and cardiovascular magnetic resonance-based diagnosis of HCM, confirmed by genetic analysis. Dual-energy X-ray absorptiometry was used to evaluate body composition and regional (trunk and appendicular) fat distribution.

Results: Age- and gender-adjusted analysis revealed that maximum interventricular septum thickness but not LV mass index was positively associated with trunk adipose tissue percentage ($\beta=0.41$, $p<0.05$). However, septum thickness showed a stronger association with male gender ($\beta=0.61$, $p<0.01$) compared to abdominal adiposity. In constrast, total and appendicular fat percentages were not related to any measure of LV mass, volume and function. Instead, BMI was associated with left atrium area ($\beta=0.45$, $p<0.05$), an indirect measure of LV dysfunction, irrespective of both age and gender.

Conclusion: In our cohort of HCM patients septum hypertrophy extent was independently associated with abdominal but not total fat. We also confirmed that male gender represents another important feature associated to the magnitude of hypertrophy.

PP128 - PHARMACOLOGICAL TREATMENT OF NONALCOHOLIC FATTY LIVER DISEASE BY METFORMIN OR ATORVASTATIN: A RANDOMIZED STUDY

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Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease, and its prevalence is rising worldwide due to the increasing prevalence of obesity. The therapeutical approach of NAFLD is currently based on lifestyle intervention, whereas there is no consensus on an effective pharmacological treatment. Both metformin and atorvastatin have been evaluated in clinical trials with variable results. Previously, we carried out a randomized 6-month trial using 1g metformin/day plus diet vs. diet alone, and observed that NAFLD improved/disappeared in a similar proportion of the two groups.

Objectives: We aimed at comparing the efficacy of three different pharmacological regimens (metformin at two different doses vs. atorvastatin) as an adjunct to dietary treatment in patients with early-stage NAFLD followed prospectively.

Methods: We enrolled 77 patients with early-stage NAFLD diagnosed at ultrasonography and with a BMI comprised from 20 to 40. Patients were randomized into three groups: the first group (n. 29 patients) was given metformin at a dose of 1g/day, the second group (n. 24) was given metformin 2g/day, and the third group (n. 22) was given atorvastatin at a dose of 20mg/day. Hypocaloric diet (1300 kcal/die) was advised to all patients. The study was terminated after 12 months.

Results: At the end of the study period, patients BMI significantly decreased in all three treatment groups ($P<0.05$). Fasting glucose, basal serum insulin and HOMA-index values decreased in both groups treated with metformin. The lipid profile improved only in the atorvastatin group. Liver steatosis, as evaluated by US, improved/disappeared in 14/29 (48.2%) patients of group A, in 11/24 (46%) patients of group B, and in 7/22 (36.3%) patients of group C.

Conclusions: In our 12-month prospective study, the treatment with metformin was associated with amelioration of insulin resistance and with a higher rate of improved/disappeared NAFLD as compared to atorvastatin treatment. However, these differences did not reach statistical significance.

PP129 - LEAN MASS AND INSULIN GROWTH FACTOR-1 CORRELATE WITH HIGHER BONE MINERAL DENSITY, BETTER BONE METABOLISM, INSULIN SENSITIVITY, AND LOWER INFLAMMATION IN OBESE ADULT SUBJECTS

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Several chronic metabolic alterations are present in obese subjects. While it is well known the detrimental effect of abdominal adipose tissue on chronic metabolic condition, less is known on the role of lean mass in obese subjects. Thus, aim of our study was to evaluate the potential correlation of muscle mass, metabolic condition, inflammation status and bone in obese individuals**.**

The study included 426 obese subjects (86 men and 340 female), mean age 44.8±14 yrs; BMI: 34.9±6.1. Exclusion criteria were chronic medical conditions or use of medications affecting bone metabolism, hormonal and nutritional status, vitamin D supplementation, recent weight loss and prior bariatric surgery. Patients underwent measurements of BMD (lumbar and hip) and body composition (lean mass, total and trunk fat mass) by DEXA and were evaluated for hormonal and metabolic profile and inflammatory markers.

Higher lean body mass (LM%) was inversely correlated with HOMA index ($p<0.0091$; r2 0.03938) and associated with lower fibrinogen levels ($p<0.0001$; r2 0.1263). Interestingly, in obese subjects LM% was associated with higher levels of vitamin D ($p<0.0001$, r2 0.1140), OSCA ($p<0.0001$, r2 0.2401) and IGF1 ($p<0.0002$, r2 0.1367). Finally, a positive correletion between IGF1 and OSCA ($p<0.0027$, r2 0.1405), IGF1 and vitamin D levels ($p<0.0004$ r2 0.1260), IGF1 and lumbar BMD ($p<0.0268$ r2 0.031), and IGF1 and femoral BMD ($p<0.0483$ r2 0.033) was obeserved.

In conclusion our results show that in obese patients higher LM% is directly linked to a lower inflammatory profile, a better insulin sensitivity, but also to higher level of Vitamin D and IGF1. Moreover our results show that in obese subjects higher levels of IGF1 correlates with a better OSCA and vitamin D profile and better BMD, both at lumbar and femoral site. These data strongly suggest the need to develop programs to facilitate weight loss in obese people, such as correct nutritional approach and an increase in physical activity.

PP130 - IGF-1 INDUCES UPREGULATION OF DDR1 COLLAGEN RECEPTOR BY SUPPRESSING MIR-199A-5P

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Discoidin Domain Receptor 1 (DDR1) is a collagen receptor tyrosine-kinase that contributes to epithelial mesenchymal transition and enhances cancer progression. Our previous data indicate that, in breast cancer cells, DDR1 interacts with IGF-1R and positively modulates IGF-1R expression and biological effects, suggesting that the DDR1 - IGF-1R cross-talk may play an important role in cancer.

In this study, we set out to evaluate whether IGF-1 may in turn affect DDR1 expression. Indeed, in breast cancer cells (MCF-7 and MDA-MB231) IGF-1 induced significant upregulation of DDR1 protein expression, in a time and dose dependent manner. However, we did not observe parallel changes in DDR1 mRNA. DDR1 upregulation required the activation of the PI3K/Akt pathway while the ERK1/2, the p70/mTOR and the PKC pathways were not involved. Moreover, we observed that DDR1 protein upregulation depends on a post-transcriptional mechanism which involves miR-199a-5p suppression via Akt activation. Accordingly, IGF-1 - induced DDR1 upregulation was inhibited by miR-199a-5p overexpression, which also impaired cell migration and proliferation in reponse to IGF-1.

These results demonstrate that this novel pathway involving Akt/miR-199a-5p/DDR1 plays a role in modulating IGF-1 biological effects. Therefore, this signaling pathway may represent an important target for breast cancers with overactivation of the IGF-IR axis.

PP131 - ENDOBARRIER: A NEW ENDOSCOPIC TECHNIQUE FOR THE TREATMENT OF TYPE 2 DIABETES AND OBESITY

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Duodenal-jeujunal bypass liner (EndoBarrier, GI Dinamics, USA) is a new nonsurgical approach to induce weight loss and to improve glycemic control in obese patients with Type 2 Diabetes (T2D). It is a 60 cm long fluopolymer sleeve delivered into and retrieved from the duodenum endoscopically. It can stay in place for 12 months and it mimics the metabolic effects of the Gastric-Bypass. Clinical experience demonstrated its safety, efficacy and ability to rapidly reduce blood glucose and weight. Metabolic effects of EndoBarrier were studied in patients ≤60 years of age, obese (BMI ≥30), with T2D for less than 10 years.Patients are selected after excluding secondary obesity, cardiological, pneumological and psychological evaluations, EGDS, blood tests and mixed meal test. Follow-up is performed every 3 months with clinically and with bloodwork. Patients are on a balanced low-calorie diet (1200 Kcal for women and 1500 Kcal for men). EndoBarrier was implanted in 7 patients (4M/3W; 51.7±8.3 years old) and 6 of them (3M/3W, 49.8±7.9 years old) finished the 12 month treatment. The data are presented as mean±standard deviation. After 12 months BMI was reduced from 42.9±9.1 to 34.2±4.9 kg/m2 and EBWL was 40±11%. We observed a reduction of 19.3±9 cm (-15±5%) of waist circumference (starting from 128.12±21.5 cm). Furthermore HbA1c lowered from 8.0±1.7% to 6.4±0.6% with a reduced number or dose of hypoglycemic agents (drug score went down from 6.4±3 to 4.75±2.5). The treatment was well tolerated and the most frequent side effect was nausea during the first days after endoscopic positioning. The only serious adverse event was a cervical esophagus erosion in one patient at the time of removal. Three months after removal 1.5 BMI points, 2.4 cm of waist circumference were gained back, while HbA1c levels remained stable with similar hypoglycemic therapy. These preliminary data suggest that EndoBarrier is a safe and effective therapy for treating obese patients with T2D. Even if after removal a small amount of weight was gained back, metabolic control remained stably optimal.

PP132 - DISCOIDIN DOMAIN RECEPTOR 1: A NEW THERAPEUTIC TARGET IN CANCERS WITH ACTIVATED IR-A/IGF-II LOOP

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Background: The insulin receptor isoform A (IR-A) is frequently overexpressed in cancer cells where it can be activated by autocrine IGF-II secretion. Indeed, over-activation of IGF-II/IR-A loop has a recognized role in cancer progression and resistance to therapies. In previous studies, using SILAC proteomics, we have identified discoidin domain receptor 1 (DDR1) as a new IR-A interacting protein. DDR1, a transmembrane tyrosine kinase, which acts as a non-integrin collagen receptor, is also overexpressed in several malignancies where it may play a role in cancer progression and metastasis.

Aims: We aimed at evaluating whether DDR1 functionally interacts with IR-A and whether DDR1/IR-A association exerts a role in breast cancer cell biology.

Results: We studied a panel of human breast cancer cells, characterized by different IR-A and DDR1 expression profile. We found that both receptors associate and co-localize after insulin or IGF-II stimulation, as assessed by co-immunoprecipitation and confocal microscopy analysis, respectively. DDR1 knocking-down by specific siRNAs resulted in marked inhibition of IR downstream signaling after insulin or IGF-II exposure. Moreover, DDR-1 specific silencing reduced breast cancer cell proliferation and migration in response to either insulin or IGF-II.

Conclusions: In human breast cancer cells, we identified DDR1 as a functionally relevant molecular partner of IR-A. These findings may provide important insights to develop new anti-cancer strategies aiming to target the activated IGF-II/IR-A loop.

PP133 - ENVIRONMENTAL NUTRITIONAL STATUS IN CAMPANIA REGION EVALUATED WITH MINI NUTRITIONAL ASSESSMENT DURING HEALTH CAMPUS EVENTS.

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Background

The diet is the environmental factor determining the nutritional status. Poor nutritional status is an important negative prognostic factor in the elderly and low weight is associated with adverse health outcomes of people >60 years. Perhaps, malnutrition is still underdiagnosed. Recently, the Mini Nutritional Assessment (MNA) has been designed and validated to provide a single, rapid assessment of nutritional status in elderly patients in outpatient clinics, hospitals, and nursing homes. It has been translated into several languages and validated in many clinics around the world.

Patients and Methods

To invastigate nutritional status in Campania region, nutritional status has been evaluated by MNA in 250 subjects (mean age 52.9±13.4; 27.6% M) undergoing health checks and screenings during Health Campus events. These subjects are representative of general population of Campania region attending the events associated with Health Campus and decided voluntarily to adhere the screening. Of these, 90 subjects were >50 yrs (F:69, M:21), 120 were >60yrs (F:91, M: 29) and 40 were >65 yrs (F:26; M:14).

Results

The sum of the MNA score distinguishes between elderly patients with: 1) adequate nutritional status, MNA > or = 24; 2) protein-calorie malnutrition, MNA < 17; 3) at risk of malnutrition, MNA between 17 and 23.5. According to the MNA, 48.4% of subjects were well nourished, 44% were at risk of malnutrition and 7.6% were malnourished.

Conclusions

In conclusion, about 50% of screened population in Campania Region is at high risk of malnutrition. The future aim of our study will be to verify in a more large number of people the impact of malnutrition, according to MNA, on mortality and on the occurrence of adverse clinical events in a 3-12 months follow-up study. In particular, the aim will be to verify whether a low MNA score could to be associate with adverse cardiovascular risk profile.

PP134 - EFFICACY OF BARIATRIC SURGERY IN AN ACHONDROPLASTIC DWARF OBESE PATIENT

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Introduction

Achondroplasia is the most common form of human dwarfism (prevalence 1/10000 e 1/30000 livebirths).

It is related to a mutation in the fibroblast growth factor receptor-3 (FGFR3), a gene encoding one member of the FGFR subfamily of tyrosine kinase receptors, which results in constitutive activation of the receptor. The clinical features of achondroplasia are macrocephaly, frontal bossing, midface hypoplasia, small chest, redundant skin folds, and extreme joint laxity. Obesity occurs between 13 to 43% of patients.

Case report

An 18 year old achondroplastic dwarf girl was admitted at our Department because of morbid obesity (weight 90 kg, height 120 cm, BMI 62.5 kg/m^2). The evaluation of obesity related complications demonstrated hyperinsulinism with acanthosis nigricans and slight obstructive sleep apnea syndrome. Bone and joint deformities associated with morbid obesity hampered the normal usual activities. Patient followed various diets associated with psychotherapy with no results. In January 2014 she underwent bariatric surgery (Roux en Y Gastric Bypass) without complications. Clinical and biochemical follow up was performed at 3, 6 and 12 months after surgery. During follow up we observed a progressive weight loss associated with improvement of the quality of life, especially related to amelioration of movement capacity. One year after surgery she weighted 65 kg and she lost 25 kg (EWL 37,5%).

Discussion

Even though our patient had a high surgical risk linked to anatomical deformities and to the severe obesity, bariatric surgery was eventful and effective.

PP135 - VITAMIN D AND INFLAMMATORY MYOPATHY: METABOLISM-RELATED ASPECTS

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~~Background Metabolic abnormalities associate with and contribute to autoimmune inflammatory myopathy (IM) pathogenesis (Galbiati et al., 2001; Rayavarapu et al., 2013; de Souza et al., 2014). Vitamin D receptor agonists (VDA) retaining anti-inflammatory effects seem beneficial both in (auto)immune disease and metabolic disturbances, as inflammation is the common link (Nasri et al., 2013; Boucher 2011). We have previously reported on VDA as immune-regulatory potential therapeutic tools in autoimmune inflammatory myopathy (IM) (Di Luigi et al., 2013).

Aim To evaluate direct metabolic VDA effects we analyzed: in human skeletal muscle cells (Hfsmc) the effect of BXL-628, less hypercalcemic VDA, on the metabolic machinery; in IM subjects, serum vitamin D, lipids - linked to early metabolism disturbance – protein C reactive (PCR) and glycemic levels.

Methods Hfsmc treated with BXL-628 or with insulin (I) were evaluated vs. control cells for: GLUT4, Caveolin1 and Flotillin1 (for membrane-associated lipid raft) localization by immunofluorescence; I-responsive paths such as Akt, mTOR, 4EBP1, by Western Blot. Sera from 21 IM subjects were assayed for vitamin D, tryglicerides, HDL, LDL, cholesterol, PCR and glycemia by routine clinical lab test.

Results In Hfsmc BXL-628 induced GLUT4 plasma membrane translocation suggesting the involvement of lipid rafts, as shown by Flotillin1 and Caveolin1 co-localization. BXL-628 phosphorylated Akt and mTOR. In IM subjects (normoglycemic) vitamin and lipids D were respectively below and over normal levels. We found that subjects with low serum vitamin D showed higher level of triglycerides and LDL; significant correlations between those parameters were found ($P<0.05$).

Conclusion Human muscle cell's metabolic machinery is direct target of VDA with an I-like effect; serum vitamin D low level might potentially be a critical index for metabolic disturbances in IM subjects before glycemia alteration: VDA could potentially be multifaceted therapeutic tools in IM.

PP136 - A RARE CASE OF GIANT INSULINOMA IN AN ELDERLY PATIENT WITH LIVER METASTASES.

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Introduction. Insulinomas are the most common hormone-producing pancreatic neuroendocrine tumors. They are typically benign, sporadic and very small (<2 cm) with clinical manifestation of hypoglycemia secondary to inappropriate insulin secretion. Only few cases of insulinoma presenting as a giant tumor have been reported up to now. We describe an exceptional case of giant insulinoma showing multiple liver metastases in an elderly patient.

Case report. A 78-year-old male presented with a history of several hypoglycemic symptoms in the last year with an acute onset of low consciousness level, light headedness and sudden confusion. Symptoms immediately disappeared after administration of glucose. On admission, laboratory tests showed serum glucose of 35 mg/dL, insulin 19 µU/mL, C-peptide 4.8 ng/mL, low ketones, chromogranin A 118 U/L (2-18). The work-up for MEN 1 was negative. A CT scan revealed a mass sized 78x52x35 mm in the pancreatic head, multiple liver lesions and no local lymph nodes involvement. Pathological examinations after surgery revealed a mass weighting 90 gr with perivascular invasion identified as a neuroendocrine tumor, with amyloid deposition, positivity for synaptophysin and chromogranine and an histological diagnosis of neuroendocrine liver metastases. Nuclear proliferation index Ki-67 was 4%. Remission of symptoms of hypoglycemia was maintained at one year follow up.

Conclusions. We describe a case of a giant insulinoma in an elderly patient with liver metastases, an extremely rare tumor of which only few cases have been reported previously. These cases presented at younger age and most of them did not have metastases at diagnosis. Our patient had a favorable outcome with recovery of symptoms achieved after surgical resection despite the size and presence of metastases. A longer follow-up is necessary to confirm the benign course.

PP137 - A CASE OF ENDOCRINE DISORDER SECONDARY TO MALNUTRITION AFTER BARIATRIC SURGERY.

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Introduction: Bariatric surgery is actually one of the most effective treatments for obesity; however, sometimes it can cause serious complications.

Case presentation: We report the case of a 34-year-old male patient (BMI 31 kg/m2) who was referred to our Division for severe asthenia, peripheral edema, lower extremity weakness, hypotension and erectile dysfunction. At presentation he showed pale skin, thinning and brittle hair, dermatitis, buccal ulcers and visual impairment that dated since 6 months. In 2004, he had performed a biliopancreatic diversion (BPD) for treatment of morbid obesity (BMI pre surgery: 62 kg/m2; excess weight loss 77.9%) and since that time he had attended a follow-up and supplementation program occasionally. His blood chemistry showed severe hypoproteinemia and hypoalbuminemia (4.9 g/dl and 1.64 g/dl, respectively), macrocytic anemia (RBC 3.12 x10^6/µL, Hb 10.4 g/dl, HCT 31.5%, MCV 100.9 fl) with apparent absence of vitamin B12 or folate deficiency (recently supplemented) and also a deficit of vitamin A (0.2 mcg/ml). Endocrine function tests showed hypotestosteronemia (total testosterone 1.9 ng/ml, LH: 4.9 mlU/ml, FSH: 6.4 mlU/ml), reduced cortisol level (at 8.00 AM: 7 mcg/dl) and normal TSH with low FT3 values (TSH: 1.3 mU/ml, FT3: 1.22 pg/ml, FT4: 0.93 ng/dl). TRH test showed normal response, excluding a secondary hypopituitarism. The patient was then fed with parental nutrition and supplements of micronutrients (e.g selenium, zinc, copper) and vitamins (e.g A, B1, B2, B6, B12, C, D, E). After one month he underwent to surgical elongation of the intestinal loop, in order to reduce malabsorption, followed by a complete remission of symptoms, anemia (Hb 12.9 g/dl), albumin and protein level and restoration of thyroid (TSH: 3.83 mU/ml, FT3: 2.45 pg/ml, FT4: 0.9 ng/dl), adrenal and gonadal function (testosterone:12.4 ng/ml).

Conclusions: This case shows that severe malabsorption is a possible but not well-known complication of bariatric surgery that may alter also hormonal status, mimicking several endocrine diseases.

PP138 - VERTEBRAL BONE MARROW FAT IS INCREASED IN OBESE WOMEN: RELATIONSHIP WITH EPICARDIAL FAT

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Introduction: Bone marrow fat is considered a risk factor for osteoporosis and fragility fracture. It is not clear if bone marrow adiposity is increased in obesity and how it relates to other compartments of adipose tissue, in particular visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT) and epicardial adipose tissue (EAT).

Aim: To evaluate bone marrow adiposity in obese as compared with normal weight women.

Design: 20 healthy premenopausal women (age 32±8 years, BMI 28±6 kg/m^2, mean±SD) underwent body composition evaluation with body impedance assessment, marrow adipose tissue (MAT) and marrow fat unsaturation measurement (L4 level) with proton magnetic resonance spectroscopy (1H-MRS), VAT, SAT (L4 level) and EAT measurement with magnetic resonance imaging, lumbar spine and femoral bone mineral density (BMD) measurement with DXA.

Results: Overweight/obese women (n=10, BMI 32±5 kg/m^2) had higher MAT content than normal weight women (n=10, BMI 22±1 kg/m^2) (40±9 vs 27±17%, $p<0.05$). MAT correlated with EAT ($r=0.473$, $p<0.05$), age ($r=0.391$, $p<0.05$), BMI ($r=0.410$, $p<0.05$), fat mass ($r=0.440$, $p<0.05$), duration of obesity ($r=0.400$, $p=0.05$) and marrow fat unsaturation ($r=-0.652$, $p<0.001$). In a multiple stepwise regression analysis including MAT as dependent variable and age, BMI, fat mass, duration of obesity, EAT as independent variables, EAT was the most significant determinant of MAT (beta=0.023±0.009, $p<0.05$). Based on medians, women with higher EAT (n=8) had higher MAT then women with lower EAT (n=12) (41±0.1 vs 28±0.2%, $p<0.05$). Marrow fat unsaturation positively correlated with lumbar spine BMD ($r=0.480$, $p<0.05$).

Conclusion: Bone marrow adiposity is increased and richer in saturated fatty acids in obese premenopausal women and it is related to fat mass and epicardial fat. This seems to confirm an increased risk for osteoporosis in obesity, possibly related to visceral adipose tissue compartments.

PP139 - CIRCULATING LEVELS OF SIRTUIN 4 IN OBESE SUBJECTS: IMPACT OF ENVIRONMENTAL NUTRITIONAL FACTORS AND RELATIONSHIP WITH ANTHROPOMETRIC PARAMETERS.

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Background: Sirtuin 4 (Sirt4) is a negative regulator of mitochondrial oxidative capacity. Circulating levels of Sirt4, mirroring its reduced mitochondrial expression, are low in obese patients, likely as an attempt to decrease fat oxidative capacity reducing the mitochondrial ROS production. Diet, a modifiable environmental factor, is a mainstay in the management of obese individuals. States of high metabolic demand, such as calorie restriction, are associated to low SIRT4 function. At present, the decreased oxidative stress is a plausible mechanism linking the Mediterranean diet (MD) to reduced cardiovascular disease risk. **Aim:** To evaluate the association between circulating levels of Sirt4, adherence of MD and anthropometric parameters in obese patients. **Patients & Methods:** Fifty patients (19 males and 31 females, mean age: 36.4±9.4 yrs) affected with moderate to severe obesity (mean BMI: 46.4±6.1 kg/m^2), were consecutively enrolled. A validated 14-item questionnaire (PREDIMED: PREvención con DIeta MEDiterránea) was used for the assessment of adherence to the MD. The dietary interview data were collected by a 7-day food record. Body composition was evaluated by bioelectrical impedance analysis. **Results:** Sirt4 levels were significantly lower in obese individuals on MD (p=0.002). Sirt4 was significantly associated with the dietary components included in the PREDIMED questionnaire: extra virgin olive oil (r=-0.369, p=0.008), vegetables (r=-0.326, p=0.021), fruit (r=-0.302, p=0.033), red wine (r=-0.31, p=0.025), legumes (-0.354, p=0.012), fish (r=-0.394, p=0.005) or with the PREDIMED score (r=-0.437, p=0.002). At multiple regression analysis, PREDIMED score was the major predictor of Sirt4 levels (r^2=0.174, β=-0.437, t=-3.4, p=0.002). Among anthropometric parameters, Sirt4 was best correlated with phase angle (r^2=0.527, β=-0.732, t=-7.4, p=<0.001). **Conclusions**: MD in obese individuals is associated with low circulating levels of Sirt4. It can be speculated that the antioxidant properties of the MD could be involved in a reduced of need of compensation by Sirt4 in obesity. Lower Sirt4 levels are also associated to higher phase angle, a direct BIA measure of cellular health and nutritional status.

PP140 - CIRCULATING SIRT1 INVERSELY ASSOCIATES WITH OBESITY AND UNHEALTHY FAT DISTRIBUTION.

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Sirtuins (SIRTs) are master metabolic regulators with protective roles against obesity and its associated metabolic disorders, including non-alcoholic fatty liver disease (NAFLD) and type-2 diabetes. We aimed to ascertain whether there is a relationship between serum SIRT1 and liver steatosis severity, weight loss or fat mass percentage (%FM) in obese patients. 72 adult obese patients (BMI 41.86±7.89 kg/m^2) with evidence of NAFLD matched with 30 healthy lean controls, and 22 obese subjects (BMI 41.82±6.28 Kg/m^2) before and 6 months after BioIntragastric Balloon (BIB), were studied. Plasma SIRT1 levels, transaminases, insulin, HOMA-index, HbA1c, BMI, body composition (DXA) and representative measures of metabolic syndrome (WC, fasting plasma glucose, blood pressure, HDL-cholesterol, triglycerides) and inflammation (ESR, CRP, fibrinogen) were evaluated.

In severe liver steatosis group, SIRT1 was significantly lower than in patients with mild steatosis, and both had lower SIRT1 plasma values compared to lean controls ($p=0.0001$). SIRT1 showed an inverse correlation with liver steatosis and HbA1c in univariate analysis ($r= - 0.386$; $p=0.001$; $r= - 0.300$; $p=0.01$, respectively). Multiple linear regression analysis showed that NAFLD was the best independent correlate of SIRT1 even after adjustment for potentially relevant variables ($\beta = -0.442$; $p=0.003$). In the follow-up study after BIB placement, concomitantly with substantial weight loss ($p=0.0002$), reduction of BMI ($p=0.0001$), WC ($p=0.0002$), trunk FM ($p=0.003$), excess body weight ($p=0.006$) and total FM ($p=0.002$), SIRT1 levels showed a significant increase ($p=0.006$). A trend toward an amelioration of the metabolic and inflammatory parameters was observed. A negative correlation between SIRT1 and %FM ($\rho= -0.537$, $p=0.017$) was also seen.

In conclusion weight loss, and %FM in particular, inversely associates with plasma SIRT1 indicating that circulating SIRT1 is stimulated by a negative caloric balance. Furthermore, serum SIRT1 might be a novel clinical/biochemical parameter associated with an unhealthy fat distribution, including liver steatosis.

PP141 - CARBOHYDRATE INTAKE AND LOW-GRADE INFLAMMATION IN A SAMPLE OF WOMEN WITH POLYCYSTIC OVARY SYNDROME LIVING IN CAMPANIA.

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Background: A growing body of evidence links obesity and chronic low-grade inflammation to the pathogenesis of polycystic ovary syndrome (PCOS), development of insulin resistance and atherogenesis. Diet-induced inflammation has been proposed as a potential mechanism for promoting chronic low-grade inflammation in PCOS, even independently of obesity. The vast majority of studies investigated the effects of a glucose load on markers of oxidative stress and inflammation. However, the literature about the association among the amount and type of carbohydrates (CHO), obesity, insulin resistance and pro-inflammatory state in PCOS is limited. Prospective methods of food records, such as the 7-day food record, are assumed to be the "gold standard" method of dietary assessment. **Aim**: Of the present study was to evaluate the nutrient intake in an ethnically homogeneous sample of treatment-naïve women with PCOS living in Campania, in association with insulin resistance and inflammatory markers. **Patients & Methods**: Dietary interview data were collected by a 7-day food record in 95 treatment-naïve women with PCOS (22.9±5.8 yrs); 32 women were normal weight (BMI 22.3±1.5 kg/m^2) and 63 were overweight-obese (BMI 33.9±6.6 kg/m^2). BMI, glucose and insulin levels, C-reactive protein (CRP) levels, and total white blood cell (WBC) were measured. Homeostasis Model Assessment of Insulin Resistance (HoMA-IR) was calculated. **Results**: Total (56.9±4.3 *vs* 55.0±3.5%; p=0.025) and simple CHO intake (24.4±4.1 *vs* 20.6±5.3%; p<0.001), HoMA-IR (3.5±2.8 *vs* 1.8±1.5; p=0.003) and CRP levels (4.3±6.9 *vs* 0.9±1.4 ng/ml; p=0.01) were higher in obese than in normal weight PCOS, while there were no significant differences in WBC. Total and simple CHO intake were correlated with CRP (r=0.402 and 0.465; respectively) and WBC (r=0.446 and 0.352, respectively), (p<0.001). After adjusting for BMI, total CHO intake remained positively correlated with CRP levels (r=0.361) and WBC (r=0.358), (p=0.013). At multiple regression analysis, total CHO remained the major predictors of CRP levels and WBC. **Conclusions**: Higher intake of total CHO was associated with higher levels of markers of inflammation, independently of BMI. Future observational studies on larger population samples and long-term dietary intervention trials will be critical for elucidate the complexive effect of macronutrient intake in PCOS pathogenesis and progression.

PP142 - RELATIONSHIP AMONG FATTY LIVER INDEX, VISCERAL ADIPOSITY INDEX AND HOMA-IR AND MEDITERRANEAN DIET AS A MODIFIABLE ENVIRONMENTAL FACTOR IN OBESE PATIENTS.

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Background: Diet, a modifiable environmental factor, is a mainstay in the management of obese individuals. Unhealthy lifestyle and obesity are the most probable causes of fatty liver (FL), a source of inflammatory factors contributing to cardiovascular and neoplastic diseases risk in obese individuals. The Mediterranean Diet (MD) can be described as the healthy dietary pattern characterized by high consumption of vegetables and low consumption of full fat dairy products and red meat. Adherence to MD is a significant predictor of changes in the fat content of the liver in overweight patients with FL. Fatty Liver Index (FLI) is a clinical and metabolic correlate of FL used as surrogate parameters for liver fat content in general population. Visceral adiposity Index (VAI) is a gender-specific index of adiposity assessment highly correlated with cardiometabolic risk. **Aim**: To evaluate the correlations between MD adherence with endocrine-metabolic indices in obese patients. **Patients & Methods**: Fifty severely obese individuals (19 M and 31 F, mean age 36.9±8.3 and 36.1±10.1 yrs, respectively) were included in this study. A validated 14-item questionnaire (PREDIMED) was used to assess MD adherence score (poor adherence 0-5; average adherence 6-9; better than average adherence ≥10). HoMA-IR, FLI and VAI were calculated. **Results**: According to PREDIMED, 46% of the sample population presented with poor adherence, 46% average adherence and 8% better than average adherence to the MD. FLI >60 and HoMA-IR>2.5 were present in all the subjects, while VAI score higher than age and gender-specific cutoff values was evidenced in 92% of the subjects. MD adherence was significantly correlated with BMI ($r=-0.541$, $p<0.001$), waist circumference ($r=-0.660$, $p<0.001$), HoMA-IR ($r=-0.531$, $p=0.014$), FLI ($r=-0.617$, $p<0.001$), and VAI ($r=-0.323$, $p=0.022$). After adjusting for BMI, MD adherence and FLI remained still significantly correlated ($r=-0.488$, $p=0.001$). At multiple regression analysis, MD adherence was the major predictor of FLI ($\beta=-0.621$, $t=-5.3$, $p<0.001$). **Conclusions**: Besides the well-known relationship between MD and insulin resistance, our data evidences the negative association among MD adherence, FLI and VAI in obese subjects, two surrogate indexes of FL and adipocyte dysfunction. The independent association between MD adherence and FLI further supports the key role of diet in FL pathogenesis.

PP143 - NUTRITION AS ENVIRONMENTAL FACTOR ON THYROID FUNCTION IN OBESE PATIENTS: EFFECTS OF DIET COMPOSITION.

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Background: Nutrition and obesity are well known adjustable environmental factors influencing the health status. In the last years, several reports indicate that obesity can influence thyroidal status. Obese euthyroid patients have a positive association between BMI and TSH and/or FT3 levels with an increase in the FT3/FT4 ratio, as an indirect index of deiodinase activity. **Aim**: To evaluate the effects of nutrient intake on thyroid function in obese patients. **Patients & Methods**: Fifty moderate or severe obese individuals (19 M and 31 F, mean age 36.9±8.3 and 36.1±10.1 yrs, respectively) were included in the study. Body weight (BW), height and waist circumference were evaluated. Body composition was assessed by bioimpedance analysis (BIA) (single-frequency 50 kHz BIA 101 RJL, Akern) and biavector analysis (BIVA software [Piccoli A, Pastori G (2002)]. Dietary data were collected using a 7-day food record. Nutritional elements were calculated with software WinFood. TSH, FT3, FT4 levels were measured and FT3/FT4 ratio has been evaluated. The results have been associated to blood pressure (BP), cardio vascular risk (CV), metabolic parameters (glycaemia [Glu], insulin and HOMA-IR), BMI, BIA and nutritional influences, including total calories (kcal) carbohydrates (CHO), lipids and protein intake. **Results**: As expected, TSH levels positively correlate with BMI ($r=0.602$, $p<0.001$), but also with CV ($r=0.750$, $p<0.001$), systolic and diastolic BP ($r=0.459$, $p=0.001$; $r=0.431$, $p=0.002$; respectively), BW ($r=0.472$, $p=0.001$) and fat mass ($r=0.596$, $p<0.001$). A negative correlation has been demonstrated between TSH and fat-free mass ($r=-0.385$, $p=0.006$), Glu ($r=0.371$, $p=0.008$), insulin ($r=0.640$, $p<0.001$) and HOMA-IR ($r=0.504$, $p<0.001$). Moreover, the FT3/FT4 ratio positively correlate with CHO% in the diet ($r=0.355$, $p=0.011$) and negatively with lipids ($r=-0.315$, $p=0.026$). No correlations have been found between FT3/FT4 ratio and protein % or total calories in the diet. **Conclusion**: In obese patients, diet can influence the thyroidal status. Since improvements in thyroid function can help the obesity treatment, we suggest that a nutritional evaluation should be performed to optimize the diet in order to ameliorate the thyroidal status.

PP144 - BODY CIRCUMFERENCES PREDICT METABOLIC SYNDROME BUT NOT ADVANCED ATHEROSCLEROTIC DISEASE: A CORONARY ANGIOGRAPHIC STUDY

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Background. The relationship between anthropometric measurements, especially waist-to-hip ratio (WHR) and neck circumference (NC), and metabolic diseases is well known. Recent data showed that wrist circumference (WC) is a marker of insulin resistance and that it is related to an increased risk of diabetes, major risk factors for cardiovascular diseases (CVD). However, whether these measures are associated with clinically significant CVD is still controversial.

Aim. In this cross-sectional study we aimed to evaluate whether body circumferences (WHR, NC and WC) could be used as easy and fast markers of advanced atherosclerotic disease.

Methods. 128 patients (73.4% males, mean age 68.1 ± 9.8 years) undergoing coronary angiography and carotid ultrasound were enrolled in the study. Advanced atherosclerotic disease was defined as ≥*70*% coronary lumen *stenosis* and/or ≥50% carotid lumen *stenosis.* Metabolic syndrome (MS) was defined according to the 2005 criteria of the International Diabetes Federation.

Results. 77.5% subjects showed advanced atherosclerotic disease. In males mean±SD body mass index (kg/m^2), WHR, WC (cm) and NC (cm) were 27.5±3.7, 1.0±0.1, 17.6±1.1 and 40.8±3.0 respectively (females: 26.6±3.7, 0.9±0.1, 15.8±1.3 and 35.1±2.6). Risk of MS increased in the highest sex-specific tertiles of WC, independently from NC and WHR (OR [95%CI] 2.6 [1.4-4.9]). WC and NC as continuous variables, but not WHR, were independently related to the presence of MS in males (2.25 [1.12-4.55] and 1.52 [1.12-2.06], respectively). WC, but not NC and WHR, was associated to higher fasting glucose in females (Adj R^2=0.18, β=3.6, p=0.037). Anthropometric measurements were not associated with advanced atherosclerotic disease. In a multivariate logistic model older age, male gender and MS significantly and independently increased the risk of advanced atherosclerosis (age: 1.05 [1.0-1.1]; male gender: 3.6 [1.3-10.0]; MS: 3.4 [1.3-8.8)]. Dyslipidemia and hypertension drove the association between MS and advanced atherosclerosis (5.04 [2.1-12.1] and 2.7 [1.0-6.9], respectively).

Conclusions. In our population no significant relationships were found between circumferences and advanced atherosclerotic disease. However, our data confirm that WC, more than WHR, is associated with precocious cardiovascular risk factors such as MS and higher fasting glucose levels; thus, further studies should clarify whether it could be a marker of early (non-advanced) atherosclerosis.

PP145 - HYPOVITAMINOSIS D IS ASSOCIATED WITH LIVER INSULIN RESISTANCE IN OBESE SUBJECTS

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Background and aims: hypovitaminosis D is highly prevalent in obese subjects. Serum 25-hydroxy vitamin D3 [25(OH)D] concentration, the best marker of human vitamin D status, has been reported to be associated with glucose status, insulin resistance (IR) and beta cell function. We aimed to specifically investigate the relationship between 25(OH)D and liver IR. **Materials and methods**: we performed a comprehensive metabolic assessment (2-h OGTT, hyperinsulinemic euglycemic clamp [HEC], body composition by DXA) in 20 obese non-diabetic subjects (42.9±2.7 yrs; BMI 37.7±0.8 kg/m2) with 25(OH)D insufficiency (<30 ng/mL). Liver IR was estimated using the index by Vangipurapu et al. (-0.091 + [log insulin AUC 0-120 min x 0.400] + [log fat mass% x 0.346] - [log HDL cholesterol x 0.408] + [log BMI x 0.435]). **Results**: there was a significant inverse correlation between 25(OH)D and the liver IR index (r = -0.514, p = 0.02). This correlation maintained its significance after adjusting for age, gender, total cholesterol, triglycerides and whole body insulin sensitivity (M value assessed by HEC) in multiple linear regression analysis. There was no significant correlation between 25(OH)D and beta cell function estimated by the Disposition Index. **Conclusion**:our data suggest that, in obese subjects, low 25(OH)D levels are independently associated with liver insulin resistance, but not with beta cell function. Further studies are needed to clarify the relationship between glucose homeostasis and vitamin D levels.

PP146 - EFFECT OF VITAMIN D SUPPLEMENTATION AND WEIGHT LOSS ON INSULIN SENSITIVITY IN OBESE SUBJECTS WITH HYPOVITAMINOSIS D

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Background and aims: hypovitaminosis D is prevalent among obese individuals and might have an independent role in the pathogenesis of insulin resistance. If so, vitamin D supplementation might increase the benefits of weight loss, i.e. the mainstay for the prevention of type 2 diabetes in overweight/obese subjects. **Materials and methods**: we conducted a double blind, randomised study to assess whether vitamin D supplementation in conjunction with a weight-loss dietary regimen has a greater impact on insulin sensitivity in obese subjects with hypovitaminosis D as compared to weight loss alone. We enrolled 18 obese volunteers (14/4 F/M; mean age±SD: 40.4±12.0 yrs; BMI: 37.7±6.9 kg/m^2) with hypovitaminosis D [serum 25(OH)D: 14.3±6.8 ng/ml]. Subjects were randomised (1:1) to hypocaloric diet + either oral cholecalciferol 25,000 I.U./week (VIT) or placebo (PLA) for 3 months. Anthropometric assessments, a standard 2-hr OGTT and a hyperinsulinemic euglycemic clamp (HEC) to measure insulin sensitivity (M value) were performed at baseline and at study end. a **Results**: anthropometric characteristics, insulin sensitivity and 25(OH)D levels at baseline were similar between groups. After 3 months, body weight significantly decreased in both groups (-7.5% and -10.5% for VIT and PLA, respectively; $p<0.05$ for both; p for interaction=0.53). 25(OH)D levels increased in the VIT group (from 14.7±5.2 to 29.7±7.4 ng/ml; $p=0.001$), but not in the PLA group (13.9±8.2 *vs*. 16.7±5.6 ng/ml; p=ns; p for interaction=0.013). No significant changes from baseline were observed in OGTT parameters. Insulin sensitivity significantly improved only in the VIT group (M pre *vs.* post: 4.6±2.1 *vs*. 6.1±3.0 mg•kg^{-1}•min^{-1}, $p=0.02$ and 4.9±2.2 *vs.* 5.1±2.0 mg•kg^{-1}•min^{-1}, $p=0.85$ for the VIT and PLA groups, respectively), but this improvement did not reach statistical significance *vs.* the PLA group (p for interaction=ns). **Conclusion**: vitamin D supplementation might potentiate the beneficial effects of weight loss on insulin sensitivity.

PP147 - THE WRIST CIRCUMFERENCE, A CLINICAL MARKER OF INSULIN RESISTANCE, IS SIGNIFICANTLY HIGHER IN OBESE CHILDREN/ADOLESCENTS WITH METABOLIC SYNDROME COMPARED TO SUBJECTS WITHOUT METABOLIC SYNDROME

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In Western countries, the prevalence of paediatric obesity and comorbidities, as soon as of the metabolic syndrome (MS) has recently increased. MS is defined as the association of obesity, insulin resistance, glucose intolerance, hypertension and dyslipidemia. We demonstrated that the wrist circumference, in particular its bone component, can be used as a clinical marker of insulin-resistance in overweight/obese children. The aim of the study was to evaluate the correlation between the presence of MS and the wrist circumference in obese children/adolescents. We recruited n=914 (M/F504/510; mean age: 9.9±2.9) obese children/adolescents at "Sapienza" University and CTO Hospital in Rome. The following parameters were evaluated for age and gender: triglycerides >95th percentile; low HDL cholesterol <5th percentile; systolic or diastolic blood pressure (BP) >95th percentile; fasting glucose ≥100 mg/dl. We defined MS as the presence of at least 2 other findings out of obesity (BMI-z score>95th percentile for age and gender). Subjects were divided into two groups according to Tanner stage (TS): prepubertal (TS 1), pubertal (TS from 2 to 5). The wrist circumference was measured using a tension-gated tape measure positioned over the Lister tubercle of the distal radius and ulna. Data were analyzed through T-test or Manny Mann–Whitney test (SAS software 9.3). The frequency of MS was 12.9% (n=107/914), with higher prevalence in males than females (8.4% vs 4.4%, p=0.017). The mean of wrist circumference was higher in obese children/adolescents with MS compared to subjects without MS, according to TS (prepubertal: 15.9 cm vs 15.2 cm, p=0.005, pubertal: 16.5 cm vs 16.3 cm, p=0.043). In male pubertal patients, the frequency of MS was higher than females (13.8% vs 4.7%, p=0.006). The obese children and adolescents with MS, showed higher median levels of: triglycerides, systolic and diastolic BP, fasting glucose, HOMA-IR, compared to patients without MS (p<0.01, for all comparisons). The identification of youths with increased risk for MS and insulin resistance could be achieved with minimal effort by measuring wrist circumference, thus avoiding testing the entire population of overweight/obese children.

PP148 - METABOLICALLY HEALTHY BUT OBESE AND METABOLICALLY UNHEALTHY OBESE PATIENTS SHOW A DIFFERENT HORMONAL PROFILE

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Obesity is an independent risk factor for the development of cardiovascular diseases and diabetes. For the definition of the degree of the cardiometabolic risk in an obese patient two phenotypes of obesity were identified: metabolically healthy, but obese (MHO) and metabolically unhealthy obese (MUO). The definition of MHO is not unequivocal, explaining the wide variability of its prevalence (6-40% of the obese population). Moreover, little is known about the determinants of this phenotype such as the role of endocrine abnormalities.

We assessed the body composition, cardiovascular risk factors, inflammatory and endocrine parameters in a population of 1950 obese patients. MHO patients were defined by normal blood glucose concentrations, normal blood pressure, normal levels of triglycerides and HDL cholesterol.

Anthropometric parameters, systolic and diastolic blood pressure, heart rate, blood glucose and fasting insulin, total cholesterol, HDL cholesterol and triglycerides, CRP, ESR, blood count, 25-OH Vitamin D, GH/ IGF-1 axis, TSH, FT3, FT4, SHBG, DHEA-S, cortisol and ACTH , urinary free cortisol, PRL, body composition by dual X-ray densitometry ray absorptiometry were recorded. Patients with history and / or symptoms and signs suggestive of hypopituitarism and GH deficiency (GHD) were tested by GHRH + arginine.

The prevalence of MHO phenotype was 17.1% (334 patients with a mean age of 40 ± 13 years, BMI 37 ± 6 kg / m2, 285 females and 49 males). MUO patients (384 males and 1232 females) were significantly older (46 ± 14 years) and more obese (BMI 39 ± 7 kg / m2). MHO were more insulin sensitive, with a lesser degree of inflammation with a peripheral distribution of body fat, improved bone density at the lumbar spine (corrected for BMI). higher levels of 25 (OH) vitamin D ($p < 0.02$), osteocalcin ($p < 0.05$), SHBG ($p < 0.001$), basal and stimulated GH and IGF-1 ($p < 0.001$), and lower levels of urinary free cortisol ($p < 0.05$) compared to MUO patients.

The MHO phenotype associates with reduction of cardio-metabolic risk factors and an hormonal profile characterized by significantly higher values of SHBG, vitamin 25 (OH) D, osteocalcin, GH (basal and stimulated) and IGF-1 and lower urinary free cortisol.

PP149 - METABOLIC PROFILE IN PATIENTS WITH PANCREATIC NEUROENDOCRINE TUMORS

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Background: It is well established that dyslipidemia and diabetes are related to cancer development. There are few data in literature about the association between neuroendocrine tumor (NET) and metabolic alterations. Aim: to evaluate the metabolic profile of patients with pancreatic NET (pNET) at the time of the diagnosis. Patients & Methods: Thirty-three patients (12 females and 21 males; mean age 55.6±13.5 yrs) diagnosed with pNET were evaluated for metabolic profile, according to tumor stage, grading, Ki67 score and Chromogranin A serum concentrations. At diagnosis, 66% and 24.2% of patients presented lymphonode and distant metastasis respectively. Results: Mean weight was 81.5±17.3 Kg. Metabolic parameters were as follows: serum concentrations of glucose 106.2±29.7 mg/dl, total cholesterol 190.7±35.4 mg/dl, LDL-cholesterol 117.8±37.7 mg/dl, triglycerides 139.5±62 mg/dl, systolic blood pressure 131±12.5 mmHg, diastolic blood pressure 80.2±7.5 mmHg. A significant correlation was found between Ki67 score and both total cholesterol and LDL cholesterol ($r=0.742$, $p<0.001$; $r=0.629$, $p<0.001$ respectively). A significant correlation was found between tumor grading and triglycerides ($r=0.442$, $p<0.002$). Higher serum glucose concentrations were observed in patients with distant metastasis than in those without distant metastasis ($p=0.0042$). Conclusions: in patients with pNET, lipid abnormalities are more pronounced in patients with high tumor grading and Ki67 score, while hyperglicemia is more frequent in patients with distant metastases. Lipid and glucose impairments seems to play a prognostic role in patients with pNET.

PP150 - APPROPRIATENESS OF STATIN THERAPY IN DIABETIC PATIENTS IN VERY HIGH CARDIOVASCULAR RISK POPULATION: DATA FROM DATABASES OF GENERAL PRACTITIONERS

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Introduction: literature data show that a large number of patients needing treatment with statins, is not on treatment or it is not properly treated. Primary aim of this study is to assess if eligible patients for treatment with statins (according to the criteria of the note 13 of the Italian Drug Agency) are indeed on treatment with statins, in a sample of general practice patients. We also describe the reasons for non-prescription of such drugs, with special attention to patients with diabetes mellitus.

Materials and methods: We analyzed retrospectively the sample of five general practitioners (GP) by using Mille GPG software, focusing on the patients at very high cardiovascular risk category (VHCRC) according to ESC/EAS guidelines. The prescription of at least one pack of statins in the 12 months before our analysis, was the criterion used to determine whether a patient was or not on current treatment. With regard to patients at VHCRC who were not on treatment, we analyzed again their clinical records to assess the completeness of patient data, evaluating age, medical history, the class of lipid disorder, allergies or adverse events, and other concomitant treatment); for patients considered suitable for treatment with statins, we suggested the initiation of the treatment.

Results: 760 out of 7340 patients were at VHCRC. 413 out of such 760 patients (54.3%) were on regular treatment, while 347 (45.7%) were not treated. Among untreated patients, 31% was diabetic. Preliminar data arising from medical records of patients on treatment showed the following: about one third of patients did not meet the inclusion criteria; in fact the medical software used (Millewin) automatically considers at VHCRC all patients diagnosed with dubious or suspected cardiovascular events, or not properly encoding as vertebrobasilar artery syndrome. By changing the code of the true diagnosis, a statistically significant variation of the results is showed. Furthermore, another third of the patients enrolled presented a cerebral or cardiac event after 80 years and, of these, about half presented a LDL value inferior to 100 mg/dl, in absence of treatment. These data show a substantial discrepancy between what is recommended by international guidelines and what really happens in the clinical practice, therefore GP should critically evaluate the multiple therapeutic choices for each patient. At last, a small percentage (5%) of patients resulted without any prescription of statins due to the poor compliance shown towards all the other therapeutic decisions, and 18% of patients was not taking statins because contraindicated.

Conclusions: Only 10% of the patients results currently lost on follow-up and should promptly start a program treatment with statins.

PP151 - TESTOSTERONE SUPPLEMENTATION AND BODY COMPOSITION: RESULTS FROM A META-ANALYSIS STUDY.

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Introduction. The effect of testosterone (T) supplementation (TS) on weight loss is conflicting. The aim of the present study is to meta-analyze, in subjects with late onset hypogonadism (LOH), all available evidence on the effects of TS on body weight and waist circumference (WC), including other parameters concerning body composition and metabolic outcomes.

Methods. We included all observational studies and randomized controlled trials (RCTs), comparing the effect of TS on different endpoints. The principal outcome of this analysis was the effect of TS, on body composition modification including fat and lean mass. Secondary outcomes included several other glycometabolic parameters.

Results. Out of 824 retrieved articles, 93 were included in the study. In particular, of those, 60 were RCTs and 33 observational studies enrolling 6866 and 2026 in TS and control groups, respectively. In observational studies, but not in RCTs, TS was associated with a time-dependent reduction of body weight, and WC. The estimated weight loss and WC reduction at 24 months were -3.50[-5.21;-1.80] Kg; -6.66[-8.12;-4.95] cm, respectively. Meta-regression analysis also indicated that results with TS on weight loss were more evident in younger and more hypogonadal subjects at enrolment. TS was also associated with a significant reduction of fat and with an increase of lean mass in both observational studies and RCTs. In addition, in observational studies, as well as in RCTs, TS was associated with a reduction of fasting glycaemia and insulin resistance. These differences in RCTs were related to differences in lean mass and not to differences in fat mass and T levels at end-point. When only RCTs enrolling hypogonadal (TT<12 moles/L) subjects at baseline were considered, a reduction of total cholesterol as well as of triglycerides levels were observed in both observational studies and in RCTs. Conversely, an improvement in HDL cholesterol levels as well as in both systolic and diastolic blood pressure was observed only in observational studies, but not in RCTs.

Conclusions. Present meta-analysis does not support the view of T as a new anti-obesity medication. However, considering the high prevalence of hypogonadism in obesity, clinicians are strongly encouraged to check T in their obese subjects, because treating hypogonadism might help in decreasing fat mass, increasing muscle mass and, therefore, and facilitating weight loss.

PP152 - BARIATRIC SURGERY OUTCOMES IN SARCOPENIC OBESITY

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Background: Several factors like gender, age, technical procedure, redo surgery have been already associated with bariatric surgery failure. Sarcopenia, defined as low skeletal muscle mass and function, was recently correlated with greater difficulty to achieve weight loss and could be a predictive factor of bariatric surgery failure.

Objective: We aimed to assess whether preoperative sarcopenic obesity could be considered as a predictive factor of weight loss failure in bariatric surgery patients.

Methods: Preoperative body composition was assessed in 69 patients (41 women, 28 men) with BMI between 34 and 48 Kg/m², operated on from 2010 to 2012. 18 patients underwent sleeve gastrectomy (26,1%), and 51 had RYGBP (73,9%). Skeletal muscle mass (SM) was calculated from BIA (bioelectrical impedance analysis) using Janssen's formula. The sarcopenic group (N = 23) was defined at the lowest tertile of SM/height² from each gender group. Surgical complications, co-morbidities and weight loss, defined as %EBMIL, %EWL and %WL were evaluated at 3, 6 amd 12 months.

Results: Despite a pre-operative lower SM (26,8vs32,8 p=0,001), the sarcopenic group did not experience a lesser weight loss during the entire follow-up (1 year %EBMIL: 75vs68 p=0,232; %EWL: 65vs69 p=0,338; %WL: 28,6vs27,4 p=0,567) even after correction for age, type of surgery and pre-operative BMI in a regression model.

Conclusions: This study suggests that bariatric surgery remains effective in sarcopenic patients. However one may question whether sarcopenia does not increase the risk of weigh regain and long-term studies are necessary.

PP153 - ROLE OF GLUCOSE VARIABILITY ON SYMPATHO-VAGAL BALANCE, IN METABOLIC SYNDROME

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Introduction: Several studies showed the impact of impaired sympatho-vagal balance on cardiovascular mortality. Metabolic syndrome (MS) is a clinical condition characterized by an increased cardiovascular risk, even befroe the onset of diabetes. The aim of our work is to evaluate the relationship between glucose variability (GV) and sympatho-vagal balance in patients with MS, but without diabetes mellitus.

Materials and methods: Thirty-five patients with MS according to IDF criteria and 16 healthy controls (C) underwent continuous glucose monitoring, from which the following indexes of GV were calculated: Standard Deviazione (DS), Mean Amplitude of Glycemic Excursions (MAGE), Continous Overall Net Glycemic Action (CONGA)-1, CONGA-2, CONGA-3, CONGA-4 and Coefficient of Variation (CV). All subjects underwent 5-minute ECG monitoring, and through spectral analysis of heart rate variability (HRV) we evaluated prevalent Sympathetic component (Low Frequency, LF) and parasympathetic component (High Frequency, HF) functions, and sympatho-vagal balance (LF/HF ratio).

Results: MS patients showed higher SD (1.1 ± 0.38 *vs* 0.9 ± 0.49 mmol/l, p=0.026) and MAGE (2.5 ± 0.71 *vs* 1.6 ± 0.62 mmol/l, p<0.001), lower HF (21 ± 13.5% *vs* 29.5 ± 13%; p = 0.041) and higher LF/HF (1.7 ± 1.4 ms^2 *vs* 1.1 ± 0.6 ms^2; p=0.044), than controls subjects. In MS patients, GV indexes (CONGA-1, CONGA-2, CONGA-3, CONGA-4) negatively correlated with HF (r = -0.39, p = 0.023; r = -0.4, p = 0.021; r = -0.416, p = 0.016; r = -0.385, p = 0.027; respectively), positively with LF (r = 0.394, p = 0.023; r = 0.399, p = 0.021; r = 0.418, p = 0.016; r = 0.383, p = 0.028, respectively) and with LF/HF (r = 0.361, p = 0.038; r = 0.418, p = 0.022; r = 0.403, p = 0.022; r = 0.396, p = 0.025, respectively).

Consclusions: In patients with MS, even before the onset of diabetes, we observed an increased glucose variability, which is correlated to an increased sympatho-vagal balance, prevalentely due to a reduction in parasympathetic component. These preliminary data suggest a possible role of glucose variability on increasing cardiovascular risk in metabolic syndrome, possibly mediated by an impaired sympatho-vagal balance, with a prevalence of sympathetic component.

PP154 - THE FTO GENE IS ASSOCIATED WITH DECREASE RATHER THAN INCREASE IN CARDIOMETABOLIC RISKS IN FRAIL, OBESE OLDER ADULTS

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Objective: To examine the association of FTO gene polymorphisms with cardiometabolic risks in the obese older population.

Methods: One-hundred-sixty-five frail, obese (BMI≥30 kg/m2) older (≥65 years) adults were genotyped for FTO (rs9939609 and rs8050136) single nucleotide polymorphisms and studied for associations with body weight and composition, components and prevalence of the metabolic syndrome, insulin response to an oral glucose tolerance test (OGTT), and levels of adipocytokines and vitamin D.

Results: Carriers of the A allele (CA/AA) of the FTO SNP rs8050136 had lower body weight, BMI, body fat, and trunk fat than those without the A allele (CC genotype) (all $P<0.05$). Moreover, genotype CA/AA was associated with lower triglyceride and higher HDL-cholesterol levels with a trend for lower waist, resulting in lower prevalence of the metabolic syndrome than genotype CC. The insulin area under the curve during the OGTT was lower in genotype CA/AA associated with higher insulin sensitivity index. Consistent with lesser obesity, leptin levels were also lower and adiponectin and 25-hydroxyvitamin levels tended to be higher in genotype CA/AA than genotype CC. No differences were observed for rs9939609.

Conclusions: Unlike results from studies in younger individuals, the risk A allele seems to confer a favorable cardiometabolic profile in obese older adults, suggesting selective survival into old age. Larger studies are needed to confirm these findings.

PP155 - EFFECTS OF SYNTHETIC GHRELIN ANTAGONISTS IN OBESE MICE

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Food intake is regulated by the interaction between neural and hormonal signals arising both from periphery and specific areas of the brain, and in particular the hypotalamus. Among peripheral mediators, ghrelin, an endogenous 28 aminoacid acylated peptide secreted mainly by the stomach, has an important role in the regulation of feeding. Ghrelin by binding to its receptor (GHS-R1a) effectively stimulates feeding behavior.

The purpose of this study was the pharmacological characterization *in vitro* and *in vivo* of new, synthetic peptidic and non peptidic molecules, analogues of ghrelin, named growth hormone secretagogues (GHS). The GHS have been screened *in vitro* for their ability to inhibit the GHS-R1a activation, by measuring the intracellular calcium mobilization using fluorescent indicator FLUO-4; *in vivo*, they have been evaluated on the capability to inhibit feeding behaviour and decrease body weight gain in a mouse model of obesity.

The *in vitro* studies were performed in CHO cells transiently transfected with the human GHS-R1a. In order to test the experimental conditions, CHO-GHS-R1a cells were treated with two effective agonists (ghrelin and hexarelin) alone or in combination with the GHS-R1a antagonist (D-Lys_GHRP6). As expected, acute ghrelin treatment induced a significant intracellular increase of calcium, superimposable to that induced by hexarelin, that was used in all the following experiments as a positive control. Co-incubation with D-Lys_GHRP6 blunted the calcium response induced by ghrelin or hexarelin. JMV 2959 and EP 80317 did not induce any calcium response, but both antagonized the calcium release induced by hexarelin, indicating that JMV 2959 and EP 80317 are antagonists of the GHS-R1a.

To induce obesity, the mice were fed for 9 weeks with a standard diet or an hypercaloric high fat (HFD) diet; the latter induced a significant increase of body weight, two-fold compared to control. The obese mice were treated with the antagonists JMV 2959 or EP 80317 or saline (ip, b.i.d.) for ten days; body weight and food intake were measured daily. EP80317 administration significantly inhibited body weight gain in obese mice in comparison to control mice, whereas JMV 2959 was ineffective. These results were confirmed also by Sky-Scan analisys, that demonstrated a reduced fat mass in EP 80317 treated mice. In conclusion, the results obtained in this research show that EP 80317 is effective in inhibiting body weight gain in obese mice and could be a useful tool to counteract obesity.

PP156 - COMPARISON OF PET-CT AND CT IN THE DIAGNOSIS OF RECURRENCE OF ADRENOCORTICAL CARCINOMA

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Adrenocortical carcinoma (ACC) is a rare tumor characterized by a high rate of recurrence following radical surgery. Surgery of recurrent ACC may increase survival; thus, it is mandatory a timely and accurate detection of recurrence, either to increase the chance of radical extirpation or to avoid unnecessary surgery. This study investigated the role of PET-CT in the diagnosis of recurrence of ACC during follow-up of disease-free patients and analyzed whether this tool may improve the therapeutic strategy. A retrospective evaluation of the use of PET-CT was done in ACC patients with suspected recurrence at CT imaging during their follow-up. Data of 57 patients followed at our center were retrieved. Recurrence was confirmed by pathology when lesions were removed (23 cases), or fine-needle biopsy (5 cases), or detection of unequivocal tumor progression during follow-up (29 cases). CT scan of the 57 patients showed a total of 153 lesions while PET-CT showed at least one focal uptake in 40 patients (70.2%) for a total of 99 lesions. For liver lesions, PET-CT showed a significantly higher specificity and a reduced sensitivity (sensitivity, CT 80% vs. PET 50%, p = 0.046; specificity, CT 89% vs. PET 99%, p = 0.057). With regard to local recurrence, the two tests had similar diagnostic accuracy (sensitivity: CT 87% vs. PET 79%, p=ns; specificity: CT 94% vs. PET 94%, p=ns). The same considerations apply to abdominal recurrences (sensitivity: CT 76% vs.PET 70%, p=ns; specificity: CT 94% vs. PET 99%, p=ns) and bone, in which CT and PET have equal sensitivity (86%) and specificity (98%). Conversely, in the lungs CT scan had non-significantly better diagnostic accuracy (sensitivity: CT 87% vs. PET 53%, p = ns; specificity: CT 91% vs. PET 95%, p = ns). In 18 patients (33%), PET findings changed the therapeutic strategy that was planned after CT as to the possibility of a radical surgery. In conclusion, PET can be considered an useful adjunct to CT for the diagnosis of ACC recurrence, increasing diagnostic specificity for suspected liver or abdominal recurrences, and improving the identification of occult lesions or multiple tumor sites. Use of PET has important clinical implications, allowing a smarter use of surgery due to improved selection of patients who can be radically resected.

PP157 - IMPROVEMENT OF ANTHROPOMETRIC AND METABOLIC PARAMETERS, AND QUALITY OF LIFE FOLLOWING TREATMENT WITH DUAL-RELEASE HYDROCORTISONE IN PATIENTS WITH ADDISON'S DISEASE

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Objective: In patients with Addison's disease (AD), the pharmacokinetic of available oral immediate release glucocorticoids (GC), hydrocortisone or cortisone, makes it impossible to fully mimic the cortisol rhythm, contributing to the increased morbidity and mortality rate. A dual-release preparation of hydrocortisone (Plenadren, PLEN) has been demonstrated to maintain cortisol levels in a more physiological range and to exert positive effects, at least in morbidity, in AD. **Subjects and Methods**: A clinical study including 19 AD was conducted in a single tertiary care university centre. Body weight, body mass index (BMI), waist circumference, fasting glucose, HbA1C, serum lipids, PRA, electrolytes and blood pressure were evaluated under conventional GC replacement (hydrocortisone, HC, 20 mg/day) and after 1, 3, 6 and 12 months of therapy with PLEN 20 mg/day. ACTH and cortisol (F) levels every 30 min for 120 min and at + 240' and quality of life (HRQoL) by AddiQoL were also evaluated under HC and after 1 and 12 moths of PLEN. **Results:** During PLEN, mean body weight and BMI showed a trend toward a decrease, while a significant decrease was found for waist after 12 months ($P=0.007$), in comparison to HC treatment. A significant decrease was observed after 12 months of PLEN treatment for HbA1C ($P=0.002$) but not for fasting glucose. Total cholesterol levels and LDL-cholesterol progressively decrease during PLEN treatment ($P<0.05$), without any significant changes in HDL-cholesterol and triglycerides. During PLEN, mean ACTH nadirs and AUCs were lower than during HC ($P<0.05$), without any differences in F peaks and AUCs, either after 1 or 12 months of PLEN treatment. No significant changes in PRA, blood pressure and electrolytes were observed during PLEN treatment. PLEN showed significant improvement in 4 out of 30 items of the AddiQoL, in particular in 4 out of 8 items assessing fatigue. **Conclusions:** Our study shows that in Addison's disease patients, PLEN reduces central adiposity, and improves glycolipid metabolism parameters and HRQoL.

PP158 - THE EFFECT OF RETINOIC ACID ON HUMAN ADRENAL CORTICOSTEROID SECRETION IN VITRO

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Retinoic acid, a derivative of vitamin A, has recently yielded promising results in the treatment of Cushing's disease (Pecori Giraldi et al JCEM 2012). Its main site of action appears to be the tumoral corticotrope as retinoic acid inhibits *POMC* transcription and corticotrope proliferation (Paez-Pereda et al JCI 2001). Studies on tumoral adrenal cell lines have revealed an additional inhibitory effect on cell proliferation and stimulated corticosteroid secretion (Paez-Pereda et al JCI 2001). Aim of the current study was to evaluate whether retinoic acid modulates corticosteroid secretion by normal human adrenals *in vitro*. Methods: Primary cultures from 9 normal human adrenals were incubated with 10 nM, 100 nM and 1 µM retinoic acid with and without 10 nM ACTH for 24 hours. Cortisol levels in medium were measured by Coat-A-Count RIA (Siemens Healthcare Diagnostics, Erlangen, Germany); *CYP11A*, *STAR* and *MC2R* gene expression were analyzed by real-time PCR (7900 HT Sequence Detection System, Applied Biosystems, Foster City, USA) normalized to *RPLP0*. Results: A clear-cut increase in cortisol secretion during retinoic acid incubation was observed in 5 adrenal specimens (10 nM: 183.9±55.9%, 100 nM: 210.1±82.6% and 1 µM: 141.3±11.7% baseline). Gene expression analysis revealed a marked decrease in *MC2R* expression (10 nM: 0.65±0.13, 100 nM: 0.63±0.09 and 1 µM: 0.55±0.08 over baseline) and an increase in *STAR* in wells treated with retinoic acid (10 nM: 1.53±0.26, 100 nM: 1.62±0.17 and1 µM: 1.63±0.16 over baseline). *CYP11A* was on average unchanged by retinoic acid. Incubation with ACTH led to a marked increase in cortisol secretion and in *CYP11A1*, *STAR* and *MC2R* expression. Retinoic acid and ACTH co-incubation resulted in a sligthly greater cortisol release (10 nM: 125.4±16.6%, 100 nM: 141.1±29.5% and 1 µM: 139.6±31.3% ACTH) and *MC2R* inhibition (10 nM: 0.76±0.13, 100 nM: 0.56±0.07 and 1 µM: 0.64±0.09 over ACTH) than ACTH alone. Conclusions: Retinoic acid exerts a stimulatory effect on adrenal corticosteroid secretion *in vitro*, activates *STAR* expression and blunts *MC2R* transcription. This paves the way for novel avenues of research in patients with Cushing's syndrome.

PP159 - TARGETING EGFR: A NOVEL ROLE FOR SUNITINIB IN ADRENOCORTICAL CARCINOMA CELL LINES

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Adrenocortical cancer (ACC) is a rare and aggressive malignancy. Currently the main therapeutic option is surgery, but due to difficult and delayed diagnosis and to early metastatic spread, medical therapy is often tried. ACC treatment is mainly represented by Mitotane alone or in association with chemotherapy, with variable results. Understanding the molecular mechanisms that regulate ACC proliferation could be useful to identify new therapeutic options. Sunitinib, a multitarged tyrosin-Kinase inhibitor, has been used in phase II trials for advanced refractory ACC, whit controversial results. Sunitinib alters steroidogenesis by down-regulating HSD3B2, while Epidermal Growth Factor (EGF) increases HSD3B2 expression in ACC cells.The aim of our study is to verify if EGF pathway could represent a Sunitinib target in ACC cells. EGF increased proliferation and reduces apoptosis not only in SW13 but also in ACC primary cultures, while had no effects on NCI-H295 cells. Sunitinib reduced cell viability in both cell lines, being counteracted by EGF in SW13 cells. Since in other settings EGF regulates cell proliferation by inducing VEGF, we investigated VEGF secretion. EGF enhanced VEGF secretion in SW13 cells while had no effects on NCI-H295. Moreover VEGF receptor blocking antibody significantly reduced EGF-induced cell proliferation.We also investigated EGFR expression, which was higher and ubiquitous in SW13 cells, while it was weaker and sparse in NCI-H295 cells. We investigated the intracellular signal transduction of EGF in ACC cells. In SW13 cells Sunitinib inhibited EGFR phosphorylation on tyrosine 1068, and counteracted EGF-induced phosphorylation of ERK1/2. In SW13 cells Sunitinib increased the expression of SAPK/JNK leading to caspase 3/7 activation. On the contrary, in NCI-H295 cells Sunitinib did not reduce EGFR phosphorylation, but attenuates PI3K/mTOR/AKT pathway.These data indicate that Sunitinib is capable of inhibiting ACC cell viability by increasing apoptosis activation and by strongly counteracting the proliferative effects of EGF. We demonstrated that EGF is important in regulating cell proliferation of ACC cell lines expressing EGFR. In conclusion our data suggest that EGF pathway could represent a new molecular target in drug design for treatment of ACC that display enhanced EGFR expression.

PP160 - THE ACUTE EFFECT OF A MINERALOCORTICOID RECEPTOR (MR) AGONIST ON CORTICOTROPE SECRETION IN ADDISON'S DISEASE

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Objective Mineralocorticoid receptors (MR) in the hippocampus display an important role in the control of hypothalamic–pituitary–adrenal (HPA)-axis, mediating the ''proactive''-feedback of glucocorticoids (GC). Fludrocortisone (FC), a potent MR agonist, has been shown to decrease HPA activity through an hippocampal mechanism. Since it has been demonstrated that FC shows a significant inhibition of the HPA-axis response to CRH-stimulus in normal subjects, also at doses usually administered as replacement therapy in patients with Addison's disease, it has been postulated an FC effect at MRs in human pituitary or a GR-pituitary agonism stronger than believed until now. **Design and Methods** Ten patients affected by autoimmune Addison's disease received: 1)placebo p.o.+placebo i.v. 2)hydrocortisone (H) 10mg p.o.+placebo i.v., 3)FC 0.1mg p.o.+placebo i.v., 4)FC 0.1mg and H 10mg p.o.+placebo i.v. in order to verify a possible GR FC-mediated effect that might display a repercussion on the GC-replacement therapy. **Results** H reduced ACTH ($p<0.01$) and increased cortisol levels ($p<0.01$) respect to the placebo session, while FC did not affect either ACTH or cortisol levels compared to placebo, and higher ACTH and lower cortisol levels ($p<0.03$ and $p<0.01$) were observed compared with the H session; furthermore the co-administration of FC+H showed an ACTH and cortisol profiles similar to what observed during H alone. **Conclusions** Our study showed a lack of FC effect on corticotrope secretion in Addison's disease, thus making unlikely the hypothesis of its GR pituitary agonism and the risk of glucocorticoid excess in primary adrenal insufficiency.

PP161 - ROLE OF SUNITINIB, A TYROSINE KINASE INHIBITOR, ON PRIMARY CULTURES FROM PHEOCHROMOCYTOMA AND PARAGANGLIOMA.

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Background: Pheochromocytoma and Paraganglioma are neurendocrine neoplasms which main treatment is represented by surgery. The high vascularisation of these tumors suggests a possible role for anti-angiogenic agent as medical therapy in patients not eligible for surgery or in malignant subtypes. Sunitinib, is a multi-targeted receptor tyrosine kinase inhibitor (TKI), mainly described to inhibit VEGFR.

Aim: To study the effects of Sunitinib on human Pheochromocytoma and Paraganglioma primary cultures.

Methods: 5 primary cultures (4 Pheochromocytoma and 1 Paraganglioma), were obtained from patients undergoing surgery in our centre. We collected data about patients age, genetic mutations, histological features, and clinical history. Cell viability and apoptosis were measured by ATPlite and Caspase 3/7 assay.

Results: Patients age was 52,6 ± 4,4 years. 2 patients were female and 3 were male. 2/5 displayed a germ-line RET mutation responsible for MEN2A phenotype (and also had medullary thyroid cancer). The main symptom of our patients was hypertension. All lesions were >1 cm in diameter. Sunitinib was capable of inhibiting cell viability (-40% vs.ct ±20%) and activates caspase 3/7 (+100%vs. ct ±20%).

Conclusion: our study shows that Sunitinib, in vitro, was able to reduce cell viability and activate the apoptotic process, independently from genetic alterations in chromaffin cells. Even if more data and experiments are needed to support the efficacy of this treatment, our preliminary data suggest a possible role for Sunitinib in Pheochromocytoma and Paraganglioma medical treatment

Keywords: Sunitinib, Pheochromocytoma, Paraganglioma

PP162 - A SINGLE-CENTRE 10-YEARS EXPERIENCE WITH PASIREOTIDE IN CUSHING'S DISEASE: PATIENTS CHARACTERISTICS AND OUTCOME

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Introduction: Pasireotide is the first pituitary-directed drug approved for treating patients with Cushing's disease (CD). We report our 10-years experience with pasireotide in CD reviewing and analyzing data about all the patients treated with pasireotide at our referral centre both in randomized trials and in clinical practice.

Patients and Methods: Twenty patients with *de novo*, persistent or recurrent CD after pituitary surgery were treated with pasireotide from December 2003 to December 2014. Four-teen patients were treated with pasireotide in randomized trials and six patients were treated with pasireotide sc (Signifor®; Novartis AG, Basel, Switzerland) in clinical practice. The mean treatment duration was 20.5 months (median 9 months; range, 3–72 months). The mean daily dose of pasireotide sc was 1333 mcg (range, 1200–1800 mcg) at the beginning of treatment as well as 1366 mcg at last follow-up (range, 600–2400 mcg).

Results: In the overall population, urinary free cortisol (UFC) levels mean percentage change (± SD) at last follow-up was –40.4% (± 35.1; range, 2–92%; median reduction 33.3%) with a normalization rate of 50% (10/20). UFC normalization occurred by months 1–3 in the majority of patients (8/10; 80%). Ten patients achieved sustained normalized late night salivary cortisol (LNSC) levels during treatment. LNSC normalization was associated with UFC normalization in 7/10 patients. Serum cortisol and plasma ACTH significantly decreased from baseline to last follow-up. Body weight decrease and blood pressure improvement during pasireotide treatment were independent from UFC response. Glucose profile worsening was observed in all the patients except one. The frequency of diabetes mellitus increased from 40% (8/20) at baseline to 85% (17/20) at last follow-up requiring to start medical treatment only in a half of patients (8/17).

Conclusions: Pasireotide treatment was associated with sustained biochemical and clinical benefit in about 50% of CD patients. Glucose profile alterations are a frequent complication of pasireotide treatment however this adverse event seems to be easy to manage with diet and lifestyle intervention in almost half of patients.

PP163 - URINARY FREE CORTISONE AS A POTENTIAL BIOMARKER IN DIAGNOSING PATIENTS WITH MILD CUSHING'S SYNDROME

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Introduction: Despite its long-term use in clinical practice, urinary free cortisol (UFC) determination presents many drawbacks due to suboptimal sensitivity (SE) and specificity (SP) especially in *mild* Cushing's syndrome (CS).

Aim: To determine the performance of UFC and its metabolite cortisone (UFE) measured using accurate assays such as HPLC and LC-MS/MS in diagnosing patients with CS.

Patients and Methods: Sixty-seven patients with CS [43 Cushing's disease (31 *de novo* and 12 persistent/recurrent); 16 Adrenal CS and 8 ectopic CS; 18 M, 49 F; mean age 46.4 ± 13.3 years] were analyzed and compared to forty-nine sex and age-matched non-CS patients. All the patients referred to our department from January 2004 to December 2014. UFF and UFE levels were assessed by HPLC until October 2009 and afterwards by LC-MS/MS. All CS patients provided almost two 24-h urine collections and the mean value was used for the analysis. Our reference range for UFF and UFE was respectively 9.2–45.2 µg/24h and 14.5–94.6 µg/24h. SE and SP were calculated at different cut-off values using ROC curve analysis.

Results: UFF and UFE values were significantly higher in patients with CS compared to non-CS. However the diagnostic performance of UFF measured by HPLC was low with 75.8% SE and 81.1% SP (cut-off value > 33.9 µg/24h). For urinary samples measured by LC-MS/MS the performance of UFF was 88.2% SE and 91.2% SP (cut-off value > 47.4 µg/24h). The performance of UFE was better than UFF by both assays. In particular, UFE by LC-MS/MS showed 97.1% SE and 91.7 SP% using a cut-off value > 88.7 µg/24h. When CS patients were stratified according to disease severity, UFE measured by LC-MS/MS showed a good diagnostic profile also in *mild* CS (UFF < 2xULN) with 92.3% SE and 91.7% SP (cut-off value > 88.7 µg/24h). Conversely, the performance of UFF in *mild* CS was low (84.6% SE 75% SP; cut-off value > 44.2). UFF and UFE showed an optimal diagnostic performance with 100% SE and 100% SP in moderate to severe CS (UFF ≥ 2xULN). The performance of UFF and UFE was not different according to age, sex, disease aetiology and disease status in CS.

Conclusions: UFE measured by LC-MS/MS seems to be a new and good biomarker in diagnosing CS especially in patients with *mild* hypercortisolism at higher risk for misdiagnosis with UFF.

PP164 - MORTALITY IN PATIENTS WITH INCIDENTALLY DISCOVERED ADRENAL ADENOMAS : THE EXPERIENCE OF SAN LUIGI HOSPITAL

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BACKGROUND Adrenal incidentalomas are found in 3–7% of radiological series and many of them are adrenal adenomas. Autonomous cortisol secretion without clinical signs of overt hypercortisolism is a common finding in these patients. Studies reported metabolic derangement and increased cardiovascular risk associated with this state of subtle cortisol excess, however scanty data are available on the natural history of this condition .

AIM OF THE STUDY To assess the rate of mortality in patients with incidentally discovered adenomas.

METHODS We studied 110 patients (39 men and 71 female) with incidentally discovered adrenal adenomas from 1998 and 2013. Metabolic and hormonal parameters were determined. We collected the following data: blood pressure, plasma glucose, lipid profile, cortisol levels after 1 mg dexamethasone suppression test (1 mg-DST), plasma ACTH and urinary free cortisol. Mortality data were obtained from the demographic registers.

RESULTS Mean age of patients was 67 yrs, with a mean follow-up of 94 months. Fourteen (12.7%) patients died: 4 (28.6%) for cancer, 7 (50.0%) for cardiovascular and 3 (21.4%) for respiratory/infective causes. Twelve of them (85.6%) had 1 mg-DST >1.8 µg/dL (4 had hypertension, 4 dyslipidemia and 4 diabetes) while 54/96 patients alive at the last follow-up (56.2%) had 1 mg-DST >1.8 µg/dL (p=0.04) . Survival probability was significantly reduced in patients with 1 mg-DST >1.8 µg/dL, with a Hazard Ratio of death of 3.64 (95% CI, 1.34 - 9.7; P=0.013). Age did not differ between patients alive or dead at the last follow-up (65.1 ± 9.8 yrs vs 65.1 ± 9.8 yrs, NS).

CONCLUSION Patients with incidental adrenal adenomas and autonomous cortisol secretion heralded by cortisol after 1 mg-DST >1.8 µg/dL may be at increased risk of mortality compared to patients with non-secreting adenomas. Excess mortality is mainly related to cardiovascular events.

PP165 - SUBCLINICAL MALIGNANT PHECHROMOCYTOMA: A CASE REPORT.

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Introduction. Pheochromocytoma (PCC) is a neuroendocrine catecholamine secreting tumour arising from the chromaffin cells in the adrenal medulla. PCC may present with a broad spectrum of signs and symptoms, depending on several factors including tumor size, type and amount of catecholamines secreted, sensivity of peripheral adrenoceptors. Subclinical PCC are incidentally discovered during radiological procedures (incidentaloma); plasma/urine catecholamine metabolite testing is recommended in patients affected by adrenal incidentalomas and is mandatory in those scheduled for surgery. Nearly 10% of PCC are malignant; malignancy is defined by presence of metastases, spreading to sites where chromaffin tissue is normally absent. **Case report.** In this report, we discuss the case of a 17-year-old girl who was referred to our clinic for the enlargement of an incidental adrenal mass discovered on radiological investigation, performed for hepatic hemangioma two years earlier. She had already undergone, in another center, an evaluation of adrenal function and plasma cathecolamines, resulted in normal ranges. Abdominal CT scanning evidenced: "modest *increase in volume of the lesion in the left adrenal gland (diam max 3,7cm)…characterized by post-contrast-enhancement,…presence of left paraadrenal lymph nodes (diam max 1,2cm)*". She had no personal or family history of endocrinopathy and was asymptomatic. Laboratory tests showed very high levels of 24-hour urinary normetanefrine, confirmed in a second test (5210ug/24h and 12276ug/24h, n.v. 162-527 ug/24h). On 123I-MIBG scintigraphy (planar and SPECT), an increased accumulation was detected only in the left adrenal gland. After a preoperative collegial consultation and adequate catecholamine blockade, the patient underwent laparoscopic excision of the mass and suspected lymph nodes, without surgical complications. Histology confirmed a malignant pheochomocytoma with 3 lymph node metastases. Genetic-analysis evaluated mutations in the subsequent genes: SDHB (exons 1-8), SDHC (exons 1–6), VHL (exons 1-3), RET (exons 10,11,13-16), TMEM127(exons 1–4), MAX (exons 1–5), FH (1-10) without identifying variants with a clear pathological significance. The patient did not receive additional therapy after surgery but underwent a strict biochemical and radiological follow-up. **Conclusion.** Since PCC may present with a broad spectrum of signs and symptoms or be asymptomatic even in malignant cases, such as in our patient, biochemical analysis is recommended in all patients with adrenal incidentaloma to confirm or rule out an excessive production of catecholamines. Measurements of metanephrines in plasma or in urine offer the best diagnostic performance and are the tests of choice.

PP166 - ADRENOCORTICAL CARCINOMA WITH LOW KI-67 INDEX

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BACKGROUND: Adrenocorticortical carcinomas (ACCs) are rare and highly aggressive tumors, arising from the adrenal cortex with a worldwide annual incidence of about 1-2 cases per million in adults. Approximately 60% of ACCs are functioning tumors. Patients can present with Cushing's syndrome alone, a mixed Cushing's and virilization syndrome, or virilization alone. Conversely, patients with non-functioning tumors usually present with local symptoms due to an enlarging abdominal mass or with an adrenal incidentaloma. Contrast-enhanced CT scan or MRI is the imaging modality of choice in suggesting the diagnosis, which is confirmed by the hystological pattern. Weiss system has gained the most extensive acceptance in clinical practice: a score > 3 is diagnostic for malignancy. Radical resection is the treatment of choice. Chemotherapy and/or mitotane are required in the event of incomplete resection or metastatic spread. Despite the generally unfavourable prognosis, there is a wide individual variation in the progression of disease and overall survival. CASE REPORT: A 32-year-old man was admitted at General Surgery Division because of epigastralgia and weight loss that had persisted for at least 3 months. Abdominal US showed a large right adrenal mass. MRI confirmed a right adrenal mass of 7x5.2x6.5 cm with heterogeneous enhancement. Adrenal hormone assessment was within the normal ranges. The patient was submitted to laparoscopic right adrenalectomy. The gross specimen presented as a well demarked, encapsulated mass of 10 cm (pT2 pNx). Despite low Ki-67 (1%) and low mitosis index, the coexistence of nuclear abnormalities, venous and sinusoidal invasion, diffuse architecture and necrosis was indicative of malignancy (Weiss score = 6). A second opinion confirmed the diagnosis of ACC with low malignancy rate. At diagnosis, brain MRI, chest CT and bone scan were negative. After 22 months, abdominal MRI and chest CT (performed every 3 months for the first year, then every 6 months) are still negative. The laboratory exams remained within the normal range. CONCLUSION: We present a rare case of ACC with a very low Ki-67 index. Among biological markers evaluated to predict the ACC behaviour, proliferative index Ki-67/MIB-1 has been demonstrated to be particularly effective. This is one of the cases of ACC that qualify as malignant according to the Weiss score, but behave in a different manner. A longer and strict follow-up in these cases is needed to better understand the outcome.

P P 1 6 7 - P R E D I C T O R S O F R E C U R R E N C E O F PHEOCHROMOCYTOMAS/PARAGANGLIOMAS: A RETROSPECTIVE STUDY ON CASES DIAGNOSED FROM 2000 AT A.S.O.U. 'CITTÀ DELLA SALUTE E DELLA SCIENZA' IN TORINO OR AT HOSPITAL 'CARDINAL MASSAIA' IN ASTI, PIEDMONT, ITALY.

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Objective. Chromaffine tissue tumors (pheochromocitomas and paragangliomas) are rare neoplasms often releasing cathecolamines, mainly originating from adrenals but occasionally observed also in parasympathetic ganglia, with a genetic base up to 25% of the cases. Therapeutic gold standard is radical surgery. Disease recurrence was believed to be under 10% but more recent studies reported a much higher rate even after many years from surgery. Apart from familiar forms, little evidence exists about the predictors of malignant forms, thus, we aimed to search for predictors of recurrence with a retrospective analysis on patients diagnosed and treated at our Hospitals. Patients and Methods. We collected clinical data of patients with diagnosis of chromaffine tissue tumors that underwent radical surgery from 2000 in our Hospitals. 69 subjects were recorded (/: 40/29, age, mean±SD: 43.6±16 years) for a mean follow up of 65.5±69.2 months. Genetic test for mutation of known susceptibility genes (VHL, RET, NF1, TMEM127, MAX, SDH and SDHAF2) was performed in 34 cases, resulting positive in 21. Results. 20/69 (29%) patients had disease recurrence. These patients were younger (30.7±14.8 vs 48.9±13.2 years; p=0.000), had higher rate of positive familiarity and genetic mutations (53.3% vs 12.5%; p=0.002 and 70% vs 14.3%; p=0.000, respectively), increased metanephrine levels (28.6% vs 63%, p=0.02), larger tumors (7.2±3.8 vs 4.5±2.0 cm; p=0.001) and lower biochemical normalization rate (66.5% vs 95.6%, p=0.008). Patients with recurrence did not differ for sex, Chromogranin A levels and score PASS values, suggestive imaging features, rate of positive MIBeG scan or complete clinical normalization after surgery. To better define predictors of recurrence, we analysis of the data of follow-up by Kaplan Mayer curves, searching for variables associated with cumulative incidence of recurrence by Log Rank test: age at diagnosis ≤ 45 years (p=0.003), neoplasm dimension > 4 cm (p=0.0097), positive familiarity (p=0.007) or genetic test (p=0.0005) and biochemical normalization after surgery (p=0.004) were associated to disease recurrence. Conclusion. Recurrences in pheochromocytomas/paragangliomas develop more frequently in young patients, with larger positive genetic testing of known susceptibility mutations. Also larger tumors, lower levels of metanephrine, incomplete normalization of biochemical markers after radical surgery are features of relapse of these neoplasms. Patients

with these characteristics should be monitored with strictly follow-up.

PP168 - EFFICACY OF LONG-LASTING TREATMENT WITH SUNITINIB IN A SDHB MUTATION CARRIER AFFECTED BY A METASTATIC ABDOMINAL PARAGANGLIOMA

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Here we describe the case of a 35 yr old SDHB mutation carrier with a metastatic recurrent abdominal paraganglioma (PGL) who was referred to us in September 2013.

He also presented a congenital right kidney hypoplasia.

Urinary normetanephrine (NMNu) resulted 8927 mcg/24h. A CT revealed a large abdominal (4.6x4.9x5.9 cm) and other smaller abdominal lesions as well as liver metastases.

Surgical removal of the primary tumor was excluded because of left kidney involvement as well as the radiometabolic therapy because of a negative MIBG scintigraphy.

In October 2013 the patient developed acute renal failure because of ureter compression and a pig tail was inserted.

In November 2013 a disease progression was documented and the patient started Sunitinib at the dose of 25 mg, later on increased at 50 mg. In February 2014 a clear reduction of the main lesion (3,7x3,5x3,6 cm) was observed as well as a significant decrease in NMNu and ^{18}FDG uptake. The occurrence of side effects obliged us to reduce the drug dose schedule (37,5 mg 1 wk on, 25 mg 1 wk on, 1 wk off) which was further reduced (25 mg/day 2 wk on, 1 wk off) in July 2014, because of stomach pain.

At the last follow up in December 2014 the main abdominal lesion was further decreased in size (2,7x3,4x3,4 cm) while at CT and ^{18}FDG-PET the abdominal lesions and liver metastases were found stable thus demonstrating the efficacy of Sunitinib therapy after 65 weeks in this *SDHB* mutation carrier affected by a malignant PGL.

PP169 - A ROLE FOR METFORMIN IN THE TREATMENT OF ADRENOCORTICAL CARCINOMA

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Adrenocortical carcinoma (ACC) is a rare and heterogeneous malignancy with a poor prognosis, mainly dependent on tumor stage at diagnosis. To date, radical surgery, possibly associated to mitotane adjuvant therapy, is considered the best option for ACC treatment. However, in the case of metastatic ACC, the mean 5-year survival rate diminishes dramatically and mitotane efficacy has not been proved. Moreover, chemo-resistance often develops. Thus, more specific and effective drugs for ACC treatment are urgently required. The antidiabetic drug metformin, used as first line therapy for type II diabetes treatment, has been proved to exert antineoplastic effects in many type of malignancies. Our study aimed to evaluate the potential anti-cancer effects of metformin in H295R adrenocortical tumor cell line, looking for a possible alternative therapeutic approach to ACC treatment. Increasing doses of metformin (0,5-250 mM), administered to cells for 24h, 48h, 72h and 7 days, inhibit cell viability and proliferation in a dose- and time-dependent manner, as observed by MTS and cell count assay. This effect on cell proliferation was further confirmed by using [^{3}H]thymidine incorporation assay. Moreover, metformin and mitotane combination reduces cell viability to a greater extent than metformin alone, indicating the presence of a synergistic effect. Since metformin actions may depend on different mechanisms, we tried to define the molecular pathways at the basis of cell growth inhibition in our cell model. Western blot analysis, performed after 6- and 24- hour metformin treatment (20, 50, 100 mM), revealed a dose-dependent decrease in the phosphorylation of ERK1/2 and m-TOR, while we observed an increase of AMPK activation. This results suggest that metformin may exert its effect on cell viability acting through these signaling pathway.

In conclusion, our data indicate that metformin is able to interfere with the *in vitro* cancer cell proliferation, showing an effect which depends on the drug concentration treatment duration. Furthermore, the synergistic effect observed in the presence of mitotane, suggests the possible use of metformin in combination with the current therapy for ACC treatment. Further *in vivo* studies are required to prove metformin efficacy in adrenocortical carcinoma.

PP170 - ACCURACY OF THE MOLECULAR DIAGNOSIS OF CONGENITAL ADRENAL HYPERPLASIA (CAH) DUE TO 21-HYDROXYLASE DEFICIENCY

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21-hydroxylase deficiency (21OHD) is an autosomal recessive disorder, due to CYP21 gene mutations, responsible of 95% of cases of CAH, in which aldosterone and cortisol synthesis pathways are impaired, and 17OHP and androgens accumulated. The classical form (CF) is rare and occurs as simple virilizing (SV) or salt wasting (SW) phenotypes, whereas the non-classical form (NCF) is much more prevalent (1:1000). In a clinical context high serum 17OHP level at baseline and/or after ACTH stimulus suggest the diagnosis of 21OHD. Molecular testing is needed to detect CYP21 mutations and confirm the diagnosis. Here we report the results of CYP21 gene analyses, performed in our Center from 2005 to 2014, on 164 subjects (including 3 prenatal testing): 85% female and 15% male, mean age 16 yrs. NCF were 80.5%, CF 19.5% (11% SW, 8.5% SV). We detected CYP21 mutations in 48.2% of clinically suspected cases; otherwise 27 (16.5%) resulted carriers and 52 (31.7%) negative. Most NCF cases (33.3%) resulted negative, but some CF subjects did not show any mutations (7 SV, 1 SW). The frequency of mutations were similar to recent literature, except for V281L, that results twice in our cohort (41.6% vs 23.9%) and I172N with a half frequency (4.3% vs 8.2%)(Table1). Our analysis shows a specificity of 83% and a sensitivity of 62%. The sensitivity improves till 73% when the clinical suspect is associated with biochemical findings and up to 78% when there is also a positive ACTH test. In conclusion, the clinical features, ACTH test and genetic counseling are important to perform correctly molecular testing.

CYP21	del8bp	Ex6	P30L	R356W	I172N	Q318X	del	I2S	V281L	other
p.st. n.(%)	3(1.6)	3(1.6)	3(1.6)	4(2.2)	8(4.3)	10(5.4)	21(11.4)	38(21.5)	77(41.6)	18(9.7)
New 2013	2.1	2.1	2.6	3.6	8.2	3.2	20	22.9	23.9	

PP171 - ADRENOCORTICAL CARCINOMA IN POST-MENOPAUSAL WOMAN: A CASE REPORT

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Adrenocortical carcinomas (ACCs) are rare with an annual incidence of 1-2 per million population, a peak incidence in the 4th to 5th decade, and a greater prevalence in women. Most ACCs in adults show a clinical presentation of Cushing's syndrome alone (45%) or a mixed Cushing's and virilization syndrome (25%). Symptoms usually develop very rapidly (over three to six months). We present the case of a post-menopausal 50-year-old woman, who had developed hypertension less than 6 months earlier, associated with hair loss and increasing growth of terminal hair. She was admitted to our Endocrinology Unit for uncontrolled hypertension despite four antihypertensive drugs (lercanidipine, bisoprolol, hydrochlorothiazide and valsartan). Physical examination showed mild hirsutism (Ferriman-Gallwey score of 10, range 0-36), mild modification of facial oval with moderate flush of the head and trunk, and increased abdominal adiposity. Hormonal assessment revealed: 1) hyperandrogenism with elevated total testosterone (1.5 ng/ml), Δ4androstenedione (>10 ng/ml), and normal DHEA-S (309 µg/dl); 2) increased estradiol for menopausal age (43 pg/ml) with suppressed gonadotropins LH and FSH; 3) primary hypercortisolism with elevated morning cortisol (27 µg/dl) and urinary free cortisol (1827 µg/24h), low-normal ACTH (5.72 pg/ml), and lack of cortisol decrease after 1 mg dexamethasone suppression test (8 a.m. cortisol: 24 µg/dl, normal values <1.8-5 µg/dl); 4) plasma aldosterone, plasma renin activity, urinary metanephrines and catecholamines at the lower limit of normal range. The MRI images showed a left adrenal mass of 9x6x7 cm, with irregular shape, inhomogeneous parenchymal signal intensity and inhomogeneous contrast enhancement with delay in contrast washout. A second mass of 3.5x2.2x3.4 cm was described with the same signal intensity and contrast enhancement features and localized to the paraaortic region under renal vessels. The F-18/flourodeoxyglucose-positron emission tomography (FDG-PET-CT) demonstrated high standardized uptake value (SUV) of both the adrenal mass and the paraaortic mass. Given the high risk of malignancy and tumor dimensions, the patient underwent an open transperitoneal adrenalectomy to avoid intra-operative capsule rupture and tumor spillage. Complete resection of the adrenal mass, the para aortic mass, and of the perivisceral adipose tissue was obtained. The histopathological examination confirmed the diagnosis of malignant adrenocortical tumor according to microscopic Weiss criteria (5-6 mitoses/50 high power fields, 20% clear tumor cells in cytoplasm, abnormal mitoses, necrosis, and capsular invasion), and identified the presence of distant metastasis, in fact the para aortic mass resulted to be a lymph node with tumor localization. The immunohistochemical study demonstrated a Ki-67 proliferation index of 15%. The CT scan performed 8 weeks after surgery excluded the presence of recurrent tumors or incomplete resection. Considering the tumor stage (ENSAT Stage III, T3N1M0) and the prognostic factors (Ki-67 expression in >10% of neoplastic cells), adjuvant mitotane and irradiation of the tumor bed have been proposed to the patient. This case emphasizes the importance of screening for secondary causes in presence of hypertension of short duration resistant to multiple drugs. Despite the mild clinical presentation, the recent onset of both hirsutism and signs of

hypercortisolism was highly suspicious for malignancy. Resection status and experienced hystological characterization are the main elements in decisional therapeutic workup after surgery.

PP172 - A RARE CASE OF HYPONATREMIA CAUSED BY SIADH IN A PATIENT WITH ADDISON'S DISEASE.

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Introduction. We describe a case of Addison's disease with superimposed syndrome of inappropriate antidiuretic hormone secretion (SIADH) leading to a difficult attempt to normalize plasma sodium with mineralocorticoid replacement.

Case report. A 40-year-old male was diagnosed with Addison's disease at the age of 18. Fludrocortisone had been discontinued because of an unspecified drug intolerance. Cortisone acetate was slowly increased to a maintenance dose of 25 mg 3 times a day. He had also thyroidectomy for thyroid carcinoma and treated with L-T4 replacement therapy. He presented with S-Na 126 mEq/L and S-K 5.3 mEq/L. In the previous year he was in a clinical situation of mild hyponatremia (S-Na 124–127 mEq/L) with modest, indistinct and nonspecific symptoms. We started treatment with fludrocortisone, with no side effects, increasing the dosage to a maximum of 0.2 mg/day. In primary adrenal failure correction of the electrolyte disturbance with cortisone and mineralocorticoid treatment is usually prompt. Therefore, because of S-K normalization (4.1 mEq/L) with only a modest effect on S-Na (129 mEq/L), a concomitant SIADH was hypothesized. Glycemia was normal (99 mg/dL), uric acid was low (3.1 mg/dL). Urine sodium and urine osmolarity were elevated, 90 mEq/L, and 395 mosm/L, respectively. No diuretics were used. On physical examination the patient was euvolemic. Extensive investigations ruled out malignancy, pulmonary, hepatic cardiac or renal disease or any other known causes of SIADH. Based on Bartter and Schwartz criteria SIADH was diagnosed. Five days fluid restriction had no effect on S-Na. Tolvaptan 15 mg p.o. daily was then administered. S-Na increased from approximately 128 to 136 mEq/L within 4 days. Fludrocortisone and cortisone acetate daily doses were gradually reduced. Tolvaptan administration was discontinued 6 days later. At 1 month follow–up S-Na was 143 mEq/L. Fludrocortisone was reduced at 0.05 mg/d and cortone acetate was administered at the usual dose of 25 mg/d. The patient reported a subjective clinical amelioration.

Conclusions. We describe a very rare association of two possible causes of hyponatremia, Addison disease and SIADH. Although no apparent cause of SIADH was found, the condition resolved quickly and completely with treatment with tolvaptan, a selective nonpeptide arginine vasopressin (AVP) V(2)-receptor antagonist approved for use in SIADH. Nevertheless, a longer follow-up is necessary to verify this observation.

PP173 - CLINICAL PERFORMANCE OF URINARY FREE CORTISOL AND MIDNIGHT SALIVARY CORTISOL IN THE DIAGNOSIS OF CUSHING SYNDROME

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Introduction: The diagnosis of Cushing syndrome (CS) represents a challenge for the endocrinologist. Urinary free cortisol (UFC) is the most reliable index of cortisol secretion and is considered the "gold standard" in the diagnosis of CS. We evaluated the diagnostic performance of UFC with LC-MS/MS in the diagnosis of CS proposing a cut-off value . We also compared the diagnostic performance with midnight salivary cortisol, proposing a cut -off value.

Patients and methods: 75 patients affected by CS who had performed at least one assessment of UFC at our center from 2010 to 2014 were included. For 56 patients midnight salivary cortisol was also evaluated. UFC was measured with LC-MS/MS. Saliva samples were collected with cotton swabs (Salivette Cortisol, Sarstedt). The samples were stored at 4°C and centrifuged at 4000 RPM for 10 minutes and assayed in electrochemiluminescence (ECLIA) using Roche kits on Modular E analyzer. Data were collected on a database of Microsof Excel. For statistical analysis the Mann - Whitney U test was used. In order to determine the cut-off values specific ROC curves were created.

Results: The mean values of UFC in CS group is 669 ± 209 µg/24h (mean ± SE). The mean values in the control group was 22,1±19 µg/24h. The Mann-Whitney U test showed a statistically significant difference between the two groups ($p < 0.01$). Based on these data , a ROC curve was created, showing an AUC = 0,98, identifying the CLU as highly predictive for the identification of patients with CS. According to this curve, the chosen cut-off value was 43,5 µg/24h with a sensitivity = 97% and specificity = 89%;

The mean values of midnight salivary cortisol in the CS group is 0,95±0,15 µg/dl. The mean values of the control group is 0,03±0,01 µg/dl. The Mann-Whitney U test showed a statistically significant difference between the two groups ($p < 0,01$). On the basis of these data, a ROC curve was created, showing an AUC = 1, identifying the midnight salivary cortisol as highly predictive for the identification of patients with SC. According to this curve, the chosen cut-off value was 0,3 µg/dl, providing a maximum sensitivity (100%) and specificity (100 %).

Discussion: In the study we evaluated the clinical performance of UFC with LC-MS/MS and midnight salivary cortisol in the diagnosis of CS. Salivary cortisol appears to be promising as first line screening test for CS. Comparing the AUC of ROC curves, although both tests have a good diagnostic performance as first level test for diagnosis the CS, midnight salivary cortisol has a greater predictive value.

PP174 - MITOTANE EFFECTS ON MATURATION OF MALE AND FEMALE GONADS

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Mitotane is the first-line treatment for metastatic adrenocortical carcinoma (ACC) and it is regularly used in the adjuvant setting after complete removal of the primary tumor. It inhibits the 3-beta-hydroxysteroid dehydrogenase and 11-beta-hydroxylase preventing the formation of cortisol. Some clinical evidences show a male hormonal hypogonadism following a mitotane treatment. The aim of our study was to analyze the effects of mitotane on the male and female gonads. We used 12 days-CD1 mice, treated for 17 days, analyzing the morphological changes between normal and treated tissues. The levels of proliferation, differentiation of the cell types in the gonadal tissue were evaluated by immunofluorescence, immunohistochemistry and Multiplex PCR. Reversibility of the process was evaluated with mating of female and male two months-mice. The morphological analysis shows a slowing-down of gonads follicular maturation in the treated-females, analyzed by level of KI-67, and gene expression of FSH receptor, AMH, 17-alfa-hydroxylase. In the treated males we found atrophy of seminiferous tubules, decreased sperm count and decreased expression of INSL3, marker of proliferation of Leydig cells. In the mating, all treated females had a pregnancy, but with a significant delay, while in all treated-males only the 50% recovered and also with a significat delay. Our data showed a decrease in the fertility following a prolonged treatment of mitotane. The gonads of both sexes are susceptible to damage in a different way: in the females we found a slow-down in the process of follicular maturation without tissue damage particularly evident. While in the male gonads mitotane induces a strong damage of the tissue with atrophy of the seminiferous tubules.

PP175 - EFFECTS OF SORAFENIB, A TYROSINE KINASE INHIBITOR, ON H295R ADRENOCORTICAL CANCER CELL LINE

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The lack of an effective medical treatment for adrenocortical carcinoma (ACC), is still a challenge for searching of new molecules for its treatment. Based on the efficacy of sorafenib, a tyrosin kinase inhibitor, in different human tumors, the aim of this study was to investigate the mechanism of action of sorafenib in ACC. The effects of sorafenib were tested on H295R adrenocortical cell line by evaluating cell viability and apoptosis and the VEGF receptor signaling, such as VE-cadherin and β-catenin complex formation, we also testing the sorefenib effects on a 3D cell culture model by using the same H295R cell line. We observed apoptosis after sorafenib treatment. Co-immunoprecipitation suggested that the treatment prevents the formation of the complex VEGFR-VE-cadherin and beta-catenin proteins. Ultrastructural analysis and 3D model demonstrated a disegregation of the single spheres.

Our finding suggest that although sorafenib induced apoptic cell death a small portion of cells survived to the treatment and showed the characteristics of a malignant phenotype. In conclusion, we suggest to avoid the use of sorafenib alone in the treatment of ACC.

PP176 - RESISTANT HYPERTENSION IN ADRENAL INCIDENTALOMA

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Introduction and aim: Resistant Hypertension (RH), associated with high risk of cardiovascular events, is defined when appropriate treatment (lifestyle measures plus 3 antihypertensive drugs) fail to control blood pressure. Patients with Adrenal Incidentaloma (AI) present a higher rate of cortisol-related comorbidities than general population, such as hypertension. We investigated prevalence of RH in outpatients with AI.

Materials and methods: We studied 134 patients with AI (27 bilateral) and hypertension; overt hormonal excess was excluded: Cushing's syndrome (clinically and on the basis of hormonal tests), primary aldosteronism (serum aldosterone/plasma renin activity ratio <30 after properly washout of therapy) and pheo (at least two normal collections for urinary fractionated metanephrines). We considered Subclinical Hypercortisolism (SH) in those patients that did not suppress serum cortisol <138 nmol/L after dexamethasone (DST), or that presented serum cortisol after DST 50-138 nmol/L and at least one other altered test among ACTH levels <10 ng/L, late night salivary cortisol (LNSC) >5.24 ng/mL or urinary free cortisol (UFC) >170 nmol/24h.

Results: In our cohort, 43 patients with AI (32%) revealed RH. Age, height, weight, BMI, waist and hip circumference, waist to hip ratio and presence of impaired glucose or lipid metabolism were not different among AI patients with and without RH. Considering testing, RH was not associated with ACTH suppression, high levels of LNSC or UFC, cortisol suppression after DST, also considering patients with or without SH or with mono or bilateral AI.

Discussion: Despite high prevalence, cortisol secretion and metabolic comorbidities are not related to RH in AI.

PP177 - CERTAIN HLA ALLELES ARE ASSOCIATED WITH GRAVES' DISEASE (GD) IN PATIENTS IN WHOM HYPERTHYROIDISM IS PRECEDED BY STRESSFUL EVENTS (SE)

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There are no studies on HLA alleles in GD patients whose initial or recurrent hyperthyroidism (RH) is preceded by ≥1 SE. RH is termed exacerbation (EXA) or relapse (REL) if occurring while on or off treatment with antithyroid drugs (ATD).

To fill this gap, we have enrolled 58 Caucasian GD patients (36 F, 22 M) in whom ≥1 SE preceded by ≤12 months the onset and the EXA/REL, if EXA/REL occurred. Based on outcomes observed over a follow-up of ≥5 years (median 13, range 5-27) after ATD withdrawal, 3 groups were formed: remission (REM, patients with neither EXA nor REL; n=15, 25.9%), EXA (≥1 EXA; n=6, 10.3%) and REL (≥1 REL; n=37, 63.8%). We performed serological HLA typing by the microlymphotoxicity test in all GD patients and in 130 Caucasians healthy controls. Differences between rates were analyzed with the χ^2 test or the exact Fisher's test.

Two HLA class I and three class II alleles were more frequent in patients compared to controls: B8 (13.8% *vs.* 3.1%, P=0.006, odds ratio [OR]=5.6), Cw7 (63.8% *vs.* 32.3%, P=0.0001, OR=3.7), DR3 (17.2% vs 7.7%, P=0.03, OR=2.9), DR4 (27.6% vs. 10.8%, P=0.002, OR=3.7) and DQ2 (36.2% *vs.* 24.6%, P=0.03, OR=2.1). In contrast, two HLA class I alleles were less frequent: B14 (1.9% *vs.* 16.1%, P=0.005, OR=0.1) and Cw8 (1.7% *vs.* 10.8%, P=0.04, OR=0.1). Among GD patients, the following alleles were over/under-represented, depending on outcome. Cw7 was more frequent in the EXA (83.3%) and REL (72.2%) groups than in the REM group (40%, df=2, P=0.05). Both A28 and DR6 were more frequent (33.3% and 50%, df=2, P=0.007 and P=0.05) in the EXA group than in the REL (3.1% and 15.6%) and REM (0% and 6.7%) groups. The Cw6 allele was more frequent in the REM group (40% *vs.* 0% of the EXA and 11.1% of the REL group, df=2, P=0.02), while B7 allele was more frequent in both the REM and EXA groups (26.7% and 33.3%) than in the REL group (3.1%, df=2, P=0.03). Among the 37 relapsers, B53 was detected only in those with ≥2 relapses (3/14, 21.4%), but in none of those with only one (0/23, P=0.05).

The association of GD with A8, Cw7, DQ2 and DR3 alleles in our patients agrees with an English study (Simmonds et al 2007). Cw7 allele confers risk for either EXA or REL, B53 for ≥2 REL, while A28, Cw6 and DR6 protects from REL. Similar to Simmonds et al, we conclude for a primary role of HLA class I-mediated response in GD, a condition assumed to be a straightforward HLA-class II-restricted disease. This role appears more prominent in SE-associated GD, in whom HLA typing may be helpful in predicting the outcome after ATD withdrawal.

PP178 - IMPACT OF MOLECULAR PROFILE ON THE CLINICAL MANAGEMENT OF PATIENTS WITH PAPILLARY THYROID CARCINOMA

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Introduction. Papillary thyroid carcinoma (PTC) accounts for 90% of all thyroid cancers. Genetic mutations constitute more than 85% of cases of BRAF, RAS (H-, N-, K-RAS), RET / PTC and PAX8 / PPARɣ (mutually exclusive). Molecular profiling is usually carried out on histological samples to characterize the tumor. BRAF-V600E is associated with an increased relapse rate in PTC. The **aims** of the study were to stratify PTC according to (histological and clinical) malignancy, to discern a pattern of genetic alterations in our population and to verify the reliability of the methods and results by comparing them with the literature data. **Subjects** We collected 93 PTC histological samples from patients (70 F, 23 M; mean age 45 ± 18 years, median 48) who had undergone total thyroidectomy from 2009 to 2011. **Methods** Genes were extracted and sequenced: real-time PCR was performed for PAX8/PPAR, RET/PTC1 and RET/PTC3 rearrangements; we amplified the specific exons containing the mutations by performing PCR and subsequent sequencing for the RAS gene family (N-RAS, H-RAS, K-RAS) and BRAF gene (using the Sanger technique). **Results** We observed 58 stage-1 tumors (including 9 papillary microcarcinomas), 4 stage-2, 28 stage-3 and 3 stage-4 (AJCC). Blood infiltration was seen in 16 samples and thyroid capsule infiltration in 31. BRAF-V600E mutation (exon 15) was present in 53 PTC and BRAF-mutation K601E in 2. In two PTC, we found an N-RAS mutation (exon 2), while H-RAS and K-RAS mutations and RET/PTC1, RET/PTC3 and PAX8/PPAR rearrangements were not found in any PTC. The mutations observed were correlated with staging; 70.6% of mutated samples were in stage 1, 75% in stage 2, 67.8% in stage 3 and 66.6% in stage 4. Blood infiltration was present in 17% of mutated cancers and 13% of wild-type (wt) cancers; this was not statistically significant (P = 0.6). Capsular infiltration was found in 35.7% of mutated PTC and in 31.4% of wt PTC. **Conclusions**. The small number of patients in this study proved to be a limitation; in our series, BRAF-V600E was the most frequently observed mutation, followed by a small percentage of BRAF-K601E and N-RAS. There were no differences in pathological presentation between PTC with mutations and those without. No association between BRAF/N-RAS mutations and blood infiltration emerged. The perspective that arises from this study is that the availability of more samples may enable a distribution pattern of genetic alterations to be discerned.

PP179 - 99MTC-METHOXY-ISOBUTYL-ISONITRILE (MIBI) SCINTIGRAPHY IS AN USEFUL AND COST-EFFECTIVE TOOL FOR ASSESSING THE RISK OF MALIGNANCY IN THYROID NODULES WITH INDETERMINATE FINE NEEDLE CYTOLOGY (FNAC).

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Background. Nodular thyroid disease is a common clinical problem. The diagnostic algorithm include laboratory test, thyroid ultrasound, thyroid scintigraphy and ultrasound-guided fine needle aspiration cytology (usFNAC), if the nodule is cold. Not rarely, the results of usFNAC are nondiagnostic (Thy 1) or unconclusive (Thy 3). This is a very important problem in the management of patients because the risk of under or over-treatment is high.The aim of our work was to verify if ^{99m}Tc-metossi-isobutil-isonitrile (^{99m}Tc-MIBI) scan can be employed in Thy1-Thy 3 patients how diagnostic test to differentiate benign from malignant thyroid nodules by qualitative and quantitative analysis. **Material and Methods.** This prospective study was conducted on 105 patients (F= 80, M= 25; mean age 47.91 ± 12.60 years) with cold thyroid nodules at ^{99m}Tc-pertechnetate scintigraphy, greater than 1.5 cm in diameter (mean size: 26.2 mm; range 15-45). The patients had underwent FNAC, with indeterminate results: Thy1, n= 5 and Thy3, n= 100. sestaMIBI scintigraphy was acquired 20 and 40 minutes after tracer administration (370 MBq) by static images of the thyroid. MIBI uptake in thyroid nodules was evaluated both qualitatively (compared with that in controlateral thyroid lobe) and quantitatively, by using region of interest that were created around nodule and outside the thyroid (background activity subtraction). All patients underwent total-thyroidectomy.**Results**. All the cold nodules were MIBI-positive, with different intensity of MIBI uptake at qualitative analysis: low (n=35 patients), moderate (n=46 patients) and high (n=24 patients). By quantitative analysis, the patients were arbitrarily subdivided in three groups: A (n=29) with a wash-out index (woi) ≥ -40%; Group B, (n=41): woi between -20 and -40%; Group C (n=35): woi ≤ -20%. We assumed that a woi ≤ -20% was suspicious for malignancy, while a woi ≥ -40% was predictive of a benign lesion. Compared to hystopathology, all patients of the group A were negative for thyroid cancer [sensitivity and negative predictive value: 100%]. In Group B were included all except seven patients affected by benign adenomas (sensitivity: 85.4%). Finally, 28 out of 35 patients of the Group C had a papillary thyroid carcinoma [specificity and positive predictive value: 80%]. All false positive patients were affected by adenoma with oxyphil cell.**Conclusions**. We suggest the use of MIBI-scan (by using quantitative analysis) in the work-up of cold nodule with indeterminate cytology to better stratify the risk patients' to have a malignant lesion, so reducing the number of patients refereed to surgery.

PP180 - THYROID AND GONADAL DYSFUNCTION IN PATIENTS WITH METASTATIC CARCINOMA TREATED WITH TYROSINE KINASE INHIBITORS. RESULTS FROM A PROSPECTIVE STUDY

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Background: Tyrosine kinase inhibitors (TKIs) may induce thyroid dysfunction, while endocrine dysfunctions have not been systematically studied.

Objective: Prospective evaluation of thyroid and gonadal function. 25 patients (23 men and 2 post-menopausal women [median age 57.7, range 51-77]) with metastatic carcinoma (24 renal cell and 1 GIST) with comparable tumor staging, normal thyroid function and no evidence of thyroid autoimmunity, were studied before and at monthly intervals after beginning TKI: Pazopanib (Votrient®) in 1, Sunitinib (Sutent®) in 24 withdrawan in three patients for progression of disease and after replaced with Axitinib (Inlyta®). Sunitinib was given at a daily oral dose of 50 mg 4 weeks on, 2 weeks off, Pazopanib daily oral dose of 800 mg and Axitinib daily oral dose of 5 mg. In all cases TSH, FT3, FT4, thyroid antibodies (TgAb and TPOAb), morphological evaluation with color doppler ultrasound, LH, FSH, Prolactin (PRL) were measured up to 12 months, associated to total testosterone (TT) in male patients.

Results. During 3-6 months of treatment 15 (60%) patients developed primary hypothyroidism (TSH increased from 1.31±0.61 to 14.35±24.72 mUI/L, [normal range 0.4-4 mUI/L]). This was associated to a decrease of thyroid volume (13.69±5.04 ml to 5.95±2.8 ml [p<0.001]) in 22 (88%) patients and to *de novo* appearance of TPOAb (84-3000 IU/ml) in 6 hypothyroid patients, who had the highest TSH values (25-114 mUI/ml). Thirteen (52%) men developed TT <3 ng/ml, (from 3.985±1.690 ng/ml to 2.472±1.149 ng/mL, [p<0.001]), while LH decreased (from 4.259±2.658 mUI/ml to 1.894±1.016 mUI/mL [p<0.001] and FSH from 6.705±4.228 to 3.049±2.604mUI/ml [p<0.001]). Finally, high serum PRL (35-68 ng/dl [normal range <20 ng/dl]) were observed in the 6 patients with severe hypothyroidism and positive TPOAb.

Conclusions. TKI interfere with the endocrine system at different levels: primary hypothyroidism (with possible involvement of autoimmune mechanisms) and central hypogonadism. Further longitudinal studies involving other endocrine axes are needed to precisely characterize TKI as endocrine disruptors.

PP181 - TSH-RECEPTOR AUTOANTIBODIES IN PATIENTS WITH CHRONIC THYROIDITIS AND HYPOTHYROIDISM: PREVALENCE, SPECIFICITIES AND CLINICAL IMPLICATIONS

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Introduction: The prevalence of TSH-R autoantibodies (TRAb) and of TSH-R-blocking autoantibodies (TBAb) in Chronic Thyroiditis (CT) were 0-48% and 0-47% respectively. These data could be due to different methods employed and different geo-demographical provenance, number and criteria of the patients recruited.

Patients: We recruited 245 consecutive patients, aged 12-89 years, F/M=3.7:1, with CT and TSH≥7mU/L, attending the Endocrine Unit in Padova. We evaluated prevalence and titres of TRAb using the Fast ELISA (kit from RSR Ltd) and correlated them with gender, age, thyroid dimensions, TSH levels, autoimmune diseases, TgAb and TPOAb. In 14 TRAb+ patients TBAb or TSAb activity was tested using a bioassay (based on TSHR transfected CHO cells and measurement of cAMP).

Results: In the overall CT group (n=245) TRAb prevalence was 30% (26.9% in females, 42% in males, $p<0.05$), 36.5% had medium/high titres. The patients were subdivided into three groups on the basis of levels of TSH: 7-9.9mU/L, 10-19.9mU/L and ≥20mU/L; TRAb were present in 27%, 28%, 32.8%, respectively and mean titres were 3.1, 4.2 and 20.5IU/L respectively. There was a significant difference ($p<0.05$) between titers of TRAb in patients with TSH ≥20mU/L and those with lower TSH levels.TRAb were present in 29% of TPOAb and/or TgAb+ patients and in 40% of TPOAb and TgAb-negative patients. In patients with reduced thyroid dimensions, TRAb prevalence was 33% and in those with normal or increased thyroid it was 28%; with mean titres 33.7 and 5.2IU/L respectively ($p<0.05$). TRAb were present in 27.6% of females ≤45 years old and in 26% of those >45 years old, with mean titres 12.9 and 12.4IU/L. TRAb were present in 44% of males ≤45 years old and in 40% of those >45 years old, with mean titres 12.8 and 11.7IU/L respectively. Frequency of TRAb+ was similar in patients with or without co-morbidities. Out of 14 TRAb+ patients with hypothyroidism, 93% were TBAb+ while 28.5% were also TSAb+.

Conclusions: TRAb are present in a third of patients with CT and hypothyroidism. Titres of TRAb are correlated to TSH levels and to reduced thyroid dimensions. In TRAb positive patients with hypothyroidism a mixture of TBAb and TSAb can be detected at the same time. We suggest to investigate the presence of TRAb in all patients with CT and hypothyroidism but especially in females of childbearing age, because they may be a marker of risk for neonatal transient hypothyroidism.

PP182 - THYROID DYSFUNCTION AND ATRIAL FIBRILLATION IN TWO GROUP OF PATIENTS RECEIVING OR NOT AMIODARONE THERAPY

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The association between heart diseases and thyroid function abnormalities is well documented. This item is often observed in an hospital setting dedicated to cardiovascular diseases. The aim of the present study was to evaluate the frequency of thyroid function abnormalities in a population of out-patients affected with atrial fibrillation, referred to the Endocrine Unit of IRCCS Policlinico San Donato, a third level hospital centre for cardiovascular diseases. We identified 144 patients with AF (69F / 75M, age: 71 ± 11 years) out of 960 out-patients (462M/498 F; aged 61 ± 19 years, mean and SE). Most of AF patients (78%) were treated with amiodarone for at least three months, while one fifth of AF patients (20%) received any dose of amiodarone. AF patients never treated with amiodarone showed the following thyroid diseases: toxic goiter (n=21, 64%), euthyroid goiter (n=8; 24%) and hypothyroidism (n=3). In AF patients treated with amiodarone hypothyroidism and hyperthyroidism occurred with the same frequency (49.5% and 50.5%, respectively). These data highlighted that: 1) AF patients frequently experienced thyroid function abnormalities and therefore serum TSH determination and clinical thyroid gland evaluation are advisable in AF patients; 2) amiodarone, even short-term, treatment is the most frequent cause of thyroid dysfunction in AF patients; 3) in the present series amiodarone-induced hypothyroidism and hyperthyroidism similarly occurred; 4) toxic goiter is the main cause of thyroid dysfunction in amiodarone-free AF patients.

PP183 - LONG TERM OUTCOME OF GRAVES' ORBITOPATHY FOLLOWING HIGH DOSE INTRAVENOUS GLUCOCORTICOIDS AND ORBITAL RADIOTHERAPY

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Introduction: Intravenous (iv) glucocorticoids (GC) (ivGC) and orbital radiotherapy (ORT) is a well established treatment for active Graves' orbitopathy (GO), with favorable outcomes in up to 80% of patients. However, little is known on the factors that may affect GO outcome in the long term, an issue that we investigated here.

Patients and methods: We studied retrospectively 96 untreated patients with GO, identified out of 787 consecutive patients who came to our GO Clinic for a follow-up visit between September 2010 and June 2013. After the first observation, patients were treated with ivGC and ORT and were then re-examined after a median period of 55.5 months. The primary end-point was the possible relation between GO outcome and the following individual variables: age; gender, smoking habits, thyroid volume, thyroid treatment, TRAb at first and last observation, individual GO features at first observation (exophthalmometry, eyelid aperture, CAS, visual acuity, diplopia) and time elapsed between first and last observation. The secondary end-point was to investigate the relation between the variables, if any, affecting the outcome of GO, and the individual GO features.

Results: Exophthalmometry, eyelid aperture, CAS, diplopia and visual acuity (the latter only in patients with an initial reduction) improved significantly after treatment. Overall, 67.7% of patients had improved and were considered as responders, whereas the remaining (29.1% stable and 4.5% worsened) were considered as non-responders. Age, smoking, thyroid volume, thyroid treatment, serum anti-TSH receptor autoantibodies and individual GO features at first observation did not affect the outcome of GO, which, in contrast, was affected by gender and by the time elapsed between first and last observation. Thus, the prevalence of responders was higher in females (76.4% vs 48% in males, P=0.02) and the time elapsed between first and last observation was greater in responders (58 mo. vs 39 mo. in non-responders, P=0.02). Whereas the prevalence of responders and non-responders was similar up to 36 months, there was an increase in responders beginning between 37 and 48 months and reaching a peak of ~80% between 61 and 72 months, to plateau thereafter.

Conclusions: Given the limitations of retrospective investigations, our study confirms that the combination of GC and ORT is effective in GO and shows that females have greater chances to respond to treatment. The notorious tendency of GO to improve spontaneously with time most likely contributes the long term outcome of the eye syndrome.

PP184 - THYROID DISEASES AND DISABILITY IN PATIENTS WITH MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is an autoimmune demyelinating disorder that often causes neurological disability in adulthood. Relapsing/remitting MS is the most frequent clinical form and shows a variable progression of clinical symptoms. Frequently, MS occurs in association with autoimmune thyroiditis (AIT): in our series of patients with AIT, MS is ranked fourth among the nonendocrine autoimmune diseases associated with AIT. Thyroid hormones play a key role in developmental myelination and their appropriate level seems to be critical also for remyelination process in MS. The aim of our study was to ascertain whether autoimmune and/or functional thyroid disorders may affect the progression of disability in patients with MS. A total of 40 patients were enrolled: 23 patients (20F/3M) with MS and a concomitant thyroid disorder [11 patients with AIT and 12 patients with non autoimmune thyroid disorder (NAITD)] were the study group. Seventeen patients (15F/2M) with isolated MS, matched for age, gender, disease duration, were the control group. All patients were in treatment with interferon beta and all hypothyroid patients were properly treated with thyroxine. Disability progression was analyzed by the score EDSS (Expanded Disability Status Scale) on a 1 to 10 scale. No evidence of progression of disability was observed in patients with AIT (EDSS = 1.0 at diagnosis and after 7 years) and in patients with NAITD (EDSS = 1.75 at diagnosis and after 9 years as well as in patients with isolated MS (EDSS= 1.5 at diagnosis and after 8 years). When the 23 study patients were subdivided on the basis of thyroid function, 9 patients were euthyroid (median TSH= 1.23 mU/l) and 14 were hypothyroid (median TSH= 5.02 mU/l). While in 8 out of 9 euthyroid patients a substantial stability of EDSS was observed (EDSS= 2.0 at diagnosis; EDSS= 1.0 after 9 years; p=ns), 9 out of 14 (64%) hypothyroid patients showed a significant disability progression (EDSS = 1.0 at diagnosis; EDSS= 2,5 after 8 years; p=0.0211). Cross-evaluation of EDSS in these 14 hypothyroid patients (5/8 with AIT vs 4/6 with NAIT) revealed that autoimmune nature of thyroid disease did not represent a bias (p=ns). These findings suggest that the presence of the AIT does not affect the disability of MS patients, when in treatment with interferon beta. On the contrary, the exposure to hypothyroidism was associated with a worse clinical outcome of MS, independent from levothyroxine treatment.

PP185 - THE THYROID CONCENTRATES SOME HEAVY METALS AND TRACE ELEMENTS MORE THAN ADIPOSE AND MUSCLE TISSUES

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Background: The residence in the Mt. Etna volcanic-area increases the risk of thyroid cancer (TC). The cause and mechanism of this association are unknown but preliminary data indicate that people living in volcanic areas can be contaminated with heavy metals and trace elements of volcanic origin. The ability of the thyroid to concentrate these compounds is unknown.

Aim: To measure intrathyroid concentration of heavy metals in comparison to muscle and adipose tissues.

Methods: Metal and trace element concentrations were measured by a quadrupole system (DRC-ICP-MS) in tissue specimens collected at thyroid surgery.

Results: Geometric means of As, Br, Cd, Hg, Mn and Se concentrations per gram of tissue were significantly higher in the thyroid in respect to the muscle and adipose tissues, while Zn and Cu were at greater concentration in the muscle. No element was found at highest concentration in adipose tissue. The concentrations of Ag, B, Co, Cr, Mo, Pb, Pd, Sn, Sr, V, W and U were not significantly different in the three tissues examined.

Conclusions: The ability of the thyroid to uptake and concentrate a variety of chemicals more than adipose and muscle tissues requires further studies to better understand the role and the mechanism of these chemicals in thyroid biology and carcinogenesis.

PP186 - TIM16 INHIBITION DECREASES CALCITONIN SECRETION AND ENHANCES SENSITIVITY TO PACLITAXEL IN A HUMAN MEDULLARY THYROID CARCINOMA CELL LINE.

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TIM 16 is a protein of the translocase complex TIM 23 situated in the mitochondrial inner membrane, encoded by Magmas, a gene overexpressed in several tumors. Magmas silencing have been associated with a greater sensitivity to apoptotic stimuli. We have recently demonstrated that in a human medullary thyroid carcinoma cell line (TT) compound 5, a TIM 16 inhibitor, was not cytotoxic but enhanced the proapoptotic effects of staurosporine.

The aim of our study is to verify if compound 5 may influence the cytotoxic effects of paclitaxel and modulate calcitonin secretion.

To evaluate cell viability we performed ATPlite assay, while Caspase 3/7 assay was used to determine apoptotic activation. ELISA test was used for calcitonin detection in cell culture medium.

Our data show that paclitaxel 10 nM was able to reduce cell viability by 40%, while compound 5 alone had no effects; on the contrary the latter was able to significantly increase the effects of paclitaxel by ~14%. In addition Paclitaxel increased caspase 3/7 activity by 130%, which was further enhanced by compound 5 (+ 130%). In addition, compound 5 was able to reduce basal and pentagastrin induced calcitonin secretion.

In summary Compound 5 could represent a tool to increase the effects of chemotherapeutic agents and to control hypercalcitoninemia in medullary thyroid carcinoma.

PP187 - MITOTANE TREATMENT IN PATIENTS WITH ADRENOCORTICAL CANCER CAUSES CENTRAL HYPOTHYROIDISM

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Introduction. Mitotane, an adrenolytic drug used to treat adrenal cortical cancer (ACC), can affect thyroid function with a reduction of FT4 levels, normal FT3 and TSH. In a murine pituitary cell line, mitotane has been shown to inhibit the secretory capacity of thyrotropic cells. Patients and Methods. In five female AAC patients (age 50.0±13.5 yrs; m±SD) treated with mitotane (dosage 1.9±0.8 g/day) we analyzed the pattern of TSH and thyroid function index (FT4, FT3 and FT3/FT4 ratio) and evaluated the in vivo secretory activity of the thyrotropic cells using the standard thyrotropin-releasing hormone (TRH) test (200 µg). Results. Basal TSH (1.63±0.42 µU/L) was in the normal range and scattered around our median reference value, FT3 levels were within the reference range (3.84±0.44 pmol/L) but below the median reference value while FT4 values were under the reference range (8.55±0.87 pmol/L) in all patients. The peak of TSH was reached at 30' after TRH administration (5.7±0.7 µU/L). The absolute TSH response (ΔTSH: peak TSH minus basal TSH) was 4.1±0.8 µU/L (range 3.5-5.3). Relative TSH response (fold TSH: peak/basal TSH) was 3.7±1.0 µU/L (range 2.6-4.7). PRL secretion was normal (fold PRL peak/basal was higher than 2.5 times). Conclusion. The result of our study represents the first demonstration in vivo that patients with AAC treated with mitotane have a condition of secondary hypothyroidism, confirming in vitro data and previous clinical observations. Furthermore, the elevated FT3/FT4 ratio of these subjects, indicator of the desiodase activity, reflects an enhanced T4 to T3 conversion rate, a compensatory mechanism characteristic of thyroid function changes observed in hypothyroid conditions. This finding may have a therapeutic implication for treatment with thyroid hormones as suggested from current guidelines for this specific condition.

PP188 - ASSOCIATION OF AUTOIMMUNE THYROID DISEASE, CRONIC ATROPHIC GASTRITIS AND GASTRIC CARCINOID

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Introduction: Autoimmune polyendocrine syndromes (APS) type III are characterized by the association of autoimmune thyroid disease (ATD) with other diseases such as diabetes, alopecia, pernicious anemia, vitiligo and cronic atrophic gastritis type A (CAGtypeA). Genetic haplotypes HLA-B8 and HLA-DR3 are frequently observed in patients affected by CAGtypeA. A strong association between ATD and CAGtypeA has been demostrated: APCA (Anti Parietal Gastric Cells Antibodies) are present in 22% of Graves Disease (GD) and 32-40% of Hashimoto's thyroiditis (HT), while CAGtypeA is present in 18% of GD and 30% of HT. Morover 10% of patients affected by CAGtypeA have a predisposition to develop gastric carcinoid tumors type I (GCT1) and adenocarcinoma as a result of chronic hypergastrinemia caused by achlorhydria and subsequent ELC cells neoplastic transformation. **Aims:** Evaluate, in a consecutive series of patients followed for ATD in our outpatients clinic, the incidence of CAGtypeA. **Patiens and Methods:** From 2004 to 2014, 242 patients (207 F, 35 M, mean age 41.3, range 12-78) with ATD underwent a screening performing APCA, Vit. B12, ferritin, iron, and hemoglobin and red cells count measurements with subsequent gastroscopy in case of APCA positivity. **Results:** we found 57/242 (23.5%) APCA + (52 F and 5 M, F:M = 10.4:1.0, mean age 47.6, range 24-67 yrs). Of these patients 31/57 (54.3%), 30 F and 1 M, were affected by GD; 4/57 (7%) 3 F and 1 M by ATD and thyroid cancer; 22/57 (38.5%) 19 F and 3 M by HT; 10/57 (17.5%) patients have anemia, 14/57 (24.5%) vitamin B12 deficiency, 9/57 (15.7%) iron deficiency. In 2/57 patients (3.5%, all females, age of 41 and 67) a GCT1 was found. **Conclusions:** Our data confirm the high association rate of CAGtypeA in ATD which frequently are not isolated disease but configure the picture of APS type III and need to be followed accordingly. A early diagnosis may avoid signs and symptoms of gastritis with anemia, reduction on L-thyroxine absorption and late diagnosis of GTC1.

PP189 - RAS MUTATIONS IN BENIGN THYROID NODULES PREDICT A FASTER NODULE ENLARGEMENT

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Context: The follow-up of small benign thyroid nodules includes repeated clinical and ultrasound evaluations until their enlargement requires surgical or alternative treatments. To date, we lack specific growth parameters able to predict the size changes of a benign nodule in a given patient. Activating mutations of the *RAS* proto-oncogene activate a signal pathway that promotes cell proliferation. *RAS* mutations have been described in thyroid nodules, including adenomas and hyperplastic benign nodules.

Objective: The aim of this study was to establish whether the volume changes of benign nodules can be predicted by the presence of *RAS* mutation.

Patients and methods: The genomic DNA obtained by fine-needle aspiration of 78 thyroid aspirates with benign cytology, were analyzed by pyrosequencing for the presence of $NRAS^{61}$and $KRAS^{13}$mutations.Ultrasonographic features were obtained.The volume of nodules at baseline and their changes after a mean follow-up of 25 months were evaluated according to the presence of *RAS* mutation.

Results: A *RAS* mutation was found in 24 thyroid aspirates (30.8%, 8 $NRAS^{61}$ and 16 $KRAS^{13}$).*RAS* mutation was not associated with ultrasonographic features, while it was significantly associated with a larger size at baseline (p = 0.017). After a 25 months mean follow-up, *RAS* mutation positive nodules displayed a faster growth (*RAS* mutation positive *vs.* negative % annual growth 27.6% +/- 32.2% *vs.* 1.0% +/- 17.0%, p <0.001).

Conclusions: Benign thyroid nodules bearing *RAS* mutation grow more rapidly than those with wild type *RAS*.Searching for *RAS* mutation in thyroid nodules with a benign cytology might be useful to the clinician in choosing a more appropriate and timely surgical management.

PP190 - SEVERAL CANCERS ARE INCREASED IN THE MT. ETNA VOLCANIC AREA IN SICILY

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Background and Aim. We previously reported a marked increase of thyroid cancer incidence in the volcanic area of Mt. Etna (Catania province, Sicily). We now investigated whether the risk of malignancy also for other tumors is increased in this area in respect to adjacent non volcanic areas where genetic background, lifestyle and access to healthcare are similar.

Methods. Five Sicilian cancer registries covering 82% of the population were analyzed for the incidence of all cancers combined and of 34 site-specific cancers in the volcanic vs. non-volcanic areas (at least 3 years in the 2002-2007 period). The crude incidence rate ratio (IRR) between the studied areas was calculated and significance evaluated on the basis of Poisson distribution adjusted by multiple not independent control test (q-value). One-sided statistical test was carried out in order to detect the excesses of cancer incidence in the volcanic area.

Results. Out of 32,980 incident cancer cases in females and 39,217 in males, overall, cancer incidence rate was increased by 6.1% in women ($q<0.001$) and 2.2% in man (n.s.) in the volcanic area. This difference was due to the significant increase of several site-specific cancers: in both genders thyroid cancer (IRR : F = 1.68, M = 1.40) and lymphatic leukemia (IRR : F = 1.48, M = 1.39); in women also Hodgkin lymphomas, stomach and breast cancers and in man prostate cancer. The increased incidence of additional cancers did not reach significance because of limited sample size. Some other cancers (liver, cervix uteri) had lower rate than in the control area.

Conclusions. The incidence of several cancers is significantly increased in residents of the volcanic area of Sicily. These data confirm that the volcanic environment can be a risk factor for some but not all cancers. No cause-effect mechanism is known. Further studies are warranted for identifying possible carcinogens and mechanisms in different tissues.

PP191 - THE CYTOLOGICAL SUB-CLASSIFICATION OF THYROID FOLLICULAR LESIONS (TIR 3) INTO TWO CATEGORIES (TIR 3A OR TIR 3B) IMPROVES DIAGNOSTIC ACCURACY.

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INTRODUCTION

We retrospectively reviewed the cytological slides of 353 patients having a FNA diagnosis of follicular lesion (TIR 3) that undergone surgery whit histopathological diagnosis.
We sub-classified these specimens as TIR 3A or TIR 3B, on the basis of cytological characteristics. Moreover, we evaluated the presence or absence of a number of cytological features to define their role in predicting the risk of malignancy.

MATERIAL AND METHODS

The cytological criteria used were as follows:

TIR 3A= follicular cells arranged in some clusters, monolayered sheets and many follicles with regular nuclei (without overlapping), scanty colloid.

TIR 3B= prevalence of follicles with big nuclei, sometimes nucleoli, often nuclear overlapping and/or irregular chromatin, some trabeculae and rarely micro-pseudoinclusions; colloid absent.

The multivariate logistic regression model was applied to assess the correlation between these morphological elements and the risk of malignancy.

RESULTS

On the basis of these cytological features, 131/353 (38%) patients were classified as TIR 3A, while 222/353 (62%) as TIR 3B. At histological examination, 299/353 (85%) nodules were benign lesions and 54/353 (15%) were carcinomas. The rate of malignancy was 9.2% (12/131 cases) in the TIR 3A group and two fold higher, 18.9% (42/222) in the TIR 3B group (p= 0.028).
The presence of trabeculae and/or micro-pseudoinclusions, significantly increased the risk of malignancy in nodules classified TIR 3B. Instead, the cytological characteristics of group TIR 3A were correlated with a low risk of malignancy.

CONCLUSIONS

In conclusion, the sub-categorization of TIR 3 follicular lesions into TIR 3A and TIR 3B

confirms that the two groups have a different risk of malignancy and is therefore useful in selecting patients to refer to surgery.

PP192 - IN PAPILLARY THYROID CANCER TERT PROMOTER MUTATIONS HAVE A WORST IMPACT ON OUTCOME THAN BRAF MUTATIONS

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TERT promoter mutations (chr5:1,295,228C>T e chr5:1,295,250C>T) were recently described in thyroid tumors, with a prevalence ranging 8-25% in papillary thyroid cancer (PTC). We and others reported that these mutations strongly associate with a poor outcome in differentiated thyroid cancers. Aim of the present study was to further investigate the prognostic role of both *TERT* promoter ($TERT^{MUT}$) and $BRAF^{V600E}$ mutations in a larger series of 216 PTCS with a long follow-up (median: 74 months). We also evaluated the possible additive effect on the outcome of the coexistence of the two genetic alterations. Genetic data were obtained by direct sequencing and were correlated with full clinical data. The prevalence of $TERT^{MUT}$ and of $BRAF^{V600E}$ was 12% and 35%, respectively. Ten cases (5%) harbored both $TERT^{MUT}$ and $BRAF^{V600E}$. Cases with a $TERT^{MUT}$ alone, but not those with $BRAF^{V600E}$ alone, were significantly associated with older age at diagnosis and poorer outcome (p=0.04 for both). No differences in the outcome were noted between cases with the $TERT^{MUT}$ alone or with the coexistence of $TERT^{MUT}$ and $BRAF^{V600E}$.

In conclusion, *TERT* mutations were found to have a 12% prevalence in PTCs and were confirmed to be a major indicator of poor prognosis. On the other hand, $BRAF^{V600E}$ was not associated with the outcome, consistent with data previously obtained in our series. Moreover, the outcome was not different among tumors with isolated $TERT^{MUT}$ and those with coexistent mutations ($TERT^{MUT}/BRAF^{V600E}$), indicating that $BRAF^{V600E}$ does not confer an additional effect in the disease persistence.

PP193 - THE CONTEMPORARY INGESTION OF METHIMAZOLE AND ACETOMINOPHEN CAUSED DRUGS INDUCED LIVER DISEASE (DILI) IN A FEMALE PATIENT WITH GRAVES' DISEASE AND GRAVES' OPHTHALMOPATHY

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Background: Graves' Disease (GD) is the most frequent cause of hyperthyroidism in iodine sufficient areas and antithyroid drugs are the first line treatment in the majority of these patients (1). Anti-thyroid drugs may cause severe adverse effects including liver failure, rare but potentially lethal. Here we present the case of a patient who suffers from Graves' disease (GD). This patient, after a course of two months of methimazole (MMI) treatment, developed severe hepatotoxicity while taking a very low MMI dose together with a large acetaminophen dose.

Patient: A 61-year-old woman was referred to our thyroid clinic because of hyperthyroidism due to GD complicated by moderate to severe Graves' ophthalmopathy (GO). She had been treated with MMI starting with 20 mg reduced by 50% every 20 days. At present the dose was 5 mg/d since 10 days and she was clinically asymtomatic for hyperthyroidism. Intravenous metilprednisolone pulse therapy (IVMP) was planned for GO. Liver enzymes, controlled before IVMP, indicated a significant increase of both ALT and AST values. Alkaline phosphatases, total and direct bilirubin were in the normal range. Five days earlier the patient had started acetaminophen 4 g/d because of severe back pain. MMI and acetaminophen were discontinued, IVMP therapy was postponed and hepatic function was monitored. Two weeks later liver enzymes returned to normal values but signs of recurrent hyperthyroidism were present. The patient started again MMI at 5 mg/d but just 4 days later the patient was symptomatic with nausea and cretaceous faeces. Laboratory tests indicated a sharp increase of ALT, AST and also GGT, total and direct bilirubin had increased. MMI was discontinued and thyroid surgery was suggested in order to resolve hyperthyroidism. Two weeks later the patient was clinically and biochemically euthyroid and liver enzymes and bilirubin were all within normal range. IVMP protocol for GO was resumed.

Conclusions: in susceptible patients, acetominophen treatment in addition to low dose of MMI may rapidly deteriorate liver function, probably because of an additive toxic mechanism. In these patients resuming MMI, even at low dose, may precipitate recurrence of liver damage.

PP194 - TYPE 2 DIABETIC PATIENTS WITH GRAVES' DISEASE HAVE MORE FREQUENT AND SEVERE GRAVES' ORBITOPATHY

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Background and aims: Due to the worldwide increasing prevalence of diabetes (DM), patients with both diabetes and Graves' disease (GD) have become more frequent. Sporadic reports indicate that Graves' orbitopathy (GO), a GD complication that affects orbital soft tissues, can be severe in DM patients. The relationship between these diseases is not well understood.

This study aims at evaluating the association of GD and GO with autoimmune and non-autoimmune diabetes (DM) and to assess diabetic features that influence GD and GO prevalence and severity.

Methods and Results: This retrospective study evaluated GD, GO and DM association in 1,211 consecutive GD patients (447 with GO and 77 with DM). A case-control study was carried out to evaluate DM relationship with GO severity by comparing at 1:2 ratio patients with GO with or without DM. A strong association was found between GD and T1DM (p=0.01) but not T2DM. Instead, the presence of GO was strongly associated with T2DM (p=0.01). Moreover, GO was more frequently severe in GD patients with T2DM (11/30 or 36.6%) than in those without T2DM (1/60 or 1.7%, p=0.05). T2DM was the strongest risk factor for severe GO (OR=34.1 vs. 4.3 p<0.049 in cigarette smokers). DM duration, obesity and vascular complications, but not metabolic control were significant determinants of GO severity.

Conclusions*:* GD is associated with T1DM but not with T2DM, probably because of the common autoimmune background. GO, in contrast, is more frequent and severe in T2DM, significantly associated with obesity, diabetes duration and diabetic vasculopathy but not metabolic control

PP195 - LONGITUDINAL STUDY OF THYROID FUNCTION IN CHILDREN WITH CH AND THYROID IN SITU

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With the improvement of newborn screening programs a progressive reduction of TSH cut-off has increased incidence of congenital hypothyroidism expecially with thyroid gland in situ. If, at recall examination, diagnosis of congenital hypothyroidism (high TSH value and low FT4) is confirmed, L-T4 therapy must be started as soon as possible. In CH children with a thyroid gland in situ, to evaluate a permanent or transient hypothyroidism, L-T4 therapy should be discontinued for 3-4 weeks after 3 years of age. When FT4 serum value is low and TSH value is elevated permanent hypothyroidism is confirmed and L-T4 therapy is reintroduced. Thyroid hormone and TSH values in the normal range identify a condition of transient congenital hypothyrodism (TCH). We have carried out a prospective longitudinal study of thyroid function in the infancy and childhood in children with congenital hypothyroidism and thyroid in situ at diagnosis (elevated TSH and low FT4 at recall) to evaluate the natural evolution of this condition. In the period 1991-2008 we recalled 699/355581 screened newborns. TSH cut-off was 20 mU/L before 2002 and 10 mU/L from 2002 to 2008. 241/699 CH were identified; 96/241 (39.8%) presented thyroid gland in situ at ultrasound examination. At re-evaluation, at 3 years of life, thyroid function testing and ultrasound were performed: 20/96 permanent CH was confermed (mean TSH value 115.8±105.8 mU/L and mean FT4 value 0.6±0.3 ng/dl) and 76/96 presented transient hypothyroidism. Genetic analysis (DUOX2) was done in 24/76 TCH. At 3 yrs of age serum FT4 values were normal in all 76 children evaluated (m 1.2±0.2 ng/dl, range 0.9-1.9). 43/76 (56.6%) showed a TSH serum value in the normal range (m 2.6±0.9 mU/L, range 1.1-3.9) and the other 33/76 (43.4%) presented TSH slightly elevated (m 5.3±1.1 mU/L, range 4.1-8.6). At 5-7 yrs of life the thyroid function was revaluated in 65/76 children: 46/65 (70.8%) showed normal thyroid function while 19/65 (29.2%) had a mild but persistent hyperthyrotropinemia. DUOX2 mutations were found in 5/24 children, all of them with normal thyroid function at 3 and/or 5-7 yrs of life.

In our population about 80% of patients with CH and thyroid in situ, diagnosed at birth, had TCH. In any case congenital hypothyroidism, althought transient, must always be treated at birth because low FT4 represent a risk factor for psycho-intellectual development. A third of TCH patients showed mild persistent hyperthyreotropinemia and none returned to overt hypothyroidism during follow-up in childhood. TSH values during early childhood, but not at neonatal diagnosis, are good predictors of thyroid function later.

PP196 - LONGITUDINAL STUDY OF THYROID FUNCTION UP TO PUBERTY IN CHILDREN "FALSE POSITIVE" AT SCREENING FOR CONGENITAL HYPOTHYROIDISM

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Screening programs have helped to identify a wide spectrum of neonatal thyroid disfunctions. A part of newborns positive at neonatal screening for congenital hypothyroidism (CH) have normal or nearly normal TSH and normal FT4 at recall examination ("false positive" or children with mild short-lasting hyperthyrotropinemia). Long-term outcome of thyroid function was already carried out in our previously longitudinal study in a cohort of 56 "false positive" children from neonatal screening up to early and advanced childhood (JCEM 2002, 2008). At 2-3 years 28/56 children (50%) presented a slightly elevated serum TSH (m 6.0±1.7 mU/L; range 4.0-10.1). At 5 years TSH serum values, evaluated in 44/56 subjects, were within the normal range in all children with normal TSH in early childhood and also in 9/28 children who had an increased TSH at 2-3 years. At 8 years 14/44 children (31.8%) still showed a slightly persistent hyperthyrotropinemia and minor morphological and/or genetic alterations. Thyroid function and morphology were now evaluated in 31/56 subjects up to puberty (Tanner stage P 3-4, age 12.5±1.4 yrs, range 9.6-15.9): 11/31 with persistent hyperthyrotropinemia and 20/31 with normal TSH values during follow-up. Hyperthyrotropinemia persisted in 7/11 children (m 6.2±2.6 mU/L, range 4.3-11.3), while 4/11 patients normalized serum TSH values in puberty (m 2.2±0.7 mU/L, range 1.2-2.9). All 20 children with previously normal thyroid function continued to show normal serum TSH values in puberty too (m 2.5±1.0 mU/L, range 1.1-3.9). Serum FT4 (m 1.0±0.2 ng/dl, range 0.8-1.6) and FT3 values (m 3.7±0.6 pg/ml range 2.5-4.5) were confirmed within the normal range in all 31 subjects. Therefore, at an average of 12.5 years, serum TSH values were normal in 24/31 (m 2.4±0.9 mU/L; range 1.1-3.8) and slightly elevated in 7/31 (m 6.2±2.6 mU/L, range 4.3-11.3). The present longitudinal study in children "false positive" at CH screening up to puberal age provides some information:

- the prevalence of subclinical hypothyroidism decrease with increasing age, but yet 23% had a persistent mild hyperthyrotropinemia with an high frequency of thyroid morphology alterations.

- all subjects with normal TSH values in early childhood or normalized in late childhood continued to present normal thyroid function in puberty and none with previously hyperthyrotropinemia evolved in overt hypothyroidism.

- in this population, therefore, puberty doesn't seem to represent a period of higher risk for thyroid disfunction's worsering or recurrence.

PP197 - EVALUATION OF CLINICAL AND HISTOLOGICAL CHARACTERISTICS OF DIFFERENTIATED THYROID MICROCARCINOMAS WITH AND WITHOUT INFILTRATION BEYOND THE THYROID CAPSULE

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INTRODUCTION AND AIM. Differentiated thyroid microcarcinoma (DTMC) is generally characterized by a good prognosis. However, in some cases metastases occur. Infiltration beyond the thyroid capsule characterizes a higher TNM stage with a higher risk of recurrence. Little is known on the differences between DTMCs which infiltrate tissues beyond the thyroid capsule (IDTMC) and those which do not (non-IDTMC). In this study we compared clinical and pathologic characteristics between these two groups of DTMCs. MATERIALS AND METHODS. We retrospectively evaluated cases from 261 patients (57 M [22%], 204 F [78%]) affected by differentiated thyroid carcinoma who were referred to our endocrine unit from January 1, 1998 through December 31, 2014. History was recorded including the causes of the first referral to a clinical visit which then led to the cancer diagnosis. A total of 115 (45%) patients were affected by DTMC, referred to as ≤1 cm carcinoma. Cases were divided according to their feature to infiltrate or not the tissues beyond the thyroid capsule. Clinical, histo-morphologic, and prognostic parameters were compared between the two groups. Events were considered as persistent non-suppressed thyroglobulin, lymphnode or distant metastases, death. RESULTS. Among microcarcinomas, 25 (22%) IDTMC and 88 (76%) non-IDTMC were found. Data were missing in 2 cases. The maximum diameter was significantly higher in IDTMCs (8.28±1.95 mm[M±SD]) than in non-IDTMCs (6.00±3.06 mm) (t-test $p<0.0001$). The area under the ROC curve was 0.719; CI: 0.613-0.824; $p=0.001$; a diameter cut-off of 6.5 mm was identified to discriminate IDTMC from non-IDTMC with a sensitivity of 80% and a specificity of 50%. Multifocality ($p<0.05$), angioinvasion ($p<0.0001$), papillary histotype ($p<0.02$), hypothyroidism ($p<0.04$), lymphnode metastases at diagnosis ($p<0.0001$), TNM stage III and IV ($p<0.0001$), and a history of radioactive remnant ablation ($p<0.0001$) were more frequently observed in IDTMCs than in non-IDTMCs. There were no differences between the two groups in age, sex, comorbidity, family history for thyroid cancer, histological findings of thyroiditis, events, and causes of first referral of these patients to our endocrine unit (ie.: i, clinical examination; ii, incidental finding during power Doppler examination of carotid vessels; iii, incidental finding at the thyroid ultrasound (US) arbitrarily performed during US study of other districts; iv, incidental finding following thyroid US because of underlying thyroid dysfunctions). CONCLUSIONS. These data demonstrate that IDTMC are significantly larger than non-IDTMC and more frequently characterized by multifocality, angioinvasion, hypothyroidism and papillary histotype. Diameter may help in distinguishing DTMCs which infiltrate tissues beyond the thyroid capsule from those which do not.

PP198 - RETROSPECTIVE CYTOLOGICAL EVALUATION OF INDETERMINATE THYROID NODULES (THY 3) ACCORDING TO THE BTA 2014 CLASSIFICATION AND COMPARISON WITH CLINICAL EVALUATION AND OUTCOME

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One out of 4 Thy 3 thyroid nodules is malignant. New cytological classifications, more refined instrumental analyses and molecular biology now enable unnecessary thyroidectomy to be avoided. In a series of 161 Thy 3 nodules with known outcome, we retrospectively evaluated the role of the BTA 2014 classification. Thy 3 nodules were divided into Thy 3a (atypical features) and Thy 3f (follicular lesion) categories. 130 pts were F and 31 M. L-T4 treatment had been undertaken in 28%. TPOAb were positive in 43%. CT was normal in all but one. US findings were uninodular (47%) or multinodular (53%) goitre. A US score (0-5) was drawn up, with 1 point being assigned to the following US findings: solid, hypoecoid, irregular margins, inner vascularization and microcalcifications. USE and CEUS were available in 58% and 53% of nodules, respectively. Histology was available as a reference in 103 nodules (64%), while 38 nodules (24%) were kept under observation after further Thy 2 cytology or following medical decision, and 13 (8%) were lost. One pt. died owing to causes unrelated to the nodule, and 6 (4%) are currently awaiting surgery. Malignant histology was found in 19% of nodules (n=10 PTC, n=5 FvPTC, n=3 FTC and n=2 other histology); another 8 mPTCs were incidentally discovered. Mutations of the BRAF V600E (n=4) and KRAS (n=1) oncogenes were observed in 11% of samples. In malignant nodules (USE: ELX 2/1 >0.95; CEUS: P index <0.99. TTP index >0.98; US score >2), ROC analysis showed sensitivity of 84%, 38%, 57% and 70%, and specificity of 80%, 75%, 75% and 100%, respectively. Cytological revision was not feasible in 5% of nodules as diagnostic slides had been used for molecular biology. Malignancy was observed in 50% of these unrevised cases. In a few cases, cytological revision upgraded the diagnosis from Thy 3 to Thy 4 (3%; 100% malignant) or downgraded it from Thy 3 to Thy 2 (5%; 100% benign). The other Thy 3 nodules were divided into Thy 3a (47%) and Thy 3f (53%) categories. The % of cases in which histology was available as a reference were similar in Thy 3a (60%) and Thy 3f (76%) nodules. No significant difference (P=0.2) in malignancy rate was noted between Thy 3a and Thy3f nodules. BRAF mutations were observed in both Thy 3 subclasses. The median US score was 2 in both Thy 3a and Thy 3f category, and USE ELX 2/1 index, CEUS P index and CEUS TTP index were similar in both cytological categories. In conclusion, in our series of Thy 3 nodules, malignancy was low and displayed no significant differences between Thy 3a and Thy 3f categories. Molecular biology and imaging techniques are now essential complementary pre-surgical evaluations

PP199 - CLINICAL IMPACT OF CLINICAL, SONOGRAPHIC AND BIOCHEMICAL PARAMETERS IN PATIENTS WITH INDETERMINATE THYROID NODULES AT CYTOLOGY

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Introduction: Thyroid nodules classified as indeterminate lesions at FNAB occur in 15-30% of all cases. The recently proposed SIAPEC reporting system includes two subclasses of indeterminate lesions with different risk of malignancy: TIR 3 A (low risk) and TIR 3 B (high risk). Immediate thyroidectomy is suggested only for TIR 3 B, while follow-up is advised for patients with TIR 3 A nodules.

Aims: We aimed to assess the clinical relevance of SIAPEC reporting system in an area of borderline iodine deficiency. We also evaluated whether the integration with clinical, biochemical and sonographic parameters, may increase the predictive value of the cytological diagnosis.

Patients and methods: The study included 125 patients, who underwent thyroid surgery for a thyroid lesion classified indeterminate at FNAB. The cytological smears of these nodules were re-evaluated and classified according to the SIAPEC reporting system. Forty-six lesions were classified as TIR3 A (36.8%) and 79 (63.2%) lesions as TIR3 B. A number of clinical (sex, age, BMI), sonographic (size, borders, echogenicity, vascularity, the AP/LL diameter ratio, multinodularity), and biochemical parameters (TSH, FT4, FT3, Ab-Tg, Ab-TPO) were also recorded and correlated with the diagnosis of malignancy.

Results: Five nodules in the TIR 3 A group and 33 in the TIR 3 B group resulted malignant at histological examination. Therefore, the rate of malignancy was 10.9% and 41.8%, in group TIR 3 A and TIR 3 B, respectively. Nodule size ≤1.5cm, irregular margins, and positivity for anti-thyroid antibodies were significantly associated with an increased risk of malignancy, but only in the TIR 3 B group.

Conclusions: TIR 3 A nodules have a rate of malignancy sufficiently low to avoid immediate surgery; in this group the evaluation of sonographic and biochemical parameters does not increase diagnostic accuracy. In contrast, the malignancy rate of TIR 3 B nodules was high enough to justify thyroidectomy, therefore, the evaluation of sonographic and biochemical parameters did not have clinical impact in this group.

PP200 - THYROID NODULES CLASSIFIED TIR 3 A AT FINE-NEEDLE ASPIRATION BIOPSY (FNAB): WHAT ROLE FOR REPEATED FNAB AT FOLLOW-UP?

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Introduction: Thyroid nodules classified as undetermined lesions at fine-needle aspiration biopsy (FNAB) represent a common and challenging problem with regard to diagnosis and management. The recently-proposed SIAPEC reporting system includes two subclasses of indeterminate lesions with different risk of malignancy: TIR 3 A (low risk) and TIR 3 B (high risk). Immediate thyroidectomy is suggested only for TIR 3 B. In contrast, in order to reduce thyroidectomy for benign lesions and minimize the false negative rate, the suggested management for TIR 3 A nodules includes echographic follow-up and repeated FNAB. However, data regarding the usefulness of repeated FNAB in TIR 3 A lesions are warranted.

Aim: We investigated the outcome of a group of patients with a TIR 3 A nodule with a focus on the usefulness of repeated FNAB in the clinical setting.

Methods: The study included 110 patients with a nodule classified TIR 3 A at the initial FNAB. Although repeated FNAB was advised to all of them, only 54 patients actually underwent a second FNAB. Nineteen patients chose to undergo surgery and 37 preferred clinical and echographic follow-up.

Results: Of the 19 patients who underwent surgery, 2 nodules (10.5%) resulted malignant, and the remaining 17 benign. However, in 6 cases the thyroid contained microcarcinoma foci outside the nodule examined. Of the 54 patients who underwent a second FNAB, only 16 (29.5%) were confirmed TIR 3 A, while 6 (11%) resulted non-diagnostic (TIR 1), 29 (54%) benign (TIR 2), and 3 (5.5%) TIR 3 B. Of the 3 patients with a TIR 3 B result, 2 resulted benign after surgery and one is waiting for histological result. No clinical or echographic sign of suspicion was noted at clinical and echographic follow-up in the remaining 37 patients.

Conclusions: In our series of TIR 3 A nodules the malignancy rate is approximately 10%. Repeated FNAB was not able to identify previously missed malignancies, but helped re-classifying TIR 3 A nodules as benign in 50% of cases, thus identifying a subgroup in which the follow-up could be less intensive.

PP201 - SERUM CT IN PATIENTS AFFECTED BY NODULAR THYROID DISORDERS WITH OR WITHOUT THYROID AUTOIMMUNITY

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The association between hypercalcitoninemia and autoimmune thyroiditis (AIT) or differentiated thyroid carcinoma (DTC) has been addressed, with conflicting results.

The aim of this study is to evaluate the influence of AIT and DTC on serum calcitonin (CT) levels.

We evaluated 1847 patients (1474 females and 373 males, mean age 57.3±14.0 years) undergoing fine needle aspiration cytology for thyroid nodules, with adequate cytology. Patients with diagnosis of medullary thyroid carcinoma (MTC) were excluded.

Mean serum CT was 3.0±3.7 pg/ml, significantly higher in males than females (4.99±5.8 pg/ml versus 2.56±2.8 pg/ml, p<0.0001). Based on the clinical diagnosis, patients were divided in two groups: nodular AIT (N-AIT, 254/1847, 13.7%) and nodular goiter (NG, 1593/1847, 86.3%). In females, the median value of serum CT was 2.25±2.6 pg/ml in N-AIT, significantly lower than that observed in NG (2.62±2.8 pg/ml; p=0.008). In males, the median value of serum CT was similar in the two groups (4.41±5.4 pg/ml in the N-AIT and 5.03±5.8 pg/ml in the NG group, p=0.27). Serum CT levels above 10 pg/ml were observed in 76/1847 patients (4.1%), with a significant higher rate in males than females (10.9% versus 2.4%, p<0.0001). The prevalence of CT levels above 10 pg/ml was not different between patients with N-AIT and NG, both in males (13% vs 10.8% p=0.72) and females (1.3% vs 2.6% p=0.34).

Based on cytological results, patients were divided in two groups: Thy2 patients (n=1546) and Thy4/5 patients (n=125) (patients with Thy3 nodules were excluded). The prevalence of patients with CT levels above 10 pg/ml was higher in Thy4/5 patients (7.2%) than in Thy2 patients (3.6%), although not statistically significant (p= 0.08). Similar results were observed when the analysis was performed in the sub-group of patients with histological confirmation (prevalence of CT levels above 10 pg/ml: 8.6% in DTC and 4.6% in benign nodules; p=0.08).

In conclusion, CT levels among patients with nodular disease are higher in males and remain higher also when analyzing CT levels above 10 pg/ml, suggesting that sex should be taken into account when interpreting serum CT levels. CT values above 10 pg/ml are not different in nodular thyroid disease with or without autoimmunity. Furthermore, CT values above 10 pg/ml are similar in nodules with benign or suspicious/malignant cytologies.

PP202 - EXPRESSION OF PDE5 AND EFFECTS OF PDE5 INHIBITORS ON HUMAN THYROID CANCER CELLS

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Normal thyroid tissues express the transcript of several phosphodiesterases (PDE4, PDE5, PDE7 e PDE8), enzymes responsible for the hydrolysis of cyclic nucleotides (cAMP e cGMP). Our preliminary data demonstrated the presence of PDE5 in a series of human papillary thyroid carcinomas (PTCs) characterized for the presence or not of *BRAF* V600E mutation and classified according to ATA risk criteria. In this study, we analyzed the expression of PDE5, both mRNA and protein levels, in three human thyroid cell lines derived from papillary (TPC-1 and BCPAP cells) and anaplastic thyroid carcinoma (8505C cells) and the effects of treatment with two PDE5 inhibitors (sildenafil, tadalafil). PDE5 gene and protein expression were analyzed in thyroid cancer cells by real-time PCR using a TaqMan micro-fluid card system and western blot. MTT and migration assay were used to evaluate the effects of PDE5 inhibitors on proliferation and migration of the cells. We found that PDE5 was expressed in all three human cell lines with the highest transcript and protein levels detected in 8505C cells. Treatment with 1, 10, 100 µM of sildenafil or tadalafil for 48 h reduced the proliferation of all three cell lines with similar trend. In particular, the strongest inhibiting effect (about 50% *vs.* control) was observed using the 100 µM concentration of tadalafil in all cell lines. A reduction of the migration was observed at 0.1, 1 and 10 µM of both inhibitors (8505C and BCPAP cells ~50% *vs* untreated cells). Interestingly, the cells carrying the *BRAF* V600E mutation (8505C and BCPAP) showed a better response to PDE5 inhibition.

Our findings demonstrate for the first time the ability of PDE5 inhibitors to block the proliferation and migration of thyroid cancer cells in culture, therefore suggesting that specific inhibition of PDE5 may be proposed for the treatment of these tumors.

PP203 - EXPRESSION OF THRB IN PAPILLARY THYROID CARCINOMAS: RELATIONSHIP WITH BRAF MUTATION, AGGRESSIVENESS AND MIR EXPRESSION

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Thyroid hormone receptors (THR) mediate the biological activities of the thyroid hormones in growth, development, differentiation and metabolism. In many neoplasia, detection of alterations in the expression levels and/or integrity of *THR* genes has made the analysis of these genes in human cancer an area of considerable interest.

In this study we analyzed *THRβ* mRNA expression in surgical specimens of a series of 36 human papillary thyroid carcinomas (PTCs), well characterized in genotypic and clinical-biological features. PTCs were divided in two group according to ATA risk (low and intermediate) and each divided in subgroups based on the presence or not of the BRAF V600E mutation. Gene expression was analyzed using fluidic cards containing probes and primers specific for the *THRβ* gene, as well as for genes of markers of thyrocyte differentiation (*TPO, NIS, Tg* and *TSH-R*) and for some miRNAs involved in thyroid neoplasia. The mRNA levels of each tumor tissue were compared with their correspondent normal counterpart.

In all PTCs examined *THRβ* transcript was down-regulated and no significant differences were found between the groups of intermediate *vs* low risk PTCs and BRAF mutated *vs* BRAF wild type PTCs. *THRβ* expression was directly correlated with *NIS, TPO, Tg* and *TSH-R* and associated with altered expression of miR -21, -146a, -181a and -221.

Our results demonstrate that down-regulation of *THRβ* is not associated with a more aggressive phenotype of PTC. However, it correlates with the reduction of all the markers of differentiation and is associated with over-expression of some miRNAs supposed to play a role in thyroid tumorigenesis.

PP204 - EFFICACY OF TWO DIFFERENT L-THYROXINE FORMULATIONS IN PATIENTS WITH SUBCLINICAL HYPOTHYROIDISM AND DYSPEPTIC SYNDROME

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Two oral formulations of l-thyroxine (LT4) are currently available: tablets (TAB) or liquid solution (SOL). LT4 TAB absorption could be affected by several gastric diseases frequently discovered in dyspeptic patients, such as Helicobacter Pylori (HP) infection or chronic atrophic autoimmune gastritis (CAG), instead no data are available on absorption of the LT4 SOL in these conditions. The aim of this study was to compare the efficacy of two different LT4 formulations (TAB or SOL) in patients with dyspeptic syndrome (DS) and subclinical hypothyroidism (SH). We included 20 naïve patients (15 females, 5 males) aged from 27 to 55 yrs, with DS and SH due to chronic autoimmune thyroiditis. All patients were firstly investigated by esophageal gastric duodenal endoscopy and urea-breath test: 4 patients presented HP infection (group A); 4 patients CAG (group B); 6 patients simple gastritis and 6 patients no organic alterations (group C). Subsequently, LT4 treatment was randomly (TAB or SOL) started at the fixed dose of 1.5 µg/kg/day; TSH and fT4 were assessed at baseline and at 3 and 6 months of LT4 treatment. On group A eradication of HP was performed at the 3th month of trial. In each groups, no difference in basal TSH levels were shown within patients assigned to different treatments (Group A: TSH_{tab}=10,1±1.8 µIU/ml, TSH_{sol}=9.6±2.2 µIU/mL; Group B: TSH_{tab}=7,1±1.2 µIU/ml, TSH_{sol}=6,9±2.1 µIU/ml; Group C: TSH_{tab}=7,9±1.7 µIU/ml, TSH_{sol}=8,1±2.1 µIU/mL). At 3th month, TSH values on group A (before eradication of HP) significantly decreased on patients treated with SOL, while on group B and C, equally decreased without difference within two formulations (Group A: TSH_{tab}=7.8±2.6 µIU/ml vs TSH_{sol}=3.1±1.8 µIU/mL, p<0.001; Group B: TSH_{tab}=2,4±1,0 µIU/ml, TSH_{sol}=2,6±1.2 µIU/mL; Group C: TSH_{tab}=1,7±1,4 µIU/ml, TSH_{sol}=2,5±1,6 µIU/mL). At 6th month, TSH levels on group A (after eradication of HP) were in the normal range and similar within two formulations; on Group B and C, TSH were also normal without significant difference within two formulations (Group A: TSH_{tab}=2,5±2.6 µIU/ml vs TSH_{sol}=3,6±1.8 µIU/mL, p=ns; Group B: TSH_{tab}=3,5±2.2 µIU/ml vs TSH_{sol}=2,1±1.8 µIU/mL, p=0.093; GroupC: TSH_{tab}=1,9±2.1 µIU/ml, TSH_{sol}=2,0±1.7 µIU/mL). In each groups, no difference were shown on fT4 values within two formulations. In conclusion this preliminary report suggest that LT4 TAB or SOL are equally efficient to treat SH in dyspeptic patients without gastric alterations or with simple gastritis or CAG, while LT4 SOL seems to be more efficient than TAB in patients with HP infection, independent of its eradication.

PP205 - ZNT8 AUTOANTIBODIES ARE NOT ASSOCIATED WITH CHRONIC AUTOIMMUNE THYROIDITIS

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Background: ZnT8 autoantibodies (ZnT8Abs) have been involved as predictive and diagnostic marker of autoimmune diabetes, in fact ZnT8 is abundantly expressed in pancreatic β and α cells. Some studies prove that ZnT8 is also expressed in the thyroid cubical epithelium, suggesting a more widespread role of this transporter. It has been also shown a higher prevalence of thyroid autoimmunity in type 1 diabetes subjects with high levels of ZnT8Abs. More recently, a study shows a high prevalence of ZnT8 epitopes in Sardinian population with chronic autoimmune thyroiditis. Here we aim to evaluate whether ZnT8Abs are a marker of autoimmune thyroiditis in subjects without diabetes.

Methods: In this cross-sectional study conducted from May to September 2014 we enrolled 81 consecutive patients with chronic autoimmune thyroiditis (CAT), defined as the presence of TPOAb +/- TGAb (Group A), and 21 age and sex matched healthy euthyroid subjects without CAT (Group B). All subjects were considered eligible if they had not a diagnosis of diabetes. Serum ZnT8Abs levels were measured in both groups by radio immunoprecipitation assay (RIA) using recombinant ZnT8 COOH-terminal or NH2-terminal proteins.

Results: In group A there were 67 females and 14 males and the age of participants was 52.1 ± 15.9 (mean ± sd); the percentage of hypothyroidism, euthyroidism and hyperthyroidism was 54.3%, 23.4%, 3.7%, respectively. In group B there were 15 females and 6 males and the age of subjects was 54.5 ± 15.7 (mean ± sd). A very low prevalence of serum ZnT8Abs positivity was found in both cases and control subjects, not differing between the two groups (2 subjects (2.5%) in group A vs 1 subject (4.7%) in group B, p=ns). The three subjects resulted positive to the test showed low ZnT8Abs titer and they were also assessed for anti-GAD autoantibodies with negative result.

Conclusion: Although it has been demonstrated the presence of ZnT8 in the thyroid cubical epithelium and recent studies show high prevalence of ZnT8 epitopes in patients with CAT, this clinical study proves that ZnT8Abs cannot be considered as a marker for thyroid autoimmunity in non diabetic subjects. Further large studies are needed to evaluate the role of this Zn transporter in thyroid disease.

PP206 - PTC REFRACTORY TO RADIOIODINE: A CROSS-SECTIONAL STUDY

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Background: Some patients affected by Papillary Thyroid Cancer (PTC) in postoperative phases are undergoing radioiodine treatment, which is commonly used for thyroid cancer follow-up. A small but not negligible percentage of differentiated thyroid cancers become refractory to radioiodine treatment either because they lose the ability to take up iodine over time or because, despite persistent uptake capacity, the effect of radioiodine is lost in terms of tumor burden reduction. Our study evaluated the prevalence of refractory cases and possible predictors at diagnosis.

Methods: We performed a cross-sectional study on 286/385 patients who received radioiodine therapy (74.3%). At present 273 patients (95.5%) proved to be disease-free and 13 (4.5%) showed radioiodine-refractory disease.

Results: In univariate analysis, the refractory patients had a higher prevalence of peri-thyroid tissue invasion [30.8 vs. 11 %; OR 3.58 (IC95%: 1.04-12.35) p=0.043), lymph node metastasis [53.8 vs. 16.8; OR 5.75 (IC95% 1.85-17.92); p=0.003], distant metastasis [23.1 vs. 2.9 %; OR 9.9 (IC95% 2.27-43.03) p=0.002]. No other significant association was found with gender, age, thyroid autoimmunity, iodine deficiency, family history of cancer, histological types, and tumor size.

Conclusion: The definition of predictor factors for PTC radioiodine-refractory could be used to select those patients suited to starting innovative therapies able to improve radioiodine sensitivity. Our data suggest the importance of the co-presence of the three factors, peri-thyroid tissue invasion, lymph node metastasis and distant metastasis, as predictors of radioiodine-refractory disease.

PP207 - THYROID METASTASES FROM RENAL CELL CARCINOMA: A CASE REPORT

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Background: Renal cell carcinoma (RCC) can metastasize to uncommon sites, for example, the thyroid gland where metastases are rarely found. Thyroid metastases of the non-thyroid malignances (NTM) are infrequently diagnosed in the clinical environment. In autopsy studies, however, metastases to the thyroid gland have a frequency of 1.25-24%. Malignant melanoma, breast carcinoma, lung, and skin cancer are the most common sources of NTM, however in agreement with previous studies, the majority of metastases to the thyroid gland derive from renal cell carcinomas.

Case: We report a case of NTM from renal cells carcinoma 15 years after radical left nefrectomy in a 66 year-old man, before treated with chemiotherapy, radiotherapy and immunotherapy. The patient was refered to our hospital because of a recent rapidly growing right-sided neck mass. Thyroid ultrasonogram showed a voluminous hypoechoic nodule with microcalcifications, well-defined margins but without halo-sign occupying the right lobe of the diameter of 6.5 cm. Thyroid function was normal and Ab anti-TPO, Ab anti-Tg and calcitonina were negative. Fine needle aspiration citology showed red blood cells, low colloid, rare groups of thyrocytes with anisokaryosis and oncocytic modifications. The patient underwent total thyroidectomy. Histology revealed the diagnosis of metastasis of clear cell renal carcinoma (CD10 + and TTF1-).

Conclusion: Isolated thyroid metastasis should be considered in patients with a previous history of cancer and newly developing thyroid mass.

PP208 - COEXISTENCE OF PAPILLARY THYROID CARCINOMA (PTC) AND DIFFUSE LARGE B-CELL LYMPHOMA IN A PATIENT AFFECTED BY HASHIMOTO'S THYROIDITIS.

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PTC is the most common type of thyroid cancer. On the contrary, primary thyroid lymphoma (PTL) is a rare disease, accounting for 2%-5% of all thyroid malignancies, and can be distinguished in two subtypes: diffuse large B-cell lymphoma (DLBCL), with a more aggressive course, and MALT lymphoma, generally indolent and associated with a better prognosis. Despite a large majority of cases of both PTC and PTL arise in the setting of Hashimoto's thyroiditis (HT), the coexistence of both tumors in HT patients is very rare.

Patients findings. A 66-yr-old woman with known hypothyroidism from HT under replacement therapy with L-Thyroxine, and long-standing nodular goiter, was referred to our outpatient clinic, because of a fast, painless enlargement in the right anterior side of the neck. A thyroid ultrasound demonstrated the growth of a hypoechoic nodule in the right lobe, measuring 32x20 mm (18 mm at the previous control). Fine-needle aspiration cytology of the right-sided nodule revealed atypical epithelial cells and lymphocytic infiltration, and the cytologist concluded for indeterminate lesion (THYR3). Total thyroidectomy was performed. Histology revealed a DLBCL (large atypical lymphocytes with irregular nuclei, condensed chromatin, small nucleoli) on a background of florid HT. Immunohistochemistry confirmed CD20, anti-BCL2 and anti-BCL6 positivity, with monoclonal lambda chains, so the neoplasia was staged as 1A. Moreover, a unifocal papillary microcarcinoma (7 mm, pT1aNxMx) was discovered. Staging studies for the PTL were performed, including total-body CT and bone marrow biopsy, showing no evidence of systemic disease or metastases. The patient was then treated with chemotherapy for the PTL, while she didn't undergo radioactive iodine ablation treatment for the microPTC, as per guidelines. Two years after surgery, the patient had no evidence of recurrence of either malignancy.

This rare case highlights the importance of monitoring of HT patients, especially if they have long-standing disease and nodular lesions, for the higher risk of neoplasia. Besides, PTL should be considered for differential diagnosis in elder HT patients whenever presenting with sudden thyroid enlargement. When the two neoplastic diseases coexist, the treatment has to prioritize the tumor with the worst prognosis and/or the worst stage at diagnosis.

PP209 - IMPACT OF BRAF V600E MUTATION ON PAPILLARY THYROID CARCINOMA PROGNOSIS: A PROSPECTIVE STUDY.

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Papillary thyroid carcinoma (PTC) is the most frequently occurring thyroid cancer, with a growing incidence in the general population.

BRAF V600E somatic mutation has demonstrated a very good diagnostic sensitivity for PTC in fine needle aspiration biopsies (FNAB), and has been shown tto associate with higher disease-related mortality and morbility in retrospective studies. Therefore, the real prognostic value of this mutation is still under debate.

We here explore the influence of BRAFV600E somatic mutation on the clinical outcome of PTC patients followed up prospectively after surgery. To this aim, we performed a prospective case-control study comparing two groups of PTC patients: the first group was represented by 80 PTC patients that displayed a BRAF V600E mutation at the pre-surgical FNAB (BRAF positive); the second group was represented by 76 PTC patients that did not display a BRAF V600E mutation at the pre-surgical FNAB (BRAF negative). In each group, 50% of the patients had lymphnode metastases at diagnosis (N1) and 50% had not (N0). BRAF negative and positive PTC patients were matched by risk factors, including sex, age, histotype, disease stage. We then evaluated pathology data (T1a, diameter, multifocality, bilaterality) at diagnosis and examined the clinical outcome. We found that T1a and microPTC were equally frequent in the two groups. However, BRAF negative N0 patients displayed a significantly higher number of T1a tumors and of microPTC as compared to BRAF negative N1 patients. The presence of lymphnode metastases was associated with a greater diameter in BRAF negative patients but not in BRAF positive patients. Multifocality was more frequent in BRAF negative patients, especially N1, as compared to BRAF negative patients, while bilateral disease was equally frequent in the two groups.

The choice to address the patient to radioiodine therapy (I131) was influenced by the nodal status but not by the presence of a BRAFV600E mutation. In all patients (both those that underwent I131 and those who did not) the rate of complete responses up to 2 years of follow-up was not influenced by the presence of a BRAF mutation not by nodal status. Therefore, these data indicate that BRAF status does not influence the stage of the disease at diagnosis nor characterizes a more aggressive disease, confirming a limited prognostic value for this molecular marker. However, a longer follow up is warranted in order to reach more solid conclusions.

PP210 - THE CLINICAL ACTIVITY SCORE IN MANAGEMENT OF GRAVES' OPHTHALMOPATHY: CORRELATION WITH ORBITAL MR IMAGING.

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Graves ophthalmopathy (GO) is a common extrathyroid disorder of Graves disease (GD) and it is unanimously considered clinically relevant to identify the acute inflammation phase at an early stage of disease.

Our aim was to investigate the correlation between GO ocular parameters obtained by MRI with contrast administration and the well-known clinical indicators of GO and to examine the relationships between the clinical course of hyperthyroidism and GO severity.

From June 2013 until November 2014, 28 consecutive patients (9 men and 19 women; mean age 48.42 years), with a diagnosis of GO were enrolled. All patients were subjected to MR on the same day as the clinical evaluation. MR examination was performed with a 1.5T scanner (3mm thin slices, FSE T2w and T1w sequences with and w/o fat saturation; pre- and post-Gd T1w sequences were obtained). Endocrine evaluation considered serum parameters (TSH, FT3, FT4, TRAb), thyroid ultrasound and medical records collection (disease duration, remission phases, smoking, therapy). Ophthalmological evaluation considered proptosis (by Hertel Exophthalmometry), ocular pressure, soft tissue signs to calculate the clinical activities score (CAS) according to EUGOGO consensus statements. All patients were divided into two groups according to their ophthalmopathy severity: 13 with mild (46.4%) and 15 with moderate to severe GO (53.6%). No cases of sight-threatening GO were included. 14/15 patients with moderate/severe disease had a activity GO (CAS ≥ 3). No difference was found for gender, age, smoking, duration of disease, serum thyroid parameters and thyroid ultrasounds between the two groups. Moderate to severe GO patients showed a significant increase in all the ocular parameters evaluated by MRI [proptosis, cross sectional area and transverse diameters of the ocular extrinsic muscles (inferior, superior, medial and lateral rectus muscles; all $p < 0.05$).

CAS confirms an optimal indicator of GO severity and activity, as demonstrated by its correlation with a greater volume of extrinsic muscles and inflammation MRI documented. MRI may be considered the technique of choice for orbital imaging because of its lack of ionizing radiation, its fine delineation of detail.

PP211 - COMPARISON AMONG LASER PHOTOCOAGULATION ABLATION AND RADIOFREQUENCY ABLATION OF BENIGN THYROID NODULES.

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Introduction. In recent years is catching on minimally invasive treatments to reduce the volume of benign thyroid nodules. The two main techniques are the radiofrequency ablation (RFA) and the laser-photocoagulation ablation (LPA). Our group started RFA in 2012 and LPA in October 2014.

Aim of the study. Compare the volume reduction and improvement of neck discomfort and quality of life at 6th month of follow-up in patients treated with LPA and RFA for benign thyroid nodules.

Materials and methods. From october 2014 we have treated 10 subjects in LPA and 10 subjects in RFA that have evaluation at the 1st, 3rd and 6th month with clinical examination with monitoring of adverse events, thyroid exams and US echography. A visual analogic scale (VAS) was completed to evaluate neck discomfort (range from 0= not discomfort to 10= maximum discomfort) and a 12-scales quality of life questionnaire (QoL) validated for thyroid diseases (ThyPRO) were compiled at each time. The patients treated with LPA were mainly females with mean age of 52±12 years old (range: 38-72) and the patients treated with RFA were mainly females with a mean age of 50±19 years old (range: 34-80). Results. Mean power during LPA was 4652±1329 J, with a mean use of 2 fibers and a mean of 2 pull-backs. 1 patient complained pain and 1 patient had a minimal bleeding during LPA, 1 patient neaded anxyolitic therapy before treatment. Afetr 1st week 4 patients complained neck discomfort (VAS 4±3), 2 patients reported a hematoma and 2 patients reported neck swelling. The volume of nodules was significantly reduced (28±15 ml at the baseline; 17±11 ml at 6th month); none had destructive thyrotoxicosis or hypothyroidism or positivization of autoantibodies. VAS score reduced significantly (5±2 at baseline and 1±1 at 6th month) and QoL didn't modify. Mean power during RFA was 29915±11868 J. All patients complained pain during RFA, none had bleeding, none need anxyolitic therapy before treatment. After 1st week 2 patients complained neck discomfort, 1 patients reported pain (VAS 2±2.1). The volume of nodules was significantly reduced (35±25 ml at the baseline; 25±18 ml at 6th month); none had destructive thyrotoxicosis or hypothyroidism or positivization of autoantibodies. VAS score reduced significantly (5±2 at baseline and 3±3 at 6th month) and QoL didn't modify.

Conclusion. LPA and RFA are effective and safe procedures to reduce the volume of thyroid nodules and neck discomforts.

PP212 - THE COMBINED USE OF ULTRASOUND AND ELASTOSONOGRAPHY PROVIDES AN ACCURATE PRE-SURGERY EVALUATION OF MALIGNANCY IN THYROID NODULES OF UNCERTAIN DIAGNOSIS AT CYTOLOGY (THY3B).

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Background: Thyroid nodules diagnosed as follicular neoplasm (Thy3B) at fine-needle aspiration biopsy (FNAB) are a matter of diagnostic uncertainty: their risk of malignancy is relevant (15-30%) but surgery will result unnecessary in most cases. Immunohistochemical and molecular markers and morphological procedures have attempted to better differentiate benign from malignant Thy3B nodules, with little success. Elastography (ES) has also been proposed to increase diagnostic accuracy but results are controversial. The present study aims at evaluating the diagnostic accuracy of the separate or combined use of Ultrasound (US) and ES in predicting malignancy of Thy3B nodules.

Methods: In fifty patients thyroidectomized because having a thyroid nodule classified Thy3B at FNAB, US and ES evaluation was carried out by the same expert operator and using an advanced equipment providing both US and strain ES imaging. Specific evaluation procedures to reduce subjective interpretation of heterogeneous nodules and two novel parameters (intra-nodule irregular blood flow at C-doppler and extra-nodule stiffness extension at ES) were also evaluated. Overall, five US parameters (echogenicity, irregular margins, microcalcifications, intra-nodule blood flow and irregular intra-nodule blood flow) and two ES parameters (intra-nodule stiffness and extra-nodule stiffness extension) were considered. Diagnostic accuracy was evaluated for either US or ES or both combined after obtaining post-surgical histological diagnosis.

Results: Sensitivity, specificity, positive (PPV) and negative (NPV) predictive values were 100%, 85%, 63% and 100%, respectively, for US and 60%, 92.5%, 67%, 90%, respectively, for ES. The newly introduced evaluation of intra-nodule blood flow irregularity increased US sensitivity from 80% to 100% while evaluation of extra-nodule stiffness extension increased ES sensitivity from 50% to 60%. The combined US and ES evaluation significantly improved diagnostic accuracy: when ≥ 4 out of 7 parameters were present the risk of malignancy was very high (sensitivity 100%, specificity 92.55, PPV 77%, NPV 100%).

Conclusions: When comparing the two imaging procedures for diagnosing Thy3B nodules malignancy, US has better sensitivity and NPV while ES has better specificity and PPV. Combining the two techniques provides a very high diagnostic accuracy.

PP213 - THE MAXIMAL STIFFNESS EVALUATION BY REAL TIME ULTRASOUND ELASTOGRAPHY, AN IMPROVED TOOL FOR THE DIFFERENTIAL DIAGNOSIS OF THYROID NODULES

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Objective: Aim of the study was to evaluate the diagnostic performance of a new ultrasound (US) elastography (USE) parameter based on the measurement of the percentage of maximal stiffness within a nodule as compared with the already established elastographic strain index (SI), and to investigate their diagnostic performance according to nodule size.

Methods: The study included 218 nodules. Each nodule underwent conventional ultrasound (US), USE evaluation and fine needle aspiration cytology (FNAC). Thyroid nodules were further stratified according to their size (G1< 1 cm, G2= 1-2 cm, G3> 3 cm). USE evaluation comprised the measurement of the percentage of the areas included in the region of interest corresponding to the maximal stiffness (% Index) and of the SI.

Results: The % Index and of the SI were significantly higher in malignant than in benign thyroid nodules, and both measurements displayed a good diagnostic performance (SI sensitivity and specificity = 0.66 and 0.90, respectively; % index sensitivity and specificity = 0.76 and 0.89, respectively). Compared with SI, the % Index was more informative, both in the whole group of thyroid nodules (OR[95%CI]=18.68[6.06-63.49], p=1.49x10-8, vs OR[95%CI]=26.15[8.01-102.87], p=3.41x10-10 respectively) and in the G1 and G2 subgroups.

Conclusion: The % Index is a stronger predictor of nodule malignancy than both the SI and the conventional US signs. This is particularly true in nodules smaller than 1 cm, which are more difficult to be explored both by conventional US and by FNAC.

PP214 - THYROID PAPILLARY MICROCARCINOMA RECURRENCE WITH FALSE NEGATIVE TG/TGAB AND NEGATIVE I-131 WHOLE BODY SCAN (WBS)

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Ultrasensitive thyroglobulin (uTg) is the most sensitive marker for persistence or recurrence of the thyroid carcinoma after total thyroidectomy and radioiodine ablation. The presence of endogenous Tg antibodies (TgAb) can cause false-negative interference and can confound the interpretation of results. We report a case of a 25-year-old patient, who presented to our Endocrine Unit in November 2011. The ultrasonosgraphy (US) revealed three large suspicious lymph nodes and a ipoechoic nodule in the right lobe of 7 mm. The hormonal evaluation revealed a euthyroid Hashimoto's thyroiditis: TSH 3.7 µU/ml, TPOAb 887 U/ml (vn <60), TgAb 86U/ml (vn<60, using radioimmunoassay). In January 2012 she underwent total thyroidectomy and right and central compartment lymphoadenectomy. The histology confirmed a follicular variant of papillary carcinoma (9 mm) in the right lobe with minimal invasion of surrounding tissues and metastases in 5 lymph nodes (pT3 pN1b). Then she received radioiodine ablation (100 mCi of I131 after L-T4 therapy withdrawal). The post-therapy WBS was negative (TSH>100 µU/ml; uTg 0.2 ng/ml, TgAb <60 U/ml). In the follow-up, during suppressive therapy, serum uTg and TgAb were always undetectable. In April 2013 she was at 15th gestational week and US showed persistence or recurrence of two large lymph nodes (>2 cm) at II b right level, confirmed by positive cytology on node-FNA and very elevated FNA-Tg wash-out levels (>5000 ng/ml) despite undetectable serum Tg. After delivery 18FDG-PET-CT was performed and there was no other uptake except in right neck lymph nodes. She underwent surgery (right lateral compartment nodes dissection) and histology confirmed the presence of metastases. At further follow-up no evidence of recurrent disease (neck US and 18-FDG-PET-CT negative; TSH<0.05 µU/ml Tg<0.2 ng/ml and TgAb <60 U/ml). The histology review revealed the presence of BRAF-V600 mutation. This is a strong independent predictor of persistence/recurrence of disease and it is associated with a poor prognosis. Pregnancy can also exert a negative prognostic role in the outcome. Current guidelines considered these patient at a low-risk of recurrence, due to negative WBS after radioiodine ablation and negative Tg and TgAb levels. However a careful follow-up with neck US (during pregnancy) and later with 18-FDG-PET-CT, allowed us to detect metastases. The histological pattern, the nonradioactive iodine avidity, the 18FDG-PET-CT uptake and the BRAF mutation, correlate with a more aggressive tumor behavior and worse prognosis. In general uTg measurement has a great sensitivity and specificity in monitoring MPTC patients, but in this case it was useless.

PP215 - ER-ALPHA AND ER-BETA EXPRESSION IN DIFFERENTIATED THYROID CANCER: RELATION WITH TUMOR PHENOTYPE ACROSS THE TNM STAGING AND PERI-TUMOR INFLAMMATION

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Objective: Thyroid cancer may express estrogen receptors (ERs) and various grades of peri-tumor inflammation. The aim of the study was to evaluate the expression of ERs in relation to the TNM stage and peri-tumor inflammatory infiltrate in differentiated thyroid cancers.

Methods: 127 patients (109 females, 18 males) with differentiated thyroid cancer (T1 = 91, T2 = 18, T3 = 11, T4 = 7) were evaluated. In tumors and in the correspondent extra-tumor parenchyma, ERs expression was evaluated by immunohistochemistry. In 114 tumors and correspondent peri-tumor tissues, the presence of inflammatory infiltration was also recorded.

Results: ER-alpha expression was higher in clinical than in incidental tumors of the T1 subgroup ($p = 0.037$), and was associated with capsular invasion in T2 tumors ($p < 0.0001$). ER-beta expression was negatively associated with vascular invasion in T1 ($p = 0.005$) and T2 tumors ($p = 0.015$). No significant relationship between ERs expression and tumor phenotype emerged in T3 and T4 subgroups. Tumors without inflammatory cell infiltrate showed a higher expression of both ER-alpha ($p = 0.035$) and ER-beta ($p = 0.026$) than the ones with inflammatory infiltrate. The relationship between tumor phenotype and ERs expression did not vary in the presence or absence of peri-tumor inflammatory infiltration.

Conclusions: ER-alpha positivity and ER-beta negativity are associated with a more aggressive phenotype in both T1 and T2 thyroid cancers, suggesting that tumor biology may be more relevant than tumor size for cancer risk assessment. Inflammatory status is also associated with ERs expression, but not with tumor growth or phenotype

PP216 - INCIDENTAL DIFFERENTIATED THYROID CANCER: REPORT FROM THE ENDOCRINOLOGY UNIT AT MAGGIORE DELLA CARITÀ UNIVERSITY HOSPITAL IN NOVARA

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Background: Incidental thyroid cancer is a malignant disease occasionally detected in patients undergoing thyroidectomy for benign disease. The aim of our study was to evaluate the behavior of this disease. Methods: We retrospectively analyzed the characteristics of patients with incidental differentiated thyroid cancer (DTC) at time of diagnosis, short-term and long-term follow-up. All cases were diagnosed from January 2000 to February 2010 at Maggiore della Carità University Hospital in Novara. Results: We enrolled 68 patients with hystological diagnosis of DTC (10 M, 58 F), with mean age at diagnosis 53.0 ± 14.1 years. All patients underwent total thyroidectomy because of: toxic multi-nodular goiter (5.9%), Graves' disease (11.7%) and compressive multi-nodular goiter, with either hypothyroidism or normal thyroid function (82.4%). Among the latter 16.1% had a previous cytological diagnosis of benignity. The 48.5% of patients underwent radioiodine remnant ablation after surgery. Regarding the hysto-pathological features: 97.1% had papillary thyroid cancer and, among them 43.9% were classical variant, 37.9% follicular variant, 7.6% classical and follicular variant and 10.6% were the more aggressive histological variants. Only 2 patients had a minimal invasive variant of follicular thyroid cancer. Regarding to tumor size, 72.1% were microcarcinomas, 13.2% small carcinomas and 14.7% were >2 cm. At the short-term follow-up, 50.0% of patients had undetectable thyroglobuline (Tg) level; 38.2% had detectable Tg level <10 ng/mL without any evidence of disease at imaging, while in the 11.8% of cases Tg level was >10 ng/mL. Among the latter, only 1.5% had evidence of disease at imaging. At the long-term follow-up, 9 patients (13.2%) showed disease recurrence: nodal metastases in 6 cases and lung metastases in 1 case. Two patients had detectable level of Tg, but <10 ng/mL and had no evidence of relapse at imaging. All cases were papillary thyroid cancers classified as low-risk carcinomas at diagnosis, in particular 7 were microcarcinomas, and instead only 2 cases were sized >2 cm and had capsular invasion. Conclusions: Our data show that incidental DTC is often indolent, and the risk of recurrence is comparable to that of thyroid cancers, non/incidental detected. However, the relapse of the disease occurred mainly in the long-term follow up and most part of patients with recurrent disease had a microcarcinoma without any aggressive hystological characteristics at initial diagnosis. This would suggest that the common staging systems for the DTC stadiation may be not enough to predict the outcome of the disease and that other markers of more aggressive behavior (genetic, environmental) should be included in the risk stratification.

PP217 - HER2 AND EPIDERMAL GROWTH FACTOR (EGFR) IMMUNOHISTOCHEMICAL EXPRESSION IN DIFFERENTIATED THYROID CARCINOMAS (DTC).

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Aim. The EGFR family members EGFR and HER2, are over-expressed in many human epithelial malignancies, including thyroid cancer, representing molecular targets for specific biological anti-neoplastic drugs. To determine the role of such receptors in the stratification of thyroid cancer, we studied their immunohistochemical expression in a cohort of DTC [45 papillary (PTC) and 45 follicular (FTC) thyroid cancers].

Methods. Immunohistochemistry was performed twice on each formalin-fixed paraffin-embedded specimen, by using firstly the monoclonal antibodies against HER2-pY-1248 (Phosphorylation site specific) (clone PN2A, Dako; w.d. 1:100) and against EGFR (clone E30, Dako; w.d.1:150), and successively the Hercep Test and EGFR pharmDx kits (Dako). Staining intensity and cellular localization in the tumour and in the adjacent non-neoplastic thyroid were evaluated. Intensity was scored as 1 (weak), 2 (moderate), or 3 (strong); and the percentage of cells stained was recorded. Low expression (1+) was defined as weak expression in less than 10% of tumour cells, and moderate (2+) to high expression (3+) as moderate or strong staining intensity (2 to 3+) in greater than 10% of tumour cells.

Results. HER2 was expressed in 70% of DTC (28/45 PTC and 35/45 FTC), with an intensity ranging from 1+ to 3+. The intensity was 2.5±0.5 in FTC (median: 3+) and 2±0.9 in PTC (median: 2+). The positivity for HER2 was diffuse throughout the section, and displayed a cytoplasmic and membranous pattern. In both histotypes, the higher the aggressiveness of the tumour, the higher the expression of HER2. In contrast, EGFR was infrequently expressed in DTC. Most tumors were negative and a few cases (5 PTC, all follicular variant and 6 FTC) showed low and patchy EGFR expression (1+). Normal thyroid parenchyma showed no immunostaining for either HER2 or EGFR.

Conclusions. Most of our DTC cases demonstrated high expression of HER2, which was absent in background thyroid tissue, while only a few cases revealed EGFR expression. Our preliminary data on a large surgical series suggest that HER2 expression may be utilized to select patients eligible for targeted therapies.

However, HER2-directed therapies may be considered individually.

PP218 - IODINE SUPPLEMENTATION AND RISK OF POSTPARTUM THYROIDITIS IN PREGNANT WOMEN LIVING IN AN AREA WITH MILD TO MODERATE IODINE DEFICIENCY

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A possible unwanted effect of increasing the iodine intake in mild to moderate iodine deficient pregnant women is an increase in postpartum autoimmune thyroiditis (PPT).

The objective of the present study was to evaluate whether iodine supplementation during pregnancy to women living in an area with mild to moderate iodine deficiency is associated to increased risk of developing PPT.

Subjects: 448 pregnant women who underwent screening and monitoring of thyroid function throughout gestation. For the purposes of the study, 191/448 women proved ineligible because they were receiving L-T4 replacement or semi-suppressive therapy for post-surgical hypothyroidism or nodular goitre prior to becoming pregnant, or because they did not complete the scheduled follow up (at least 6 months after delivery). The remaining 257 made up the study group. The study protocol included: i) FT4, TSH and TPO-Ab determination at early pregnancy and in the post-partum; FT4 and TSH at 6 weeks intervals throughout pregnancy; thyroid ultrasonography (US) at enrolment.

Results: PPT developed in 34/257 (13.2%) of the participants [25/34 (73.5%) hyper- and hypothyroid phases, 5/34 (14.7%) hypothyroidism only, 4/34 (11.8%) hyperthyroidism only]. Twenty-eight/34 (82.3%) women diagnosed with PPT were TPO-Ab positive at early pregnancy. Univariate regression analysis showed significant association for PPT occurrence and TPO-Ab positivity at early pregnancy ($p<0.0001$; OR 12.1 – 95%CI 4.78-30.7), first-trimester serum TSH >2.5mU/L (p 0.014; OR 2.75 – 95%CI 1.22-6.18), hypoechoic pattern (p 0.03; OR 1.71 – 95%CI 1.05-2.79) and pseudonodules (p 0.002; OR 4.95 – 95%CI 1.83-13.41) at thyroid US. Use of iodine containing (150-225 μg) supplements was not associated to increased risk of PPT, whereas a weak association was found for long-term iodised salt consumption ($p<0.011$; OR 1.11 – CI 95% 1.03-1.21). Nonetheless, in a multivariate regression analysis, the independent significance of TPO-Ab positivity was the only confirmed (p 0.002; OR 9.48 – CI 95% 2,25- 40.05).

In conclusion, in the current study we found that iodine supplementation during pregnancy to either TPO-Ab positive or negative women living in an area with mild

to moderate iodine deficiency is not associated to increased risk of PPT. Screening for TPO-Ab in early pregnancy can predict women at high risk of developing PPT.

PP219 - EFFECTS OF L-THYROXINE AND/OR IODINE PROPHYLAXIS ON NEUROINTELLECTUAL PERFORMANCES IN CHILDREN BORN TO MODERATELY IODINE DEFICIENT MOTHERS: RESULTS OF A LONGITUDINAL PILOT STUDY

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Background. Adequate iodine intake during pregnancy is essential to maintain maternal and fetal euthyroidism and to guarantee normal brain development in the fetus.

Objectives: To verify the effects of iodine prophylaxis by iodized salt on neurointellectual outcome of children born to moderately iodine deficient mothers.

Subjects: Sixty children (6-12 years) born to: i. mothers regularly using iodized salt prior to pregnancy (I group, n=15); ii. mothers on LT4 treatment and regularly using iodized salt prior to pregnancy (I+T4 group, n=15); iii. mothers who had never used iodized salt (no-I group, n=15); iv. unsupplemented mothers on LT4 treatment prior to pregnancy (no-I+T4 group, n=15). Methods: Wechsler Scale of Intelligence Scale for Children 3th ed. (WISC-III), 13 subscales. Results: Children born to I and I+T4+ mothers had similar total IQ scores (tIQ), which were 12 and 15 points higher than that of unsupplemented mothers (no-I and no-I+T4). The overall prevalence of borderline or defective cognitive function was more than 3-fold higher in children born to unsupplemented than to supplemented mothers (66.6% vs 20.7%, χ^2 10.85, p 0.0004). Univariate analysis for explicative variables and confounders showed maternal nutritional iodine status, assessed either as long-term iodized salt consumption or as maternal urinary iodine excretion as a continuous measure, to be statistically related to children's cognitive outcome (tIQ *OR* 7.67, *95%CI* 2.36-24.86; verbal IQ (VIQ) *OR* 7.3, *95%CI* 2.3-23.2; performance IQ (PIQ) *OR* 4.78, *95%CI* 1.3-17.2), Notably, no relationship was found between suboptimum tIQ, VIQ, and PIQ and both maternal serum FT4 and TSH levels, at each considered gestational interval, neither when the whole group was considered, nor when analysis was restricted to untreated women (I and no-I groups) only.

Conclusions: Neurointellectual outcome seems to be affected by maternal iodine status rather than by maternal thyroxine levels, likely because of a more adequate fetal thyroid function. Nonetheless, the high rate of cognitive deficit also observed in children born to mothers who regularly used iodized salt indicates this mean of iodine prophylaxis to be insufficient during pregnancy.

PP220 - MORPHOLOGICAL AND FUNCTIONAL ALTERATIONS OF THYROID GLAND DURING TREATMENT WITH TYROSINE KINASE INHIBITORS IN ADVANCED RENAL CELL CARCINOMA

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Background: Sunitinib (SUN) is a novel oral multitarget tyrosine-kinase inhibitor (TKI) that has demonstrated its efficacy in the treatment of metastatic renal cell carcinoma (mRCC). The thyroid dysfunction is one of the most common side effects of SUN. The mechanisms inducing thyroid dysfunction are still poorly understood.

Aim: Identify the incidence, severity, ultrasonographic changes and pattern of response of thyroid function tests during treatment with SUN.

Methods: This ongoing prospective observational study to date has completed the evaluation of 25 mRCC patients: 10 women (59±18 yrs) and 15 men (65.5±7 yrs). 5/25 patients received LT4 replacement therapy and 11/25 had thyroid nodules at enrollment. SUN was administered at daily dose of 50 mg (schedule 4/2). Thyroid function tests were assessed at baseline and at week-4 and -6 of each cycle, ultrasound at baseline and after the first and the third SUN cycle.

Results: we observed an increase in TSH values, most frequently after the second cycle of SUN (mean-TSH 17.05±43.56 µUI/mL) and in older men (mean-TSH 91.95±106.4 µUI/mL). TSH rose above normal range (0.35-4.94 µUI/mL) only in patients which were not on LT4 replacement at enrollment. Half of untreated patients had an TSH elevation requiring LT4 replacement after the first cycle; all of them required a further dosage increase after the second cycle. No dose-adjustment was needed in patients already on LT4 at enrollment. In all patients, a volumetric reduction of thyroid lobes occurred at week-6. Ultrasound at week-18, detected the appearance of a hypoechoic solid nodule in one patient and a volumetric increase of pre-existing nodules in two other patients.

Conclusion: SUN is associated with thyroid functional and morphological changes occurring rapidly, within few weeks, in most but not all patients. Distinct individual patterns of response to TKI are identified allowing a better prognosis and

management.

PP221 - MEN 2B AND KARTAGENER SYNDROME IN THE SAME FAMILY, ARE THEY ASSOCIATED?

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Background: Multiple Endocrine Neoplasia 2B (MEN2B) represents 8-15% of MEN2 (prevalence 1:30000) and is characterized by predisposition to medullary thyroid carcinoma (MTC) (85%), pheochromocytoma (50%), mucosal neuromas (100%) and intestinal ganglioneuromas (98%); megacolon and marfanoid habitus (MH) are variably associated with the syndrome. MEN2B is due to a mutation in the intracellular domain TK2 RET (chromosome 10). Kartagener Syndrome (KS) is a subgroup of primitive ciliary dyskinesia, characterized by congenital defect in the functioning of cilia in the airways, situs inversus totalis, chronic sinusitis and bronchiectasis. It is an inherited autosomal recessive disease (prevalence of 1:20-40000).

Case report: a 36 years old woman with dextrocardia and MH come to our out-patient clinic complaining hypertension, diarrhea, and malaise. Her family history was negative for endocrinopathy, including thyroid disease; she had a sister with KS.CT scan revealed bilateral pheochromocytoma and thyroid ultrasound evidenced a multinodular goiter. 24-hour excretion levels of catecholamines and serum calcitonin level were markedly increased. The patient underwent to bilateral adrenalectomy and subsequent total thyroidectomy and central lymphadenectomy: the histology showed a multifocal MTC (pT3mN1b). Genetic test was positive for RET mutation (M918T codon 16) and diagnosis of MEN2B was done. Her sister, affected by KS,was negative for RET mutation.

Discussion: the coexistence of two rare syndromes in two sisters and the presence of some phenotypic characteristics of KS in the patient with MEN2B (dextrocardia) may have a common genetic mutation. To date are known 27 different types of mutations at the origin of the primary ciliary dyskinesia. KS can occur with each of these even if it's more frequently associated with the DNAH11 (7p21) mutation. At the origin of MEN2B there is a point mutation in 95% or a double mutation in 5% of cases (amino acid substitution in the chromosome 10q11.21, M918T or A883F). Furthermore 10 rearrangements of RET gene are known, most of them with PTC and one with PMC1 gene (translocation 8-10), the last one is involved in the ciliary genesis and in the function of the centrosomes.

Conclusions: To our knowledge this is the first report in the literature describing the coexistence of MEN2B and KS in the same family. This observation raised the question as to whether both diseases have a common underlying genetic basis. One or more common mutations involving RET gene and other genes that control neuroectodermal tissue development may be involved. Further studies are needed to assess if the coexistence of these two rare syndromes is not incidental but genetically determined.

PP222 - EVALUATION OF THE GENOTYPE DISTRIBUTION OF VITAMIN D RECEPTOR (VDR) GENE POLYMORPHISMS IN HASHIMOTO'S THYROIDITIS (HT) PATIENTS.

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Besides its effect on bone metabolism, vitamin D exerts immunomodulatory effects via its nuclear receptor. The VDR gene, located on chromosome 12q12–14, harbors several polymorphisms (SNPs), namely FokI, BsmI, TaqI, ApaI, which were reported to be associated with autoimmune diseases, such as type 1 diabetes and Addison disease. Concerning thyroid autoimmunity, conflicting data are available from the literature. In this case-control study, we investigated the distribution of TaqI and ApaI SNPs in HT patients compared to healthy controls. We enrolled 100 unrelated HT patients (87 F, 13 M; mean age±SD 42±16 yr) and 100 sex- and age-matched healthy individuals (88 F, 12 M; mean age±SD 40±13 yr) from the same geographic area. DNA was extracted from whole blood of each subject. The genotype for the two VDR SNPs was determined by the digestion pattern of the amplified DNA fragments using the restriction enzymes TaqI and ApaI,according to the presence (t or a) or absence (T or A) of restriction site. Appropriate pairs of primers were used to generate a fragment of 740 bp of the gene harboring both the TaqI and ApaI SNPs (rs731236 SNP and rs7975232, respectively). The digestion revealed genotype TT , Tt , tt for TaqI and AA, Aa and aa for ApaI. For all DNA datasets, genotype frequencies were in Hardy–Weinberg equilibrium. Genotypic analysis for the two SNPs showed the following distribution in HT patients *vs* controls: for TaqI T/T=38% *vs* 30%; T/t=42% *vs* 49%; t/t=20% *vs* 21% and for ApaI, A/A=31% *vs* 35%; A/a= 53% *vs* 45%; a/a=16% *vs* 20%. Thus, no differences emerged in the genotype distribution of the two SNPs (TaqI p=0.471; ApaI p=0.512). No significant difference between the two groups was found in the allelic frequency distribution too (*cases/controls:* f(T) p=0.617; f(t) p=0.617; f(A) p=0.999, f(a) p=0.999). Finally, linkage disequilibrium between markers and haplotype associations analyses were performed by means of the Haploview. The risk Haplotype "AC" (TaqI: T/T or T/t) + (ApaI: a/a or A/a) was not statistically significant (cases 39.3% *vs* controls 37.8%; p=0.75). These preliminary data seem to rule out a role of the two analyzed SNPs as predisposing factors to HT. However, further studies are needed to evaluate the full pattern of VDR SNPs (including also FokI and BsmI) and the corresponding haplotypic variants.

PP223 - IMMUNOHISTOCHEMICAL EXPRESSION OF ESTROGEN RECEPTOR ALPHA (ERALPHA) AND PROGESTERONE RECEPTOR (PR) IN TWO SERIES OF PATIENTS WITH PAPILLARY THYROID CANCER

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Introduction. Papillary thyroid cancer (PTC) prevalence is nearly three-times higher in females than in males, decreasing after menopause thereafter. The gender difference suggests that growth and progression of PTC might be influenced by female sex hormones.

Objective. To analyze the expression of both estrogen receptor alpha (ERalpha) and progesterone receptor (PR) by immunohistochemistry in 203 patients from Catalonia (Spain) and Sicily (Italy).

Material and methods. Both ERalpha and PR expression was evaluated in paraffin-embedded tumoral tissues of 45 males (M) and 158 females (F) aging 48.2±14.3 yrs and followed up for 7.1±4 yrs.

Results. ERalpha was expressed in 52 (25.6%) patients (41 F, 11 M), PR in 94 (46.3%) patients (75 F, 19 M). ERalpha and PR were co-expressed in 31 (15.3%) patients (27 F, 4 M). There was no gender difference in terms of ERalpha and PR expression (F 25.9% vs M 24.4%; p = NS, and F 47.5% vs M 42.2% p= NS, respectively). The positivity of the Eralpha expression (Eralpha+) was associated, only in women, to a higher PTC size: 22.8±11.8 mm in positive cases versus 15.1±12.4 mm in negative cases; p = 0.02). Among the 148/158 women and the 44/45 men still followed up in our reference centers, 30 women (20.3%) and 9 men (20.4%) had persistence/recurrence (P/R) PTC. Eralpha + was associated, again only in women, to a lower risk of P/R risk. Thus, only 2/30 (7%) patients with P/R were Eralpha+, while 37/118 (31%) of those who did not have P/R were Eralpha + (Chi-square 7.5, p < 0.01). PR expression was not associated with any of the parameters analyzed.

Conclusion. Eralpha expression was found to be increased in tumor in larger diameter but was related with a reduced risk of persistence and/or recurrence of the disease.

PP224 - AGGRESSIVE MEDULLARY THYROID CANCER WITH BILATERAL BREAST METASTASIS: A CASE REPORT

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Medullary Thyroid Cancer is a tumor derived from parafollicular cells. It usually metastasizes to the liver, lungs, and bone. Breast metastases from MTC are very rare and only few cases have been reported in the literature.

Case report: A 76year-old woman underwent total thyroidectomy for sporadic MTC. During the follow up she had persistently elevated calcitonin and CEA levels and developed lung and liver metastases. 12 years after the diagnosis, integrated positron emission tomography with 2-[(18)F]fluoro-2-deoxy-d-glucose (FDG-PET/TC) revealed further progression of the disease. New focal FDG-avid lesions were discovered in the brain and bilaterally in the breasts. Mammography and ultrasonography confirmed the presence of multiple lesions in both breasts. Ultrasound-guided fine needle aspiration cytology (FNAC) showed the presence of malignant cells with an MTC cytomorphology . Immunochemistry confirmed the diagnosis of bilateral breast metastases form MTC.

Clinicians should consider the possibility of breast metastases from MTC in patients with history of aggressive medullary thyroid cancer.

PP225 - L-T4 IN SOFT GEL CAPSULE AND IN ORAL LIQUID FORM IS BETTER ABSORBED COMPARED TO TABLET IN A PATIENT WITH BILIOPANCREATIC DIVERSION.

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Background. Bariatric surgery is a treatment for obesity that along with substantial weight loss causes malabsorption of vitamins, minerals and drugs. In biliopancreatic diversion (BPD) two third of the stomach is removed. The remaining portion is re-connected to the ileum significantly shortening the distance between the stomach and the colon. Reduced drug absorption, especiallly L-thyroxine (L-T4), cyclosporine, phenytoin and rifampin may occur post-bariatric surgery. Individual dose-adjustment and therapeutic monitoring may be required.

Aim and Methods. In a 34-year-old male with subclinical hypothyroidism who underwent BPD 10 years before, we assessed absorption of L-T4 using different formulations of the hormone: soft gel capsule (A), liquid solution (B) and tablet (C), manufactured by IBSA, Lugano, Switzerland. Baseline samples were collected and 150 µg of L-T4 were administered. Blood samples were collected at 1, 2, 4, 8 and 24 hr. The pharmacokinetics parameters were assessed measuring the time to peak FT4 concentration (Tmax), the increase FT4 (ΔFT4) calculated subtracting from the baseline value and the area under the curve (AUC).

Results. All L-T4 preparations reached Tmax at 2 hrs. However, maximum ΔFT4 was much higher for A and B compared with C (2.4, 1.8 and 0.25 pmol/L, respectively). AUC of FT4 were also higher for A and B in comparison to C, although to a lesser extent (AUC=305.1, 301.7 and 275.8, respectively).

Conclusions. In a patient treated with BPD the pharmacokinetic parameters of L-T4 absorption are improved using soft gel capsule and liquid preparations compared to tablet. The stomach, duodenum and the upper part of the jejunum are not sites for L-T4 absorption and, as a consequence, tablets of L-4 may show a delay in this process in BPD patients. Soft gel capsule and liquid formulations show better and faster absorption of L-T4 and should be considered for treatment of hypothyroid subjects undergoing BPD. However, the clinical significance of this finding must be assessed in larger studies.

PP226 - MUCINOUS VARIANT OF FOLLICULAR CARCINOMA OF THE THYROID GLAND: A CASE REPORT

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Mucinous variant of follicular carcinoma of the thyroid gland is a very rare neoplasm of thyroid gland distinct by other histological types of carcinoma showing mucin production such as mucinous variant of medullary carcinoma, primary mucinous carcinoma of thyroid gland and thyroid metastasis of other mucinous tumor. We present a case of a 63-year-old male patient, with incidental evidence of subclinical hyperthyroidism (TSH 0.26 µUI/mL, normal free thyroid hormones). His family history was negative for thyroid diseases and he did not have history of neck radiation. Thyroid ultrasonography showed an homogeneous parenchyma with the presence, at the isthmus, of a solid, hypoechoic, dishomogeneus nodule of 27x12x31 mm, with an internal macrocalcification and poor vascularization. Subsequent blood tests showed negative thyroid antibodies and normal calcitonin value. Then, the patient performed thyroid scintigraphy with 99mTc, which showed a cold nodule with high and dishomegeneus distribution of 99mTc into the parenchyma. At last, the patient performed fine-needle aspiration which documented a cytology compatible with papillary carcinoma, THY5. For this reason, the patient underwent total thyroidectomy with a definitive histological diagnosis of encapsulated microfollicular proliferation and epithelial elements with abundant extracellular basophilic mucinous material. Immunohistochemistry was positive for thyroid-specific-transcription factor 1 (TTF1) and thyroglobulin and negative for calcitonin and chromogranin A, compatible with a minimally invasive mucinous variant of follicular carcinoma. Until now, only seven cases of primary mucinous carcinoma of thyroid gland have been reported and only one case of mucinous variant of follicular carcinoma. Our case of mucinous variant of follicular carcinoma is the second one described in literature and the first one described in a man. This diagnosis highlights the importance of the introduction of this variant of follicular carcinoma on future histopathological classification of the thyroid cancer.

PP227 - RELATIONSHIP BETWEEN CIRCULATING ANTI-THYROGLOBULIN ANTIBODY (TGAB) AND TUMOR METABOLISM IN PATIENTS WITH DIFFERENTIATED THYROID CANCER (DTC): DIAGNOSTICS AND PROGNOSTICS IMPLICATIONS

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TgAb concentrations respond to changes in the mass of Tg-secreting thyroid tissue. It has been suggested that TgAb concentration may have clinical value as surrogate DTC tumor-marker. Recent evidence suggest a link between TgAb titers and DTC aggressiveness. DTC metabolism as assessed by 18F-FDG PET is a well-recognized prognostic indicator.

AIM: to evaluate the relationship between TgAb levels and tumor glucose metabolism in patients with DTC.

Methods: 71 patients with DTC who underwent 18F-FDG PET/CT scan were included present study. Indications for PET were negative iodine-131 whole-body scan and elevated TgAb level and restaging in high risk patients for diagnostic and prognostic purposes. Radioiodine avidity of DTC lesions was established on the bases of recent patients' history (post-therapeutic 131I scan was available for all patients). TgAb levels were measured with ELISA within 15 days from FDG-PET scan. On the bases of the results of FDG-PET, previous post-therapy 131I scans and Tg levels, patients were divided in two groups according to the evidence (ED) or absence (NED) of disease. ED patients were further divided in three subgroups: 1. radioiodine avid with positive FDG PET (PET+/131I+). 2. Radioiodine refractory with positive FDG PET (PET+/131I-) 2. Radioiodine avid with negative FDG-PET (PET-/131I+). Finally mean standardize uptake values of FDG-avid lesions was assessed. T-test was performed to assess the difference between TgAb levels in ED and NED patients as well as between ED one side and PET+/131I+, PET+/131I- and PET-/131I+ subgroups respectively. Linear regression was performed to assess relationship between TgAb on one side and SUVmean in the three subgroup of ED patients.

Results: TgAb levels (IU/L) were: 33 (range 9-117) in NED, 37 (range 2-416) in ED, 94 (range 79-416) in PET+/131I+ ($p<0.003$ with respect to NED) and 24 (range 2-59) in PET+/131I- ($p<0.04$). Only one patient belonged to PET-/131I+ subgroup (Tgab were 47 IU/L). Only in PET+/131I+ subgroup, SUVmean was directly correlated with TgAb levels.

Conclusions: The present results support the role of TgAb levels as surrogate tumor marker in DTC patients (at least in patients with preserved Tg-secreting DTC/radioiodine avidity). Significantly higher TgAb in radioiodine avid DTC with positive FDG-PET and the direct relationship of Tgab with tumor metabolism in this group further suggest that increased TgAb level may have also prognostic implications in the follow-up of DTC patients.

PP228 - HYDROXYTYROSOL EXERTS ANTI-PROLIFERATIVE EFFECTS AND REDUCES EMT PROCESS IN THYROID CANCER CELLS

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Olive oil is a common component of Mediterranean dietary habits. Epidemiological studies have shown how the incidence of various diseases, including certain cancers, is relatively low in the Mediterranean basin compared to that of other European or North America countries. Current knowledge indicates that the phenolic fraction of olive oil has antitumor effects because of its capacity to inhibit proliferation and promote apoptosis in several tumor cell lines, by the activation of diverse molecular signaling pathways. The highest concentrations of phenolic compounds, widely noted for their anticancer properties in olive oil, are the glycoside-Oleuropein, Hydroxytyrosol and Tyrosol. In this study, we evaluated the activities of Hydroxytyrosol (HT) against papillary (TPC-1, FB-2), follicular (WRO) and anaplastic (ARO) thyroid tumor cell lines. MTT assay revealed that in our experimental models, HT inhibited cell growth in a dose dependent manner. Concomitantly, by real-time RT-PCR and western-blot analysis (WB), we observed a reduction of cyclin D1 expression and an up-regulation of cell cycle key modulators p53 and p21 levels. Further experiments lead us to establish that HT treatment reduced cells vitality inducing apoptotic cell death as revealed by PARP cleavage observed by WB analysis and DNA laddering assay showing the presence of DNA fragmentation. Finally, motility assays showed that in our experimental models, the exposure to HT decreased cell motility and induced the up-regulation of key markers of EMT process as Vimentin, N-cadherin and FSP-1 and a down-regulation of epithelial marker E-cadherin. Taken together our in vitro results underline the possibility of include HT in the class of targeted therapeutics to test alone and/or in combination for treatment of refractory thyroid cancers.

PP229 - SELENIUM SUPPLEMENTATION FOR HASHIMOTO'S THYROIDITIS AND SUBCLINICAL HYPOTHYROIDISM

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Hashimoto's thyroiditis is now considered the most prevalent autoimmune disease, as well as the most common endocrine disorder. It was initially described in 1912, but only rarely reported until the early 1950s. The main feature is infiltration with hematopoietic cells,mainly lymphocytes, organized in lymphoid follicles that often show prominent germinal centers. Lately it has been observed an inverse correlation between concentration levels of selenium and the incidence of autoimmune thyroiditis. Selenium deficiency could trigger and maintain autoimmune thyroidits in patients predisposed to the development of the disease. An important deficiency of selenium is associated with increased intraglandular necrosis and increased macrophage invasion.

Aim of our study to evaluate in patients with autoimmune thyroiditis, with and without replacement therapy, the effect of selenium supplementation after one month of treatment on antibody concentration and TSH. Without adjusting the levothyroxine therapy in the group of patients under treatment, it was added a tablet of selenium daily. We enrolled 40 patients, including 8 men and 32 women; mean age 46,6 (± 17,1) 27 patients under Lt4 therapy and 13 untreated. Without making any changes to levothyroxine therapy, one tablet of 100 mg of selenium daily was added. When we divided the patients in group regarding the outcome of TSH after one month, we noticed that in the group with TSH reduction, the mean of age was 46,6 years (15.5 DS), lower Sent from my iPhone of them was assuming farmacological therapy. In the 7 patients in treatment only with Se-Met, the mean of age was lower, suggesting that perhaps the assumption of Semet in monotherapy can be more efficient in younger people. If our findings can be confirmed in large population, selenium supplementation can be a new therapeutic approach for chronic autoimmune thyroiditis. In conclusion, the reduction of selenium in the diet will remain an important factor in the defense mechanisms of oxidative damage associated with autoimmune thyroiditis.

PP230 - STRAIN RATIO ON ULTRASOUND-ELASTOGRAPHY IN THE MANAGEMENT OF TIR 3A NODULES

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Introduction The new classification SIAPEC 2014 subdivided the category of thyroid nodules with indeterminate (TIR 3) fine-needle aspiration cytology (FNAC) to reduce the percentage of surgery indication, given the wide variability in risk of malignancy. TIR 3A, low (5-15%) risk follicular lesions are heterogeneous group, which includes microfollicular pattern or Hurtle cells, poor colloid and cytological abnormalities too slight to be included in the TIR 4 category. TIR 3B (follicular proliferation or suspected follicular neoplasm) had a high-expected risk of malignancy (20-30%). While the management for TIR 3B involves surgery, in the case of TIR 3A is indicated to repeat FNAC and reassess the case according to clinical and imaging data. **Objectives** To assess whether Elastography increases the characterization of TIR 3A cytological class in thyroid nodules aspirates, indicating the proper management. **Methods** 15 thyroid nodules with FNAC consistent with low risk follicular lesions (TIR 3A) were evaluated with color-Doppler ultrasound (US) and Elastography software (Philips) with a 5-12 MHz linear array probe. Strain ratio (SR) between the unaffected parenchyma and the nodule was calculated setting two identical regions of interest. Diagnosis on surgical specimen was available only in 9/15 cases. Nodules were excluded from this study when raw data were unavailable. **Results** 10/15 TIR 3A nodules were included in the analysis. Elastography showed a mean SR of 1.51±0.85. US defined 37.5% as hypoechoic nodules, 37.5% as isoechoic, 25% as dishomogeneous; 50% of nodules presented the anechoic halo. There was an inverse correlation between SR and microfollicular structures ($r=0.52$, $p=0.044$), suggesting that in our sample a lower SR provides a sensitive estimate of this cytological pattern. 12.5% of patients had Hashimoto's thyroiditis. 5/10 nodules had also histological diagnosis: 3 follicular variant of papillary thyroid carcinoma (PTC): all presented cytological abnormalities consistent with a suspicion of malignancy, 2 had lower SR (0.88) and a diameter <2 cm, while 1 with diameter >3 cm showed a higher SR (2.17). The others two cases were 1 Hashimoto's thyroiditis and 1 benign hyperplasia both with higher SR (2.71 and 2.20 respectively). **Conclusions** Elastography could be a useful additional tool improving diagnostic accuracy of TIR 3A thyroid nodules < 2 cm in diameters. The contemporary presence of several cytological features of suspicion in combination with a lower SR in a small nodule should indicate a higher risk of malignancy in TIR 3A.

PP231 - PREVALENCE OF THYROID CANCER IN PATIENTS WITH HASHIMOTO'S THYROIDITIS WITH OR WITHOUT THYROID NODULES AT THE FIRST CLINICAL EVALUATION AND IN THE SUBSEQUENT FOLLOW-UP

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BACKGROUND: Several surgical and pathological studies suggest a significant association between Hashimoto's thyroiditis (HT) and thyroid cancer, but this relationship is still elusive and often not confirmed by non-surgical series. In this study we compared the prevalence of thyroid cancer in HT associated to thyroid nodules *vs* HT without thyroid nodules at the first diagnosis and during the subsequent follow-up.

PATIENTS AND METHODS: A total of 484 patients with a diagnosis of HT at their first clinical evaluation were subdivided into two groups: 243 HT patients without thyroid nodules (HTN-) and 241 with one or more nodules (HTN+). FT3, FT4, TSH Tg/TPO-autoantibodies and thyroid ultrasound were performed in all cases; fine needle aspiration cytology (FNAC) were performed in 155 patients with thyroid nodules and 74 patients underwent surgery.

RESULTS: HTN+ patients displayed a high prevalence of suspicious/malignant cytology (Tir 4-5) (44/152 = 28.9%); indeterminate (Tir-3) cytology was found in 47/152 (30.9%). In the 74 HTN+ patients submitted to thyroidectomy, a high rate of malignancy was documented (48/74 = 64.9%). The histotype distribution was 44 papillary (PTC), 2 follicular (FTC), 2 medullary (MTC) thyroid carcinomas, PTC being the only tumor significantly associated to HT. A high prevalence of multicenter PTC was also found (14.4%) in this group. In the HTN- group, a minority (22/130 = 17%) of patients with available follow-up (1-9 years) developed one or more thyroid nodule, but only 3 of them required FNAC which resulted benign (Tir-2) in all cases. None of these patients was submitted to thyroidectomy.

CONCLUSIONS: This study confirms a high prevalence of thyroid cancer (mostly PTC) in patients with HT associated to thyroid nodules at the first clinical evaluation. Conversely, no evidence of increased prevalence of thyroid malignancy was found in patients with HT without associated nodules at the first clinical evaluation during the first years of follow-up. Although this observation requires further investigations, it might provide a clue to understand the marked differences in the association rate between HT and thyroid cancer reported in surgical and not-surgical studies.

PP232 - THYROID CANCER STEM CELL RENEWAL IS REGULATED BY P63

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Background. *p63* is a member of the *p53* tumor suppressor gene family. p63 is a transcription factor involved in stem cell renewal, cell lineage choices, and maintaining the balance between cell proliferation and differentiation. p63 expression is up-regulated in thyroid cancer where it increases cancer cell survival and resistance to p53 driven apoptosis.

Objective. This study aimed at evaluating the role of p63 in regulating human thyroid cancer stem cell biological proprieties (cancer stem cells - CSCs).

Method. Thyroid cancer stem cells derived from human thyroid cancer specimens and cell lines were cultured as three-dimensional spheres under stem-cell conditions (CSCs). Standard monolayer cultures were used as controls. In those cell model p63 expression and function was investigated.

Results. qRT- PCR revealed that p63 expression was significantly up-regulated in CSCs spheres compared to the non-CSCs counterpart by 48,50 ± 3,2 fold. To determine whether the changes in expression levels of p63 were affected by epigenetic mechanisms, we performed Chromatin immunoprecipitation (ChIP) experiments. We observed that the expression levels of p63 were correlated with higher H3 acetylation levels. Interestingly, treatment of CSCs with histone deacetylases (HDAC) inhibitors strongly decreased levels of p63. Furthermore, we observed that down regulation of p63 expression by targeted siRNA or HDAC inhibitors reduced sphere-forming efficiency by 20% and 50%, respectively.

Conclusions. 1) p63 expression is up-regulated in thyroid cancer and its expression is associated with cancer stem cell propierties. 2) p63 expression in thyroid cancer stem cells is positively regulated by histone H3 acetylation. 3) p63 plays a crucial role in self-renewal maintenance of thyroid CSCs.

PP233 - HASHIMOTO'S THYROIDITIS AND THYROID CANCER: A CASE-CONTROL STUDY OF UNSELECTED PATIENTS WITH THYROID NODULES SUBMITTED TO CYTOLOGICAL AND HISTOLOGICAL EXAMINATION

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BACKGROUND: The association between thyroid autoimmunity or Hashimoto's thyroiditis (HT) and thyroid cancer has been often suggested in many reviews but the relationship of this phenomenon is still debated. Aim of this study was to confirm this association in a retrospective series of patients who underwent fine needle aspiration cytology (FNAC).

PATIENTS AND METHODS: The study group consisted in 152 patients with thyroid nodules associated to HT (HT+) diagnosed on the basis of ultrasound, serological (positive serum Tg-/TPO autoantibodies) and functional (increased serum TSH) criteria and 161 patients with thyroid nodules matched for age and gender and selected for the absence of any evidence of associated thyroid autoimmunity (HT-), representing the control group. Thyroid ultrasound and FNAC were performed in all cases. A subgroup of 111 patients underwent total thyroidectomy.

RESULTS: HT+ patients had higher prevalence of suspicious/malignant cytology (Tir 4-5) (HT+ 44/152 = 28.9%; HT- 12/161 = 7.4%, $p<0.0001$) compared to HT- patients. A similar prevalence of indeterminate (Tir 3) cytology was found in HT+ (47/152 = 30.9%) and in HT- (45/161 = 28%).

In the group submitted to thyroidectomy, malignant histology was found in 48/74 (64.9%) HT+ and in 16/37 (43.3%) HT- patients ($p<0.05$). The histotype distribution was 44 papillary (PTC), 2 follicular (FTC), 2 medullary (MTC) thyroid carcinomas in HT+ and 13 PTC, 2 FTC, 1 MTC in HT- patients, PTC being the only tumor significantly associated to HT. Taken together, a significantly higher number of histologically proven PTC was detected in HT+ (44/152 = 28.9%) vs HT- (13/161 = 8,1%, $p<0.0001$) patients originally submitted to FNA. Interestingly, the prevalence of multicentric PTC was significantly higher (14.4%) in HT+ than in HT- (5.6%) patients ($p=0.0085$).

Finally, in the 70 surgical specimens with significantly diffuse or perinodular lymphocytic thyroid infiltration, a higher prevalence of PTC (46/70, 65.7%) was observed as compared to benign nodules (24/70, 34.3%; $p<0.05$).

CONCLUSIONS*:* This study strongly confirms a significant association between HT and PTC based on both clinical and pathological results.

PP234 - UNEXPECTED ELEVATED FREE THYROID HORMONES IN PREGNANCY

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We present the case of a 35-years woman referred to us at 26 week of her second pregnancy. She had a previous diagnosis of autoimmune chronic thyroiditis about 5 years before; a physiological pregnancy with levothyroxine replacement therapy and natural childbirth about 2 years before; no other significant previous medical history. During our visit the patient was clinically euthyroid as expected by TSH value (0,79 mcIU/ml), but laboratory tests showed elevated values of free thyroid hormones, both fT3 (13,2 ng/l; normal range: 1,8-4,6) and fT4 (65,6 ng/l; normal range: 9,3-17) determined by 1-Step chemiluminescent assay. The patient was taking 125 mcg/daily levothyroxine replacement therapy and the previous blood tests (18 week of gestation) showed concordance between TSH and free thyroid hormone values. Anti-thyroid peroxidase antibodies and anti-TSH receptor antibodies were not detectable. Based on clinical and TSH value we confirmed the current levothyroxine treatment. We followed up the patient during all the pregnancy: free thyroid hormones were constantly elevated (in 2 controls, up to 34 week of gestation) using one-step methods: however, the values of total thyroid hormones did not exceed the normal range, in agreement with TSH values. Post partum fT4 and fT3 values returned progressively to normality (in agreement with TSH value), almost completely in about 1 year. We hypothesize the presence of thyroid hormone autoantibodies (THAb) that can interfere, although to a variable extent, with thyroid hormone one-step assays; this explanation was supported by the determination of both fT4 and fT3, on stored patient sera, by a 2-Step chemiluminescent assay (Architect), which gave invariably normal results (at 30 week of gestation fT4=1,17 ng/dl normal range 0,7-1,48; fT3 2,08 pg/ml normal range 1,71-3,71). Despite their relative rarity, autoantibodies causing interference may be suspected when laboratory data are not consistent between each others, nor compatible with the clinical picture. In literature different conditions have been associated with transient THAb (therapy with interferon alpha; viral infections), but to our knowledge no previous case of transient appearence of THAb in pregnancy has been described.

PP235 - ROLE OF ULTRASONOGRAPHY, FINE NEEDLE ASPIRATION BIOPSY AND BRAF ANALYSIS IN THE MANAGEMENT OF INDETERMINATE LESIONS DURING A 5 YEARS FOLLOW-UP.

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The management of cytologically indeterminate nodules is still a matter of great debate. A better characterization is needed to associate the correct risk of malignancy to Bethesda class III and IV lesions in order to follow the best clinical approach, possibly avoiding unnecessary surgery. To explore this issue, we evaluated 366 indeterminate nodules (296 class III and 70 class IV lesions), all tested for BRAFV600E mutation. Among these, 140 underwent a second fine needle aspiration biopsy (FNAB), that allowed to categorize as benign 70% and as malignant 3% of class III lesions, respectively; 75% of class IV lesions were confirmed as such, with 25% benign lesions. In total, 239 patients underwent surgery. Risk of malignancy (RM) was calculated among class III lesions, that had been divided into 9 cytological sub-categories, as follows: microfollicles in a sparsely cellular aspirate with scant colloid (group 1, n=97, RM=30%, BRAF+ = 6.2%); Hurtle cells in a sparsely cellular aspirate with scant colloid (group 2, n=10, RM=50%, BRAF+ = 40%); mild follicular cell atypia with drying or clotting artifacts (group 3, n=1, BRAF+ =0); Hurtle cells in a cellular aspirate (group 4, n=26, RM=15.4%, BRAF+ = 0); focal features of papillary carcinoma in an otherwise predominantly benign appearing specimen (group 5, n=45, RM=65%, BRAF+ = 40%); cyst-lining cells with nuclear atypia in an otherwise predominantly benign-appearing specimen (group 6, n=12, RM=50%, BRAF+ = 33.3%); follicular cells with regenerative atypia (group 7, n=2, RM=50%, BRAF+ =0); atypical lymphoid infiltrate (group 8, n=1, RM=100%, BRAF+ =0); not otherwise categorized (group 9, n=3, RM=100%, BRAF+ =0). The 127 nodules (99 class III and 28 class IV) that did not undergo surgery were followed up for 5 years with yearly ultrasound (US) and TSH levels. Most of the nodules (86.8% class III, mostly group 6, and 75.5% class IV) did not change or decreased in diameter at the last US control. Among the 13 class III nodules which diameter increased, 70% belong to group 1. US features and TSH levels did not correlate with growth pattern over time. In conclusion, a second FNAB is not useful to better categorize class IV nodules, that tend to grow, suggesting that surgery is an appropriate approach. On the contrary, BRAF analysis and repeated FNAB are useful to select class III nodules displaying a low RM, since they do not tend to grow and may be followed conservatively over time.

PP236 - CONGENITAL HYPOTHYROIDISM (CH) WITH PROPERLY LOCATED GLAND AND ISOLATED HYPERTHYROTROPINEMIA (IH) IN CHILDREN AND ADOLESCENTS: CLINICAL, BIOCHEMICAL AND MOLECULAR ANALYSIS AT DIAGNOSIS AND AFTER RE-EVALUATION

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In recent years increasing cases of CH with properly located and normal sized thyroid are identified. When slightly higher TSH values are found after perinatal age, in absence of anti-TG and anti-TPO Ab in patients with normal thyroid gland, IH is diagnosed. About the need to treat with L-thyroxine (LT4) these mild forms there is no complete agreement.

We evaluated 131 patients aged under 18 years, 64 with CH with normal thyroid gland and 72 with IH. We evaluated the need for LT4 therapy, after discontinuation of it, and performed perchlorate test in most patients. We evaluated the evolution of thyroid function during controls. In these patients was also performed genetic analysis of DUOX2 and TSHr genes.

At the end of follow-up, 65 patients (49.6%) assumed LT4 therapy, while 66 (50.4%) were not taking it. Among the 59 patients with CH, at the end of follow-up, 37 patients were receiving therapy with LT4 (62.7%), and 22 patients (37.3%) had discontinued therapy; of all patients with IH, 28 were receiving therapy with LT4 (38.9%), while 44 (61.1%) were not receiving it. Among patients with CH, 51 performed clinical reassessment: 11 had overt hypothyroid (21.6%), 25 showed hyperthyrotropinemia (49%) and 15 were euthyroid (29.4%). Among patients with IH who had started treatment with LT4, 27 underwent to revaluation: one showed an overt hypothyroidism, 14 patients confirmed hyperthyrotropinemia and 12 were euthyroid. 2.2% of the patients showed the appearance of TG-Ab during checks.

Genetic analysis revealed: 4 polymorphisms, a mutation in compound heterozygosity, a point mutation and a deletion of the DUOX2 gene; 1 point mutation and 2 polymorphisms of TSHr gene. The patients with the same mutation showed a different phenotype.

In conclusion, a half of patients in our study were taking LT4 therapy at the end of follow-up. A high percentage of patients with CH underwent to discontinuation of therapy with LT4, and only a minority of cases of IH, thyroid function evolved towards hypothyroidism. Mutations in DUOX2 gene were the most frequent, however, children carrying the same mutation had a different phenotype, suggesting that other factors are responsible of the clinical characteristics.

PP237 - IN PATIENTS WITH AUTOIMMUNE THYROIDITIS AND ANTI-GASTRIC PARIETAL CELL AUTOANTIBODIES, SERUM PEPSINOGEN I AND GASTRIN-17 LEVELS ARE PREDICTABLE OF ATROPHIC GASTRITIS

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Background and aim: Chronic autoimmune thyroiditis (CAT) can be associated with autoimmune atrophic gastritis and approximately 30% of patients affected by CAT exhibit anti-gastric parietal cell antibodies (APCAs). However, level of APCAs does not represent a marker of disease. The diagnostic gold standard for atrophic gastritis is histology from gastric body/fundic mucosa. Recently, ELISA test to evaluate blood levels of pepsinogen I (sPGI), pepsinogen II (sPGII) and antral gastrin (sG17) has been considered promising

to predict atrophic gastritis.To evaluate the correlation between serum PGI, PGII, G17 levels and the histological diagnosis of atrophic gastritis in patients with CAT and APCAs.

Material and methods: Twenty-three patients with CAT were enrolled. All patients reported medical history. All patients underwent: blood sampling to evaluate thyroid hormones and antibodies (TPO-Ab and TG-Ab), APCAs, sPGI, sPGII, sG17 and Hp antibodies (ELISA test); urea breath test; upper endoscopy with biopsy of gastric antrum, corpus, fundus and mucosal breaks or other abnormalities if present.

Results: 6 males and 17 females, mean age 48.3±14.9 yrs. 21 patients were APCAs positive and 2 were APCAs negative. APCAs negative patients had normal gastric mucosa; APCAs positive patients were divided into two groups according to histological examination outcome: patients with body/fundic atrophic gastritis (CAG; n=15), and patients with non-atrophic gastritis (NAG; n=6). sG17 levels were significantly higher in patients with CAG (median 60.2 pmol/L, interquartile range 55.3-63.8 pmol/L) than in patients with NAG (median 0,9 pmol/L, interquartile range 0.4-3.5 pmol/L) (p=0.006). sPGI levels were significantly lower in patients with CAG (median 7.0 µg/L, interquartile range 62.9-94,5 µg/L) (p=0.002). sPGII levels were not significantly different between the two groups.

Conclusions: sPGI and sG17 levels allow identifying atrophic gastritis in patients with CAT and APCAs. Given the high association frequency of these two conditions, these markers could select patients, more likely affected by atrophic gastritis, to undergo upper endoscopy and gastric histological examination.

PP238 - RESULTS OF THYROGLOBULIN MEASUREMENT AFTER RECOMBINANT HUMAN THYROTROPIN DURING THE FIRST YEAR OF FOLLOW UP IN LOW-RISK WELL DIFFERENTIATED THYROID CANCER

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Background: In low risk thyroid cancer patients who have had remnant ablation and are clinically free of disease under suppressive L-T4 therapy , there is good evidence that a Thyroglobulin (Tg) level >2 ng/ml following rh-TSH stimulation is highly sensitive in identifying patients with persistent disease.

Objectives: The aim of this study was to verify whether rh-TSH stimulated Tg levels <2 ng/ml during a 12 month follow-up accurately predict the absence of detectable disease in 166 consecutive low risk papillary thyroid carcinoma patients treated with surgery and radioiodine ablation (RAI).

Patients and methods: All patients (124 F, 42 M, mean age 52 yr, range 23-77 yr) were clinically free of disease with serum Tg levels <1 ng/ml on suppressive L-T4 therapy. Patients underwent US neck examination and anti-Tg antibody (ATG) measurement.

Serum Tg levels were obtained at baseline and on day 3 and 5 after the first of 2 consecutive injections of 0.9 mg of rhTSH, in accordance with standard protocols. Patients were considered as disease –free when they did not have RAI uptake outside the thyroid bed in post-therapy whole-body scan and had a normal neck US and a stimulated Tg <2 ng/ml after 1 year.

Results: Tg levels <2 ng/ml after rh-TSH in the absence of ATG were observed in 97% of the patients. Among these 161 patient, however, cervical lymphnode metastases were detected by US in 1 patient displaying rh-TSH stimulated Tg levels <1 ng/ml. Five patients (3%) had Tg levels >2 ng/ml after rh-TSH. In 3 of them US examination identified cervical lymphnode metastases, then confirmed by cytology and by pathology. The other 2 patients had a negative neck US, negative imaging for distant metastases and are under follow-up.

Conclusions: Serum rh-TSH stimulated Tg levels <2 ng/ml accurately predict the absence of disease recurrence or persistence during follow-up of thyroid cancer patients treated with surgery and RAI. Neck US examination plays a pivotal role, since it is highly sensitive in detecting node metastases also in patients with undetectable basal and rh-TSH stimulated Tg levels. Both evaluations are mandatory for an accurate follow-up of low risk papillary thyroid carcinoma patients.

PP239 - EFFICACY AND DURABILITY OF RADIOFREQUENCY ABLATION FOR BENIGN THYROID NODULES: A 2 YEARS FOLLOW- UP

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Objectives: Percutaneous radiofrequency thermal ablation (RFA) was reported as an effective tool for the management of thyroid nodules (TNs) but only few data on long term follow-up is available. The aim of this study was to prospectively evaluate the volume reduction of benign thyroid nodules two years after a single session of RFA performed by “moving-shot technique”.

Methods: 40 patients with large thyroid nodules (median volume 21,7 ml, IQR 14,8-35,4) were enrolled. In all patients, a thyroid malignancy was ruled out by repeated fine-needle aspiration cytology. All patients underwent clinical, biochemical and US evaluation at baseline and 3,6,12 and 24 months after RFA; 5 of them were hyperthyroid at baseline, receiving treatment with methimazole.

Results: a significant volume reduction was observed since the first follow-up visit (median volume at 3 months 11,8 ml, IQR 6,7-21-4, $p < 0,001$); most interestingly, a trend towards a progressive reduction was shown at 6,12 and 24 months (median volume respectively of 9, 8,7 and 7,7 ml). The overall volume reduction at 1 year was 64,5% (IQR 54,4-79,1). The volume reduction at 24 months was statistically significant when compared to that recorded at 3 months (p 0,039). At 2 year follow-up all hyperthyroid patients were still taking methimazole, although the dose was half or less than that before RFA. RFA was safe and well tolerated in all patients without any significant side effect.

Conclusions: This trial shows good efficacy of RFA in reducing volume of benign solid thyroid nodules, with improving effects over time. RFA may represent a valid therapeutic approach in patients with TNs not receiving conventional treatments.

PP240 - EXPRESSION OF P53 ISOFORMS IN MULTINODULAR THYROIDS: THEIR IMMUNOREACTION IN BENIGN LESIONS CORRELATES WITH SUBCELLULAR PHENOTYPES AT ELECTRON MICROSCOPY.

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P53 isoforms modulate cell proliferation and cell fate outcome in response to DNA damage. There are 12 P53 isoforms as a result of alternative initiation of P53 translation through use of an internal promoter (P2) located in intron 4 and alternative splicing of intron 9. To examine the immunoistochemical expression of P53 isoforms in benign proliferative lesions occurring in multinodular thyroids and to assess the subcellular phenotype of P53 isoforms distribution by electron microscopy (EM), we examined 38 thyroid glands showing a goitrous appearance associated with one single lesion in 12 cases and with two lesions in the remaining 26 cases. By immunohistochemistry (IHC) and EM, a total of 102 lesions were analyzed: 38 nodular goiters (NG), 52 follicular adenomas (FA) and 12 Hashimoto's thyroditis-associated oncocytic hyperplasias (OHHT). FA was classified in normo-follicular (NF-FA, n=10), macro-follicular (MaF-FA, n= 9), micro-follicular (MiF-FA, n=28) and solid (S-FA, n=5) variants. Immunoreactions for P53 isoforms was observed in near 50% of multinodular glands. EM analysis of individual nodular lesions revealed different subcellular phenotypes corresponding with the expression of P53 isoforms. NG: P53 isoforms were expressed in 60% of colloid nodules and 56% of parenchimatous lesions. Reactive NG showed increased irregularity of nuclear membrane, dispersed chromatin, increased amount of cytoplasmic organelles and rough endoplasmic reticulum. Cell basal borders were prevalently infolded. FA: P53 isoforms were expressed in 50% of NF-FA, 33.4% of MaF-FA, 57.1% of MiF-FA and 60% of S-FA. Nuclear membrane was irregular in Ma-FA, Mi-FA and S-FA, whereas it did not show irregularities in N-FA. The chromatin was prevalently dispersed in all cases. Except for N-FA, subcellular organelles and RER were increased in all FA. Cell basal borders were infolded in about 50% of cases. An increased expression of P53 isoforms was observed in the majority of OHHT. The ultrastructural phenotype was similar to FA. In conclusion, immunodistribution of P53 isoforms in NG, FA and OHHT suggests their role in the development of these lesions. The ultrastructural findings support the hypothesis that P53 isoforms immunoexpressions correlate with reactive proliferative changes in the thyrocytes.

PP241 - EFFECT OF A VERY LOW DOSE OF RITUXIMAB ON ACTIVE MODERATE-SEVERE GRAVES' ORBITOPATHY (GO): AN INTERIM REPORT.

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Previous studies have shown that Rituximab (RTX) is effective as a disease modifying drug at doses of 500 mg or 1000 x2 mg in active GO. We have conducted a pilot study (EUDRACT 2012-001980-53) in which patients with active moderate-severe GO were treated with a single infusion of low dose RTX (100 mg). Ten patients were enrolled of whom seven completed the study at 52 weeks. Five patients did not respond to a previous treatment with i.v. methylprednisolone, whereas five had newly diagnosed GO. Disease activity was assessed with the clinical activity score (CAS) and severity with NOSPECS score. The primary endpoint was the decrease of the CAS of 2 points or CAS≤3. **Results**: of the seven patients who completed the study, six had inactive disease at 12 weeks (ANOVA P=0.01); one was submitted to surgical orbital decompression because of signs of optic neuropathy (ON). Another patient, although who had disease inactivation at 12 weeks, underwent surgical orbital decompression at 22 weeks because of suspected subclinical ON. At 24 weeks four patients were inactive (ANOVA P=0.001); one had a transient disease reactivation with stabilization at 40 weeks. The treatment was well tolerated with only minor infusion-related reactions.

Conclusion: very low dose of RTX seems effective in disease inactivation but may not modify the natural course of disease as has been observed with higher therapeutic doses.

PP242 - THYROTOXYCOSIS INDUCED BY IODINATED CONTRAST MEDIA IN EUTHYROID PATIENTS WITHOUT KNOWN THYROID DISEASE: BURDEN, PREDICTORS AND FOLLOW-UP OF HYPERTHYROID PATIENTS

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Aim of the study. We studied the occurrence of thyroid functional changes after administration of iodinated contrast media (ICM) for coronary angiography in euthyroid patients without known thyroid disease. We then studied the evolution of thyroid function in the group of subjects become hyperthyroid (TSH <0.3 μU/ml) up to 7 years after the administration of ICM.

Subjects and methods. Since August 2006, 931 consecutive cardiac patients with indication for coronary angiography, without known thyroid disease or interfering treatment on thyroid function, not submitted within the three months before the study to ICM procedures, underwent the evaluation of thyroid function (TSH, fT3, fT4), and morphology (ETG). At 30 days after the procedure measurement of TSH, fT3, fT4 was repeated. The 829 euthyroid patients, 608 men (73.3%), aged 65.7 ± 10.3 years, were admitted to the study.

Results. After ICM 54 subjects (6.5%) developed thyrotoxicosis (50 subclinical and overt 4). Nodular thyroid disease (OR: 4.7, 95% CI 2.5-8.5), the amount of ICM greater than 340 ml (2.1, 1.1-3.9) and male sex (2.8, 1.2-6.6) were independent predictors of development of thyrotoxicosis. The follow-up (average 55.32 months) in order to assess the cumulative incidence of return to euthyroidism was possible in 43 patients, 38 (88.4%) males, 67.1 ± 8.0 years; months "at risk" were 1272, 38 (88.4%) patients normalized thyroid function; 8 died.

At follow-up, thyrotoxicosis was almost basically silent. Three patients were transiently treated with methimazole for symptoms and comorbidities; these cases were considered thyrotoxic throughout the treatment period. The cumulative incidence of euthyroidism during follow-up was 50% at 12 months. The return to euthyroidism was significantly (log rank test, $p<0.03$) more slowly in the female population, and (but not statistically significant) in patients either younger or with thyroid nodules.

Conclusions. Our data suggest that after ICM by coronary angiography the appearance of thyrotoxicosis in patients previously euthyroid without known thyroid disease, affects a small but not negligible number of cases. Thyrotoxicosis is usually asymptomatic but the exposure to increased thyroid hormone levels seems quite long (especially in women) to constitute a potential clinical problem in patients with heart disease.

PP243 - STUDY OF THE INTERFERENCE BY DRUGS ON THE INTESTINAL ABSORPTION OF LT4 AND METABOLIC/CARDIOVASCULAR CONSEQUENCES IN THE SETTING OF GENERAL PRACTITIONERS (GP)

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Monitoring of LT4 therapy for both replacement (RT) and suppressive (ST) purposes is based on periodic assays of serum TSH. The higher is serum TSH the higher are the chances of associated metabolic syndrome/diabetes mellitus (DM) and/or cardiovascular risk. Certain orally-ingested medications may impair the intestinal absorption of LT4 (IAT).

In the setting of the GP Medicine, we wished to assess the magnitude of: a) patients (pts) under LT4 therapy; b) co-ingestion of drugs that interfere with the IAT (DIAT); c) consequences of TSH for not being on target (outcomes).

We screened the clinical records of 4330 pts and found that 375 (8.7%) had been under RT or ST with tablet LT4 for ≥1 year. Of these 375, 155 (41.3%; 3.6% of 4330) co-ingested ≥1 DIAT. Of these 155, 65 (59 F, 6 M; age=63.9±11.6 yr) had: a) repeat TSH measurements from the same lab in each patient; b) a minimum of 2-yr follow-up after the first documentation of DIAT-induced LT4 malabsorption. Statistics was by ANOVA and χ^2 tests.

The DIAT were: proton-pump inhibitors (n=55, 84.6% of 65), ranitidine only (n=1, 1.5%), ferrous salts only (n=4, 6.1%), calcium salts only (n=3, 4.1%), nonabsorbable antacids only (n=2, 3%). In the 65 pts, TSH averaged 1.17±1.4 mUL when off DIAT, but 3.15±4.4 (P<0.0001) when on DIAT; when on DIAT, 21% of TSH measurements were ≥4.0 mU/L. Within the 2-yr follow-up, any of the outcomes [hypertension, DM, dyslipidemia, cardio/cerebrovascular (CCV) accidents, or worsening of any of them] occurred in 40 (61.5%). In contrast, upon matching the 65 pts with 74 thyroid disease-free controls (66 F, 8 M; age 63.1±12.0 yr), outcomes occurred in 29/74 (39.1%, P=0.009). During follow-up, TSH was 3.57±5.1 on DIAT (*vs.* 1.25±1.6 off DIAT, P<0.0001) in the 40 pts who had the outcomes. By contrast, in the 25 pts with no outcomes, TSH was less increased (2.07±1.6 on DIAT *vs.* 1.03±1.0 off DIAT, P=0.0002). TSH levels ≥4.0 mU/L were observed in 27% of the measurements in the 40 pts compared with 6% in the 25 pts (P=0.009).

If confirmed nation-wide, ~4% of the pts under the care of GP take both LT4 and DIAT. Even a serum TSH of 3.0-4.0 mU/L carries important metabolic and CCV consequences, which will occur in almost 2/3 of such pts in 2 years. In contrast, TSH levels <2.5 mU/L protect from those consequences. GP should promptly manage failure of TSH to be fully normalized by, for instance, switching to or already starting with novel DIAT-refractory formulations of LT4.

PP244 - PRECONCEPTIONAL TSH AND MISCARRIAGE IN INFERTILE WOMEN SUBMITTED TO IN VITRO FERTILIZATION

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It is known that high levels of TSH are associated with higher miscarriage risk, though the precise TSH cut-offs are debated. Aim of the present study was to evaluate if pre-conceptional TSH levels associate with increased risk of early miscarriage in a large series of infertile women submitted to in vitro fertilization (IVF) and to determine the threshold of TSH associated with the highest prevalence of pregnancy loss. We retrospectively studied 1484 infertile women (mean± age 36.7±4.1 years, mean± SD BMI 22.7±4) submitted to IVF in a single center from 2004 and 2014. Overall, 371/1484 (25%) patients had a biochemical pregnancy and 152 of them experienced a pregnancy loss. Mean TSH levels of women with regular pregnancy were significantly lower than mean TSH levels recorded in patients with a pregnancy loss (1.8±0.8 *vs* 2.2±1.2, P=0.01). Interestingly, the miscarriage rate was progressively higher for increasing TSH cut-off levels (≤2.5 *vs* >2.5 P=0.08; ≤3 *vs*>3 P=0.001; ≤4.5 *vs* ≥4.5 P=0.004). Moreover, among the 152 women with pregnancy loss, 59 (39%) were clinically pregnant and had a miscarriage in the first trimester, while in 93 patients (61%) a biochemical pregnancy without clinical evolution was documented, but no significant differences in mean TSH levels were recorded between women with different time of miscarriage. In conclusion, in women undergoing IVF, lower TSH levels reduce the risk of early pregnancy loss. These data strongly indicate the need for TSH screening prior to IVF procedures, and suggest the treatment of women with TSH levels >3 mU/l.

PP245 - EFFECT OF THE ADMINISTRATION OF IODINIZED CONTRAST MEDIA ON THYROID FUNCTION OF HYPERTHIROID VS EUTHYROID SUBJECTS. A STUDY ON A LARGE POPULATION OF PATIENTS UNDERGOING CORONARY ANGIOPLASTY

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It is known that the administration of iodinated contrast media (ICM) is able to determine significant alterations of thyroid function, although in a small percentage of euthyroid patients. Instead, little evidence exists on the effect induced by the ICM in patients with thyrotoxicosis.

Aim of the study. Impact of pre-existing thyrotoxicosis on changes in thyroid function of patients with heart desease who underwent coronary angiography.
Subjects and methods. Since August 2006, in 931 patients with heart disease undergoing coronary angiography, without known dysfunctional thyroid disease, not receiving ICM in the three months prior to enrollment, we evaluated thyroid function (TSH, fT3, fT4), and thyroid morphology by US. After 30 days from the procedure TSH, fT3, fT4 was measured again.

Results. At baseline 829 patients (89%) were euthyroid (EUT, 608 males (73.3%), age 65.7±10.3 years), 67 were hyperthyroid (IXT, 7.1%, 49 males (73.1%), 68.6±7.7 years), 61 subclinical, 6 overt. 35 hypothyroid subjects were excluded from the study.
After ICM, 8 IXT showed overt thyrotoxicosis, even if poorly symptomatic; 26 returned to euthyroidism. In IXT there was an increase in circulating levels of fT4 in 27 subjects (40%) vs 289 (34.8%) in the EUT; mean increase of fT4 was not significantly greater than EUT (mean ± SD: 0.7 ± 3.6 vs 0.5 ± 5.3 ng/L; p=0.73). The number of post-ICM IXT with increase of fT4 levels ≥ 1 ng/L or ≥ 2 ng/L was not elevated (27 and 15 patients, 40 and 22% respectively) and was not significantly (p=0.71 and p=0.37) different than that recorded in the EUT (289 and 170 patients, 34.8 and 20.5% respectively). In IXT male sex and the amount of ICM were positively related to 'increase fT4≥2 ng/L, but at multivariate analysis the only independent significant predictor was male sex (OR: 7.9; 95% CI: 1.4-43.1, p = 0.01).

Conclusions. Coronary angiography with iodinated contrast induces a significant increase in thyroid function in a small percentage of patients with hyperthyroidism. Moreover, this increase is not significantly different from that observed after the procedure in the population of euthyroid patients

PP246 - CLAUDIN 1 AND 7 IN PAPILLARY THYROID CARCINOMA: INTEGRATING GENE AND PROTEIN EXPRESSION DATA.

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Introduction: Claudins are a large family of integral membrane proteins crucial for tight junction (TJs) formation and function. TJs are involved in the maintenance of cellular polarity and homeostasis and by their capacity to recruit signaling proteins may regulate cell proliferation, differentiation and tumorigenesis. The abnormal regulation, either increased or decreased expression levels, of claudins has been reported in many human carcinomas. Recently, Claudin1 (CLDN1) was found to be up-regulated in papillary thyroid carcinoma (PTC) compared to normal parenchyma and other thyroid tumors. Conversely, claudin7 (CLDN7) has been reported in both normal and neoplastic thyroid tissue, suggesting a possible role of this protein in the architectural stability of follicular cells. Aim: To analyze and to compare the gene and protein expression levels of CLDN1/7 in a cohort of PTCs. Materials and Methods: We tested 85 PTCs by immunohistochemistry, using the polyclonal antibody anti-CLDN1 and the monoclonal antibody anti-CLDN7 (Zymed, San Francisco, CA). In a subset of 20 PTC samples, we analyzed CLDN1/7 mRNA expression through qRT-PCR. Finally, we evaluated CLDN1/7 protein expression by Western Blot analysis. Results: In the majority of PTCs, CLDN1 showed a strong and diffuse, linear membranous positivity. The staining tended to decrease in the tall cell variant PTC showing a weak and incomplete membranous pattern. Overall, CLDN7 exhibited a high reactivity similar to normal parenchyma, with linear/fragmented membranous pattern. In the poorly differentiated/solid areas of PTC, the CLDN1/7 expression were decreased, as at the invasive front of the tumor. We found a correlation between CLDN1 mRNA expression, immunohistochemical data and Western Blot results, proving the up-regulation of this membrane protein in tumor samples, compared to healthy parenchyma ($P < 0.05$). We did not find any significant correlation between mRNA and protein expression for CLDN7. Conclusions: Our study confirms that CLDN1 is frequently up-regulated in PTC, supporting the hypothesis of an involvement of TJs in thyroid tumorigenesis. The decreased CDLN1/7 expression in the “aggressive” variants of PTC could represent a prognostic index and could be one of the morphological indicators of epithelial-to-mesenchymal-transition in PTC.

PP247 - MOLECULAR DETECTION OF PPARG REARRANGEMENTS IN FOLLICULAR PATTERNED-THYROID LESIONS: A BREAK-APART FISH-BASED STUDY.

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Introduction: The differential diagnosis between follicular thyroid carcinoma (FTC) and the other follicular patterned lesions represents to date a big challenge for the clinicians and pathologists. The chromosomal rearrangement PAX8/PPARg is a common mutational event in FTC, with a wide range of the reported prevalence among different studies (from 11% to 63%) and it is considered a molecular marker of FTC. However, this alteration has also been detected in some follicular adenomas (FA) and in a small proportion of the follicular variant of papillary carcinoma (fvPTC). The fluorescence in situ hybridization (FISH) assay represents the gold standard method for detecting gene rearrangements on histology sections with the possibility to separate the clonal (driver mutation) from subclonal event (passenger mutation) and to quantifying intratumoral genetic heterogeneity. Moreover, FISH analysis seems to be suitable on thyroid tumors due to their low growth rate. Aim: To investigate the prevalence of PAX8/PPARg by FISH in a cohort of follicular patterned lesions of the thyroid and to evaluate its diagnostic application. Materials and Methods: Ten FTC (8 minimally invasive and 2 widely invasive), 10 FA and 10 fvPTC $BRAF^{V600E}$ and RET/PTC wild-type, were analyzed by a dual-color interphase FISH assay using the commercially available Poseidon™ Repeat Free™ PPARg (3p25) Break probe (Kreatech Diagnostics, Amsterdam Netherlands), optimized to detect translocations/amplification involving PPARg gene. For each samples, 100 non overlapping tumor cell nuclei were scored for the presence of PPARg split FISH signal (separated red and green signals). Results: PPARg rearrangement was observed in 1 of 10 (10%) of FTC and in 1 of 10 (10%) of fvPTC, showing 40% and 44% of positive nuclei respectively. All 10 AF were negative for PPARg translocation. Conclusions: Although limited by a small sample size, our study confirm that the detection of PPARg translocation may be of diagnostic value because it occurs mainly in malignant follicular patterned lesions. As already suggested, the possible clinical application of this observation is to provide a presurgical test of malignancy on cytological specimens.The identification of a precise FISH cut-off appears to be a pivotal prerequisite in the interpretation of the presence of PPARg rearrangement in order to identify clonal vs subclonal events.

PP248 - INTRAVENOUS METHYLPREDNISOLONE FOR GRAVE'S OPHTHALMOPATHY IN A PATIENT WITH CHRONIC HEPATITIS C: A CASE REPORT.

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BACKGROUND: Glucocorticoids (GC), alone or in combination with orbital radiotherapy, represent the first-line treatment for active and moderate-to-severe Graves' Ophtalmopathy (GO). Intravenous glucocorticoid (ivGC) pulse therapy is significantly more effective and better tolerated than oral steroids. Intravenous GC therapy has been associated with hepatotoxicity in patients with preexisting hepatic diseases. The role of ivGC on viral hepatitis is unknown.

CASE PRESENTATION: In 2013, a 52-year-old Ukrainian woman was admitted to our clinic for moderate active GO (Clinical Activity Score 5/10). In 2010, she was diagnosed with hepatitis C and treated with interferon for the two years. The initial liver assessment showed low activity hepatitis C with scant fibrosis. Methylprednisolone pulse therapy was administered for ten weeks at a cumulative dose of 3.5 g. During the ivGC therapy and at 3 and 6 months after treatment, neither adverse events nor serum increases in liver enzymes and viral load were observed. Ophthalmology examination at the end and 6 months after treatment discontinuation, showed significant improvement of GO (Clinical Activity Score 0/10); the patient experienced a marked clinical improvement.

CONCLUSION: We report a case of a middle-aged woman with chronic hepatitis C treated with ivGC for the management of moderate active GO. Hepatotoxicity related to methylprednisolone should be considered. In this case ivGC therapy resulted effective and safe, with no adverse reactions and alterations liver function test requiring withdrawal of medication. Chronic hepatitis may not necessarily be an exclusion criteria to ivGC therapy upon ascertainment of individual risk.

PP249 - IODINE INTAKE IN PREGNANT WOMEN FROM A MODERATE IODINE DEFICIENT AREA OF TUSCANY

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Introduction: Thyroid function is crucial during pregnancy.

Aim: Epidemiology of iodine intake and its effects on thyroid function in pregnant women.

Patients/Methods: 211 consecutive pregnant women from a moderate iodine deficient area of Tuscany were recruited. Thyroid function and autoimmunity, urinary iodine [UI] and iodine supplementation(s) (iodized salt [S], multivitamins including iodine [M], both [SM] or none [N]) were evaluated at 10th, 15th, 20th, 25th and 35th weeks of gestation.

Results: 108 women were healthy [H], 69 had chronic autoimmune thyroiditis [CAT], 24 had thyroid nodules [TN], 8 ha Grave's disease [GD] and 2 had a thyroid carcinoma in clinical remission at the time of pregnancy [Ca].

39 women were using S, 65 M, 64 SM and 41 N. UI values were consistent with iodine supplementation: at 10 weeks median UI was 40 µg/l in N group, 70 µg/l in S, 98 µg/l in M and 105 µg/l in SM. When including only untreated women, median UI was 33 µg/l in N, 48 µg/l in S, 117 µg/l in M e 85 µg/l in SM. Differences among groups were significant: 1_ in all women, in N *vs.* SM (p-value 0.014) and in S *vs.* SM (p 0.041); 2_ in untreated women only, in N *vs.* M (p 0.026) and N *vs.* SM (p 0.029).

At 10 weeks, 79% of women had a poor iodine intake (UI < 150), 9% adequate (150<UI<250) and 12% excessive iodine intake (>250). In H women only, 71% of women had a poor iodine intake, 7% adequate and 22% excessive iodine intake.

Among H women, no significant correlations were found between UI and FT4, FT3, TSH, FT3/FT4 at any time point.

Conclusions: The majority of pregnant women, both healthy and affected by thyroid diseases, have a poor iodine intake. UI positively correlates with iodine supplementation. M seem to be more efficient respect to S in order to increase iodine intake in pregnant women. So far, no correlations were found between UI and thyroid function during pregnancy.

PP250 - THYROGLOBULIN RESPONSE TO RH-TSH TEST IN PATIENTS WITH DIFFERENTIATED THYROID CANCER: RISK STRATIFICATION AND THERAPEUTIC IMPLICATIONS

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Introduction. After initial treatment, differentiated thyroid cancer (DTC) follow-up includes periodic cervical ultrasonography (US) and measurement of serum thyroglobulin (Tg). Recombinant human TSH (rhTSH)-stimulated Tg evaluation should be performed in patients without evidence of disease in absence of anti-Tg antibodies (Tg-Ab). Prognostic significance of minimally detectable Tg levels is still debated. The aim of this retrospective study was to evaluate the ability of stimulated Tg to predict disease recurrence and whether the first rhTSH test result had influenced subsequent follow-up and therapeutic decision making. **Patients.** 166 DTC patients (F=127; median age at diagnosis= 45,2 years) diagnosed between 2003 and 2010 were included. After total thyroidectomy and radioiodine ablation of residual tissue, all patients were considered clinically and biochemically disease free and underwent rhTSH-stimulated Tg test, in absence of Tg-Ab. Histological examination had shown 143 papillary carcinomas, 21 follicular e 2 poorly differentiated carcinomas; 70% of tumours were stage I or II according to TNM system. For this study, patients were divided into three groups based on the stimulated Tg levels: ≤ 1, 1.1 to 2, >2 ng/ml. After rhTSH test, all included subjects were followed by periodic neck ultrasonography and measurement of serum Tg while on l-thyroxine; in the case of suspected recurrence, further investigations (FNA, ^{131}I WBS, ^{18}FDG PET) and/or treatments (^{131}I administration e/o surgical removal of metastasis) were performed. **Results.** rhTSH Tg ≤ 1 ng/ml was detected in 90% and Tg=1.1-2 ng/ml in 6% of cases, while only 4% of patients showed stimulated Tg > 2 ng/ml. Recurrence was found in 2% of patients with rhTSH Tg < 1, and in 25% of patients with Tg > 1 ng/ml. In 6/7 patients the recurrence occurred within 4 years after initial treatment; sites of recurrence were loco regional lymph nodes (4 cases), lung (2 cases) or liver (1 case). rhTSH test showed an elevated negative predictive value (98%) but a low sensitivity and poor positive predictive value (25; 33%). The management of patients with stimulated Tg > 1 ng/ml was not homogeneous: 7/16 cases underwent further investigations and in 6/16 cases a surgical and/or radiometabolic treatment were performed. Mean time between two consecutive follow-up visits was significantly different in patients with rhTSH Tg ≤ 1ng/ml and patients with Tg > 1ng/ml (11,7 vs 8,9 months; p = 0,001). **Conclusion.** Patients with stimulated Tg ≤ 1 ng/ml can be considered disease free. Tg stimulated values between 1 and 2 ng/ml should be a marker of possible recurrence. RhTSH Tg > 1 ng/ml may contribute to increase attention of clinicians; nevertheless, rhTSH test result cannot be considered the only determinant of subsequent follow-up.

PP251 - EPIDEMIOLOGY OF ANAPLASTIC THYROID CANCER IN SICILY

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Introduction: Anaplastic thyroid cancer (ATC) is a rare, aggressive and undifferentiated tumor with poor prognosis, representing 1-2 % of all thyroid cancer arising from thyroid follicular cells, with an incidence of 1-2 cases per million inhabitants/year. Because of its aggressive behaviour this cancer is classified by the American Joint Committee on Cancer (AJCC) as T4 category and stage IV and contributes up to 15-40% thyroid cancer related mortality with 1-year and 10-year survival rates of 10–20% and less than 5%, respectively.

Aims: Analysis of epidemiology, incidence, mortality and histopatological and prognostic factors in ATCs reported in the Sicilian Register of Thyroid Cancer (SRTC) database in the 2002-2007 period. **Data Sources and Methods:** SRTC was set up by systemic, active survey of all pathology centers in Sicily. Mortality rate of ATC was obtained from the Sicilian Regional Epidemiology Observatory (OER, Palermo). Death certificates indicated age, sex, residence and cause of death.

Results: Among 4206 diagnosed thyroid cancers in Sicily in 2002-2007, 34 were ATCs (0.8%) with an overall age-standardized incidence rate for the world population (ASR(w) of $0.14/10^{5}$ inhabitants/year (95% CI: 0.12-0.15). No different incidence was observed among the nine Sicilian provinces. F/M ratio was 1.4:1.0 (20 F, 14 M) with a median age at diagnosis of 71.4 yrs (range 31-95). Median tumor size was 6.65 cm, larger in patients older than 70 yrs (6.2 vs 4.9 cm in younger patients; p= 0.14). ATC histopatologic features coexisted with differentiated thyroid carcinoma histotypes in 56% of cases (papillary 55%, follicular 30% and tall cells and insular 15%). ATC histologic patterns included giant-cell subtypes in 33%, spindle-cell in 22%, squamoid-cell tumors in 17% and mixed variants in 28%. Median survival was 4.1 months (range 0.2-20) with 1-year overall survival of 27%.

Conclusions: Data from SRTC confirm the ATC aggressiveness with incidence and survival rates similar to those reported in other countries. No difference between female and male rates and the hystopatological subtypes was observed. In our series age at diagnosis is higher than that reported in the literature. Further studies are warranted to identify prognostic factors, markers for precocious diagnosis and more efficacious therapy in this rare but highly aggressive tumor.

PP252 - THYROID HORMONE INACTIVATION IS ESSENTIAL FOR THE PROLIFERATION OF MUSCLE STEM CELLS AND THE REGENERATION PROCESS

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Thyroid hormones (THs) are important regulators of growth, development, and metabolism. Most of the active thyroid hormone (T3) is generated by peripheral TH metabolism mediated by the iodothyronine deiodinases. The type 2 deiodinase (D2) is the key TH-activating enzyme, while the type 3 deiodinase (D3) exerts an opposite effect, namely, it inactivates T3 and T4 via specific deiodinase reactions. Thyroid hormone is a major determinant of skeletal muscle functions in vivo. We analysed the role of D3-mediated thyroid hormone inactivation in the regulation of muscle stem cells (satellite cells) physiology and in the repair process. We found that D3 is highly expressed in vitro in proliferating muscle stem cells and in vivo in the early phases of the regeneration process. Loss of D3 in vitro in proliferating satellite cells induces cell death due to the thyrotoxic state. Specific genetic ablation of D3 in vivo impairs skeletal muscle regeneration. This impairment is due to massive satellite cell apoptosis, caused by aberrant exposure of activated satellite cells to the physiological, but spatio-temporally excessive, TH concentrations in the circulation. Thus, we conclude that D3 is essential for the activation and proliferation of the stem cells compartment during the early phases of muscle repair. Moreover, we discovered that a dynamic muscle-specific surge in D3 activity during the proliferative phase of muscle regeneration leads to reduced T3-dependent transcription, a previously unrecognized but essential component of the satellite cell activation program, and an absolute requirement for efficient muscle repair.

Our data provide novel insights into the role of deiodinases in the myogenic program in physiological and pathological settings. In addition it set the stage to use hormonal regulation as a tool to manipulate at will the physiology of muscle stem cells, modulating their expansion and differentiation in a therapeutic context.

PP253 - UNUSUAL METASTASES OF DIFFERENTIATED THYROID CANCER: A CLINICAL STUDY OF 38 CASES

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Background: DTC has a favorable prognosis in most patients. However approximately 7-10% of patients show distant metastases (DM) and experience progression of disease with a negative cancer outcome.The most frequent sites for DM are lung and bone but a subgroup of patients will develop end-organ disease to unusual sites which are often expression of disseminated advanced disease. **Objective and Methods:** Retrospective analysis of DTC patients with unusual metastases followed in our Thyroid Clinic in the 1974-2014 period highlighting histopathologic features,clinical manifestation,treatment and outcomes. **Results:** Among 320 DTC patients with DM, 38 (11.9%) developed DM in unusual sites (15 M, 23 F,M/F=1:1,5).Median-age at diagnosis was 56,5 yrs, follow-up of 108,6 mths (range 6-524). Histotype was papillary in 20/38 (53%), follicular in 18/38 (47%;Hurtle-cell variant in 3/18 and insular in 6/18).Tumors were in T3/T4 categories in 22/38 (58%). Most patients (n=33,87%) had multiple metastases. In our series unusual metastases were diagnosed in the liver (N=7,18%),brain (N=6, 15%),kidney (N=5, 13%),larynx-trachea (N=5,13%),skin (N=4,10%),eyes (N=3,7%),surgical scar (N=3, 7%),parotid gland (N=2,5%),retroperitoneum (N=2,5%),muscles (N=2,5%),lymph nodes (inguinal,axillary, N=2, 5%),adrenal (N=1,2%),submandibular gland (N=1,2%),pleura (N=1,2%),bronchial cysts (N=1,2%),spinal cord (N=1,2%) and ovary (N=1,2%). Metastases caused clinical symptoms/signs in 17/38 patients (45%). In the other cases were diagnosed at I^{131} whole body scan (N=6, 16%),CT scan (N=11,29%) or FDG-PET (N=4,11%). A total of 27 patients (71%) received I^{131} therapy (median dose 300 mCi,range 50-1310) and most of them underwent also surgical metastasectomy (N=21,55%) and/or external radiotherapy (N=11, 29%).A minority received systemic chemotherapy (N=4,11%) or chemo embolization (N=2, 5%).Disease free time survival was 21 months (range 1-216, DS ± 48,5).At last control visit, 28 (74%) patients were alive,all with persistent disease, and 10/38 (26%) have died within a period of 36-91 mths after diagnosis. **Conclusions:** In our series unusual DM represented 11.9% of all DM,frequently coexisted with multiple metastases and were expression of advanced disease with poor outcome. Increasing importance should be given to rare metastases in DTC patients, especially with the presence of multiple metastases, unexpected site of I^{131} uptake or aggressive histology. In these patients multimodal initial staging modality,such us the use of I^{131} SPECT/CT or FDG-PET/CT could be a useful tool for modifying therapeutic strategies.

PP254 - A "FORGOTTEN" ECTOPIC MEDIASTINAL HYPERFUNCTIONING GOITER AFTER TOTAL THYROIDECTOMY MIMICKING A THYMOMA: A CASE REPORT

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Background: "Forgotten" goiter is a rare disease which is defined as a mediastinal thyroid mass found after total thyroidectomy. In the presence of a mediastinal mass, it is necessary to rule out other conditions such as thymoma, lymphoma and other cancers. **Case report:** a 67-year-old woman was referred to our department for tachycardia and increasing shortness of breath on exertion. In 2002 she had undergone total thyroidectomy for multinodular goiter, benign at histology, and received levo-thyroxine therapy for hypothyroidism. In 2014 a routine examination of thyroid stimulating hormone (TSH) showed persistently suppressed levels of the hormone so that a gradual dosage reduction of l-thyroxine until complete withdrawal was done. After two months of l-thyroxine withdrawal, suppressed levels of TSH with free T4 and free T3 increase persists. An ultrasound of the neck showed an empty thyroid bed. A computed tomography (CT) evidenced a large anterior right paramedian mediastinal mass, 72x65 mm; the trachea was slightly deviated to the left. She was sent for cytological investigation of the mass whose findings appeared compatible for thymoma. Normal serum chromogranin and calcitonin. The relief of detectable serum thyroglobulin with positivity of anti-thyroglobulin associated with the presence of uptake of Tc-99m-pertechnetate by the mediastinal mass confirmed the presence of mediastinal ectopic thyroid tissue. She underwent thoracotomy excision of the mediastinal mass (85x85x50 mm), which resulted in continuity with both the thymic tissue and the thyroid bed. Histological examination showed thymus involuted and thyroid tissue with diffuse nodular hyperplasia. The postoperative course was uneventful. After surgery clinical hypothyroidism was founded and l-thyroxine replacement therapy was introduced. **Conclusions:** "Forgotten" goiter is a rare condition which can be prevented if particular attention is paid to preoperative imaging and intraoperative management: in particular a CT and eventually a Tc99-pertechnetate scintigraphy with mediastinal extension may allow identify, in the case of voluminous goiter, the true extent of thyroid mass to perform a radical removal of goiter. Moreover the relief of TSH levels persistently suppressed after total thyroidectomy requiring significant reduction up to discontinuation of l-thyroxine treatment, should always put the suspected presence of ectopic thyroid tissue.

PP255 - THE ARYL HYDROCARBON RECEPTOR (AHR) IS OVEREXPRESSED IN PAPILLARY THYROID CARCINOMA

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Purpose: AHR is a ligand-activated transcription factor that is best known for mediating the toxicity and tumor-promoting properties of the carcinogen dioxin. It influences the major stages of tumorigenesis and physiologically-relevant AHR ligands are introduced with the diet or are formed at the site of disease processes or immune responses including the tumor microenvironment. In detail, the products of tryptophan transformation by Indoleamine-2,3-dyoxygenase 1 (IDO1) are known AHR agonists. Conversely, *IDO1*, as *cytochrome P4501B1* (*CYP1B1*), is a known AHR target gene. Purpose of the present study was the evaluation of AHR relevance in papillary thyroid carcinoma (PTC) tumorigenesis.

Methods: *AHR*, *IDO1* and *CYP1B1* expression levels were evaluated by QPCR in 90 PTC samples and compared with those of a normal thyroid control. All PTCs were also analyzed for the presence of *ret/PTC 1* and *3* rearrangements and *BRAF* (exon 15), *H-RAS* (exon 3) and *N-RAS* (exon 3) mutations. Statistical analysis included one-sample t-test, the Mann-Whitney nonparametric test and Spearman's rho, as appropriate.

Results: *AHR*, *IDO1* and *CYP1B1* expression resulted higher in PTC compared to normal thyroid (respectively 26.91±15.45 folds [P<0.00], 10.75±40.9 folds [P=0.02] e 2.31±3.44 folds [P=0.01]). A close to statistical significant correlation could be found between *AHR* and *CYP1B1* expression levels (R=0.28, P=0.07), but not between *AHR* and *IDO1* or *IDO1* and *CYP1B1*. *AHR* expression resulted higher in *BRAF* mutation-positive PTC than in the *BRAF*-negative counterpart (29.22±16.37 *vs* 20.91±10.9, P=0.03).

Conclusions: These data indicate that *AHR* and two of its target genes, namely *IDO1* and *CYP1B1*, are overexpressed in PTC. The absence of a statistical significant correlation between *AHR* gene expression levels and those of *IDO1* gene can be ascribed to the complex regulation of the latter whose expression is also under the control of NF-*k*B and inflammatory cytokine signaling. Conversely, the close to statistical significant correletion between the expression of the *AHR* gene and its exclusive target gene *CYP1B1*, in a cell type not devoted to xenobiotic detoxification, indicates the functional relevance of AHR in PTC cells. *BRAF* mutation-activated signaling appears as one of the possible pathways regulating *AHR* expression. In summary, these data indicate a possible role of AHR in PTC tumorigenesis and a potential for therapeutic modulation of its activity in this tumor type.

PP256 - DIFFERENT LEVO-THYROXINE ADMINISTRATION SCHEDULES: CARDIOVASCULAR EFFECTS, COMPLIANCE AND QUALITY OF LIFE

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Introduction: L-T4 treatment at TSH-suppressive doses may be associated with symptoms such as palpitations, diarrhea and irritability. There are few data on the use of alternative schedules to reduce the side effects secondary to TSH suppression.

Objective: To evaluate the effect of different L-T4 administration schedules on the cardiovascular response, quality of life and compliance to therapy in patients with post-surgical hypothyroidism in treatment with L-T4 at TSH-suppressive doses.

Methods: We enrolled consecutive patients with differentiated thyroid carcinoma treated with total thyroidectomy and complementary radioiodine therapy with ^{131}I and receiving a once daily TSH-suppressive treatment (0.05-0.5mUI/l) with L-T4. At the enrollment (T0) we evaluated TSH, FT3 and FT4 at different time-points during the first 24h after taking a once/daily L-T4 (h 7:00). Such assays were repeated after 90 days (T1) of a twice/daily L-T4 schedule (h 7:00-19:00). Both at T0 and at T1, all patients underwent a Holter ECG assessment and filled a specific questionnaire on quality of life, compliance to L-T4 therapy and presence of cardiovascular symptoms (QoL-C-CS).

Results: We included 13 patients (2M/11F, 44±5 years). The mean 24h TSH levels at T0 and T1 resulted 0.5±0.45 and 0.25±0.03 mIU/L, respectively (p=0.432), remaining in the range of suppression. Similarly, there were no significant changes between T0 and T1 in the mean 24h FT3 (2.93±0.2 vs 2.66±0.81 pg/ml, p=0.255) and FT4 (1.52±0.11 vs 1.41±0.28 ng/dl, p=0.199) levels. Holter ECG assessment showed a 25.67% reduction in the mean QRS/24h between T0 and T1 (103396±12740 vs 31127±76584, p=0.008) and a 91% decrease in mean heartbeats in tachycardia/24h (19595±1696 vs 26060±1404, p=0.021). In parallel, a significant improvement from T0 to T1 in the QoL-C-CS score occurred (2022.5±275.5 vs 2267.9±245.7, p=0.025).

Conclusion: The twice/daily L-T4 administration schedule is a valuable treatment option for patients with thyroid carcinoma. Compared to the once/daily schedule it guarantees a similar TSH suppression throughout the day, lower cardiovascular impact and a greater compliance, resulting in an overall improvement in the quality of life of patients.

PP257 - DESCRIPTION OF TWO MEN2A UNRELATED FAMILIES: CLINICAL VARIABILITY AND LOW AGGRESSIVENESS ASSOCIATED WITH RET MUTATION AT CODON 618.

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Background: Multiple endocrine neoplasia type 2 (MEN-2) is an hereditary syndrome characterized by medullary thyroid carcinoma (MTC), pheochromocytoma (pheo) and hyperparathyroidism (HPTH) due to a germline mutation of the proto-oncogene RET; in literature has been demonstrated the association between genotype and corresponding fenotype of the disease. Aims: We describe the clinical and molecular characterization of two large families with MEN 2A syndrome associated to the same RET mutation at codon 618. Results: Family 1: The index cases was a 66 yrs old man, with multinodular goiter associated to elevated calcitonin levels (722 pg/ml) that underwent to total thyroidectomy (TT)+central and bilateral neck dissection with histological confirmation of MTC (T3N1bMx, Stage IVa). Family 2: the index case was a 44 yrs old female with incidental diagnosis of MTC after TT for nodular goiter (T2NxMx, Stage II). In both index cases genetic test revealed the same germline RET mutation (Exon 10, p. 1853G>A, Cys618Tyr) with no apparently kinship relations between the two patients. Then, 65 family members were examined for RET gene mutation and 35 of them (20/42, 48% in family 1 and 15/23 , 65% in family 2) harbored the same mutation. All RET+ patients were negative for pheo and positive for HPTH in 2 of them (1 in each family) both treated surgically during TT. A total of 23 RET+ patients (4-53 yrs, median 21yrs) with elevated CT levels (range 13.7-282 pg/ml, median 23.5 pg/ml) underwent surgery (8 TT, 15 TT+central/lateral neck dissection). Histology identified isolated C cell hyperplasia in 2 cases and MTC in 21 patients (median tumor size 0.5 cm, range 0.1-3.0); 13 tumors were stage I, 1 stage II, 3 stage III e 4 stage IV (3 stage IVa e 1 IVc). In both families MTC was diagnosed by age 10. Post operatively only 4/21 (19%) MTC patients showed detectable CT levels with evidence of morphological persistent/recurrent disease in one of them. At the time of last control 1 patient was dead for disease. Conclusions: In this report, we describe two large families with MEN 2A syndrome associated to the same RET mutation at codon 618 (Exon 10, p. 1853G>A, Cys618Tyr), identified in 53.8% of screened family members; according to literature we observed a low penetrance of HPTH and pheo. Despite the age at time of the screening (in most cases patients were >10 yrs old), in our series MTC at diagnosis and during follow-up showed a low aggressiveness: 67% MTC were stage I-II with biochemical persistence of disease in 19% and low rate of morphological recurrent disease.

PP258 - THE PRESENCE OF MACROPHAGES CORRELATES WITH THE MOST AGGRESSIVE HISTOLOGICAL VARIANTS OF WELL DIFFERENTIATED PAPILLARY THYROID CANCER AND WITH THE BRAFV600E MUTATION

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Papillary thyroid carcinoma (PTC) accounts for about 85% of differentiated thyroid tumors and can appear with different histological variants that differ in their degree of aggressiveness. The presence of macrophages (TAM), is a common feature in many human tumors; in thyroid tumors, the presence of TAM has been reported in both PTC and anaplastic thyroid carcinoma. The aim of our study was to evaluate the presence of TAM in PTC and to verify the correlation with tumor aggressiveness and BRAF mutation.

The study was performed on 98 PTC patients (73 females and 25 males). The research of TAM was performed by immunohistochemistry using a monoclonal antibody directed against the CD68. In all cases we evaluated the presence of intra- and peritumoral TAM. CD68 positivity was correlated with the clinic-pathological features of the tumor (TNM, stage, variant). In 44 cases the presence of BRAFV600E mutation was also evaluated.

Forty-six out of 98 PTC patients had a classical variant (CV), 37 had a follicular variant (FV) and 15 had a (TCV). Overall, 79 samples were CD68 positive (CD68+) in the intratumoral tissue and 47 samples were CD68+ in the peritumoral tissue. In particular in the peritumoral tissue TAMs were present in 88% (n=13/15) of the PTC with TCV, in 47% (n=21/46) of the PTC with the CV and in 35% (n=13/37) with FV of PTC. In the intratumoral tissue TAMs were present in 93% (n=14/15) of the PTC with TCV, in 80% (n=37/46) of the PTC with the CV and in 75% (n=28/37) with FV of PTC. The statistical analysis demonstrated that the presence of peritumoral TAM was positively associated with the TCV(p=0.0031). We also evaluated the intensity of positivity and we observed that an increased staining intensity, both in the intra- and in the peritumoral tissue, was significantly associated with the tall cell variant (p=0.0144 and p=0.0074, respectively). No significant correlation was observed between TNM, stage of disease and the presence of intra- and peritumoral TAM. The BRAFV600E mutation was found in 20/44 (45%) samples. The presence of the BRAF mutation correlated with the presence of TAM in the peritumoral tissue (p = 0.02) and with the staining intensity in the intratumoral tissue (p=0.0087).

Our study confirmed the presence of TAM in PTC but demonstrated for the first time that the presence of TAM is significantly associated with more aggressive variants (TCV) and with the presence of the BRAFV600 mutation.

PP259 - THE MAJORITY OF SPORADIC ADVANCED MEDULLARY THYROID CANCER (MTC) HAVE A SOMATIC MUTATION OF RET AND, ONLY RARELY, OF RAS GENE.

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All series of sporadic MTC reported so far show a prevalence of *RET* somatic mutation of about 45%. A quite different range of prevalence has been reported for *RAS* somatic mutation varying from 15% to 80% in *RET* negative cases.

In the present study we focused our attention on a series of advanced cases that, because of their aggressiveness, were filling the criteria to participate in clinical trials with tyrosine kinase inhibitors.

A total of 50 MTC cases were analyzed, 27 were paraffin embedded tumoral sections and 23 were fresh tissues collected at surgery. *RET* exons 11,14,15 and 16 and H/K Ras exons 2, 3 and 4 were analyzed by direct sequencing.

Overall 45 cases (45/50, 90%) were found to harbour a somatic mutation 2 of whom (2/50, 4%) affected the *RAS* genes (K117N and Q61R in H-RAS) and 43 (43/50, 86%) affected the *RET* gene. Only 5 patients were found to be negative for the presence of a mutation both in *RET* and *RAS*. Among the *RET* mutations we found that 34 patients (33/43, 79.1%) were affected by the classical M918T mutation in exon 16, 5 patients (5/43, 11.7%) were found to have a small deletion (3 cases in exon 11 and 2 cases in exon 15), 2 patients (2/43, 4.6%) had the V804 mutation in exon 14 and 2 patients (2/43, 4.6%) had a mutation in exon 15 (A883F and S891A). No patients showed 2 simultaneous mutations. The presence of a *RET* germline mutation was excluded in all cases thus confirming the true sporadic origin of the disease.

In conclusion, the present study demonstrates that a) the prevalence of somatic mutation, mainly *RET* mutation, is very high in very aggressive and advanced MTC; b) the most frequent *RET* somatic mutation is the M918T mutation in exon 16 but also deletions and mutations in alternative exons are present; c) *RAS* mutations are rare in the group of advanced MTC and, as expected, mutually exclusive with RET.

PP260 - ECHOGRAPHIC CLASSIFICATION OF THYROID NODULES ACCORDING TO THE RISK OF MALIGNANCY (ECON-ARM)

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Introduction: Thyroid Imaging Reporting and Data System on thyroid nodules proposed in 2009 (TIRADS, Thyroid Imaging Reporting and Data System), comprehend several ultrasound (US) patterns to define the risk of malignancy. The US patterns associated with malignancy are: presence of microcalcifications, hypoechogenicity, absent halo sign, irregular margins, solid pattern, shape more tall than wide, chaotic vascularisation, low elasticity. **Aim of the study:** to validate a classification which modified and simplified the TIRADS and which included 6 categories **ECON-ARM** (Cat) with an increased risk of malignancy: Cat 0-1 very low risk; Cat 2-3 intermediate risk; Cat 4-5 high risk. The same risk category included nodules with different US patterns (example in cat 0-1 cystic or mixed nodules and iso/hyperchogenic solid nodules), but with a similar risk of malignancy. An image series with standard categories was used in the clinical practice to classify each case. **Methods:** the study included 522 nodules of consecutive patients come to our center to perform cytology on fine needle aspiration (FNA). The ultrasound was performed using a real time instrument (Esaote SpA, MyLab 70, 7,5-13 MHz probe). The cytological diagnosis was reported according to the Italian Consensus (Nardi et al. 2014), which includes 5 classes (TIR 1-5).

		TIR-1		TIR-2	TIR-3		TIR-4	TIR-5
		1	**1C**		**A**	**B**		
ECON-ARM	n°							
0A-1B	338	20	16	264	28	10	0	0
2-3	166	13	1	108	29	7	3	6
4-5	18	1	/	0	1	2	3	11
Total	**522**	**51**		**372**	**58**	**19**	**6**	**17**

Results:338 (64,75%) nodules were included in Cat 0-1 (low risk): none had TIR 4-5 cytology and 38/338 (11,2 %) TIR 3 cytology. 166 nodules (31,8%) were included in

Cat 2-3 (intermediate risk): 9/166 (5,4%) had TIR 4-5 cytology and 36 (21,7%) TIR 3 cytology. 18 (3,4%) nodules were included in Cat 4-5 (high risk): 14 (78%) had TIR 4-5 cytology and 3 (1,6%) TIR 3 cytology. **Conclusions:** this new US classification of the risk of malignancy in the thyroid nodule allows to establish the strength of the indication to perform FNA in each thyroid nodule.

PP261 - IS THE CLINICAL BEHAVIOUR OF PTC HARBOURING RARE BRAF MUTATIONS DIFFERENT FROM THOSE HARBOURING BRAFV600E OR BRAF WILD TYPE?

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Up to now *BRAFV600E* mutation is the most common mutation described in papillary thyroid cancer (PTC). This mutation was described to be associated to advanced clinico-pathological features of PTC at diagnosis. Moreover, patients with PTC tumors harbouring this mutation had a decreased survival rate and a higher risk of recurrence. The systematic screening of PTC in the primary tumors led to the discovery of rare *BRAF* mutations whose behavior is unknown.

From 2006 to 2012 a total of 2961 PTCs were analyzed for the presence of *BRAF* mutation. The clinico-pathological features of these tumors were collected in a computerized database and analyzed for the classical clinico-pathological features of aggressiveness.

BRAFV600E was present in 1131 (38.2%), 1775 (60%) were "wild type" (*BRAF Wt*) while 55 (1.8) had a rare *BRAF* mutation.

Age, sex and size did not differ between the three groups. *BRAFV600E* mutation was associated with advanced clinico-pathological features of PTC at diagnosis compared to *BRAF Wt* PTC. Advanced clinico-pathological features of PTC were more frequent in *BRAFV600E* than in rare *BRAF* mutations. In particular, aggressive histologic PTC variant ($p=0.004$), absence of tumoral capsule ($p<0.0001$), extrathyroid extension ($p<0.0001$), "advanced" pT (T3+T4) ($p<0.0001$), presence of lymph node metastases ($p=0.001$) and advanced AJCC stage ($p=0.003$) were more frequent in *BRAFV600E* tumors compared to *BRAF Wt* PTC. The clinico-pathological features of rare *BRAF* mutations at diagnosis were comparable to *BRAF Wt* PTC tumors.

In conclusion, the prevalence of rare *BRAF* mutations is extremely low. We found 6 mutations never described before. The fact that the majority of these mutations were never described before and that the clinico-pathological features of PTC harbouring rare *BRAF* mutations present with a less advanced features at diagnosis suggest that these mutations have a low transforming activity.

PP262 - A PHASE 3, MULTICENTER, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL OF LENVATINIB (E7080) IN PATIENTS WITH 131I-REFRACTORY DIFFERENTIATED THYROID CANCER (SELECT)

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Background: Based on efficacy results of the phase 2 study of patients (pts) with ^{131}I-refractory differentiated thyroid cancer (RR-DTC), this phase 3 **S**tudy of (**E**7080) **LE**nvatinib (LEN) in Differentiated **C**ancer of the **T**hyroid (SELECT) was developed.

Methods: This randomized, double-blind, placebo (PBO)-controlled study enrolled pts with RR-DTC with documented disease progression within 13 months (mo). Pts were stratified by age (≤65, >65 years), region and ≤1 prior VEGFR-targeted therapies and randomized 2:1 to LEN or PBO (24mg/d, 28-d cycle). The primary endpoint was PFS assessed by independent assessment; secondary endpoints included overall response rate (ORR; complete response [CR] + PR) and safety.

Results: 392 pts were randomized. Pts on LEN had a significantly prolonged PFS vs PBO (hazard ratio 0.21, 99% confidence interval [CI] 0.14–0.31; *P* <.0001); median PFS was LEN: 18.3 mo (95% CI 15.1–not evaluable), PBO: 3.6 mo (95% CI 2.2–3.7). A LEN PFS benefit was observed in all predefined subgroups; median LEN PFS for pts with prior vs no prior VEGF-therapy was 15.1 mo (n=66) and 18.7 mo (n=195), respectively. Rates (n) of CRs were LEN: 1.5% (4), PBO: 0; PRs were LEN: 63.2% (165), PBO: 1.5% (2). Deaths per arm were LEN: 71 (27.2%), PBO: 47 (35.9%). The 3 most common LEN treatment-related adverse events (TRAEs; any Grade) were hypertension (68%), diarrhea (59%), and reduced appetite (50%). Dose was reduced in 78.5% of pts and discontinued due to adverse events (AEs) in 14.2% of pts.

Conclusion: LEN significantly improved PFS compared with PBO in pts with progressive RR-DTC. There were no unexpected toxicities and AEs were manageable.

ClinicalTrials.gov identifier: NCT01321554

Funding Source: Eisai, Inc.

PP263 - ROLE OF VITAMIN D LEVELS AND VITAMIN D SUPPLEMENTATION ON BONE MINERAL DENSITY IN KLINEFELTER SYNDROME

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PURPOSE Decreased bone mineral density (BMD) in Klinefelter syndrome (KS) is frequent and it has been traditionally related to low testosterone (T) levels. However, low BMD can be observed also in patients with normal T levels and T replacement therapy does not necessarily increase bone mass in these patients. Nothing is known about vitamin D levels and supplementation in KS. In this study we determine vitamin D status and bone mass in KS subjects and compare the efficacy of T therapy and vitamin D supplementation on BMD.

METHODS A total of 127 non-mosaic KS patients and 60 age-matched male controls were evaluated with reproductive hormones, 25-hydroxyvitamin D, PTH and bone densitometry by DEXA. Patients with hypogonadism and/or 25-hydroxyvitamin D deficiency were treated with T-gel 2% and/or calcifediol and re-evaluated after 24 months of treatment.

RESULTS 25-hydroxyvitamin D levels were significantly lower in KS patients with respect to controls, and they had significantly lower lumbar and femoral BMD. The percentage of osteopenia/osteoporosis in subjects with 25-hydroxyvitamin D deficiency was higher with respect to subjects with normal 25-hydroxyvitamin D and was not related to the presence/absence of low T levels. Subjects treated with calcifediol or T + calcifediol had a significant increase in lumbar BMD after treatment. No difference was found in T-treated group.

CONCLUSIONS These data highlight that low 25-hydroxyvitamin D levels have a more critical role than low T levels in inducing low BMD in KS subjects. Furthermore, vitamin D supplementation seems to be more effective than T replacement therapy alone in increasing BMD.

PP264 - PROXIMAL FEMUR STRENGTH, CORTICAL THICKNESS AND BONE STRUCTURE IN KLINEFELTER SYNDROME

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Introduction

Klinefelter Syndrome patients (KS) frequently show low bone mass, which could have multiple etiologies. The structural basis of low bone mass and its consequences on bone strength are almost not known, but analogies in bone microstructure and strength between KS and aging women have proposed by studying distal tibia by HRpQCT. The aim of this study was to compare proximal femur strength and bone structure of KS with elderly women and men.

Patients and Methods

Proximal femur QCT analysis was performed on 18 KS (mean age 44±8 years) and compared with 89 elderly women (76±6 years) and 39 elderly men (79±5 years). QCT-based estimates of proximal femur strength were obtained with a personalized Finite Element procedure previously validated in-vitro and in-vivo under loading conditions corresponding to 10 fall directions to span accidental conditions. Bone structure analysis included trabecular and cortical volumetric bone mineral density (Tb.vBMD, Ct.vBMD), and cortical thickness (Ct.Th.), mapped to 18 sectors covering the whole femoral neck. Femoral neck length and cross-sectional area were calculated.

Results

KS and women had similar bone strength (KS: 2981±514 N, W: 2822±627 N, Mann-Whitney P=0.14), both significantly lower (P<0.001) than elderly men (4176±985 N). Bone cortex was significantly thinner in KS patients with respect to women (P<0.05 in 13 out of 18 sectors). Ct.vBMD was equivalent in KS and women, whereas Tb.vBMD was instead higher in KS (P=0.003). Femoral neck was significantly larger in KS patients (CSA 25% higher, P<0.001).

Conclusion

We showed for the first time that, at proximal femur, KS and elderly women are similar in terms of bone strength. This similarity emerged however from different structural traits: KS had thinner femoral neck cortex, partially compensated by a denser trabecular compartment and larger bone dimensions (i.e. higher moments of area and bone mass).

PP265 - PARATHYROID CARCINOMA WITH BROWN TUMORS AND CONCOMITANT PAPILLARY THYROID CANCER: A CASE REPORT

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Background: Parathyroid carcinoma (PC) is a rare tumor with a propensity to multiple recurrences in spite of the best surgical effort. It is frequently functional, causing nearly 1% of the cases of primary hyperparathyroidism (HPT) and in some cases, as it occurs in other forms of severe HPT, it may be complicated by brown tumors, mimicking bone metastases. Synchronous parathyroid and papillary thyroid carcinomas are rare.

Case report: A 42-year-old female was admitted to our Clinic after two surgical procedures for severe and persistent HPT due to enlarged right parathyroid. At the second surgery, in addition to the parathyroid mass, also the right thyroid lobe was excised and the histopathological diagnosis was PC with thyroid invasion. No history of neck irradiation or familiar endocrinopathy was reported. CT scan and 18FDG-PET showed multiple hypermetabolic, lytic and destructive lesions in the dorsal column, suggesting bone metastases. Their regression after PTH reduction and under Ca++ and vitamin D treatment suggested the diagnosis of brown tumours due to severe HPT. Given the persistence of HPT, with recurrent hypercalcemia, and the growth of the local disease with invasion of the left thyroid lobe/isthmus, a third neck surgery was performed (resection of the right parathyroid mass and left thyroid lobe/ isthmus). The PC diagnosis was confirmed and an incidental 4 mm papillary microcarcinoma was found in the left thyroid lobe with a nodal metastasis in the central compartment. A genetic test discovered a never before reported mutation of the CDC73 (HRPT2) gene, codifying the parafibromin and determining a premature stop codon (c.580A>Tp.Arg194). Because of the persistence of HPT, cinacalcet therapy was started to control hypercalcemia.

Conclusion: This is a peculiar patient, with a newly discovered variant of the CDC73 gene and a phenotype characterized by a recurrent PC, brown tumors and a N1a metastasized thyroid microcarcinoma. This case confirms that PC may not exhibit clear malignant properties at the first assessment, contributing to an inadequate initial surgical treatment. Although infrequently, PC can be associated to papillary thyroid cancer. Therefore, thyroid and neck imaging before parathyroid surgery for HPT should be advised. The diagnosis of brown tumor should be considered in patients with severe HPT and multiple destructive bone lesions mimicking metastases on PET/CT images.

PP266 - OSTEOPOROTIC FRACTURE AND BONE ULTRASONOGRAPHY IN TYPE 2 DIABETES MELLITUS

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Diabetes mellitus type 2 (DM2) is a disease that may be associated with osteoporosis and fractures. The X-ray bone densitometry (DXA) has given disappointing results in measuring the risk of fracture in patients with DM2.

In the general population, bone ultrasound (QUS) has demonstrated a predictive power of osteoporotic fracture equal to that of DXA.

The aim of our study was to evaluate the risk of osteoporotic fracture by QUS of the calcaneal bone in patients with DM2.

A group of 108 diabetic patients (55 men and 53 women), consecutively seen in our clinics, was compared with a population of 287 healthy subjects (102 men and 285 women), matched for age and sex. All patients were subjected to ultrasonography (QUS) of the heel (Achilles GE Lunar Expert II). The QUS parameters analyzed were the Speed Of Sound (SOS), the Broadband Ultrasound Attenuation (BUA), the Stiffness Index (SI) and the T-score.

People with diabetes compared to the healthy controls showed values for T-score significantly lower (-0.56 ± 1.38 vs -0.16 ± 1.18, p = 0.004) and higher BMI (28.44 ± 5.89 kg/m^2 vs 27.26 ± 4.94 kg/m^2, p = 0.047). Within the diabetic population, individuals with fractures (Fx) showed values of all QUS parameters significantly lower compared to non-fractured (NFx) (Fx vs. NFx, respectively: BUA 103.2 ± 16.32 dB/MHz vs 112.94 ± 12.04 dB/MHz, P = 0.007; SOS 1538.67 ± 36.99 m/s vs 1569.94 ± 37.94 m/s, p = 0.004; SI 79.73 ± 19.02 % vs 94.77 ± 16.71%, p = 0.002; T-score - 1.73 ± 1.28 vs - 0.38 ± 1.31, p = 0.0001).

In conclusion, our data demonstrate that QUS values are lower in diabetics than in controls. Within the diabetic population, QUS values are significantly lower in subjects with fractures, compared to non-fractured. The ultrasound of the heel appears to be a promising method for screening for fracture risk in patients with DM2.

PP267 - THYROID STATUS IN A COHORT OF ADULTS WITH DOWN'S SYNDOME AND ITS RELATIONSHIP WITH OSTEOPOROSIS AND INDEX OF OXIDATIVE STRESS

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Down'syndrome (DS) has been demonstrated both as an independent risk factor and the most significant predictor of low bone mineral density (BMD) in persons with intellectual disabilities: DS people show lower BMD compared to general population, without differences between sexes, and the prevalence of osteoporotic fractures in DS over 50 has been reported as high as 85%. Low BMD in DS is an incompletely understood multifactorial condition. Many known secondary causes for low BMD in general population are common in DS including Hypovitaminosis D, Hypothyroidism, hypogonadism and early menopausal age. Hypothyroidism is already associated with higher oxidative stress (OS) as DS itself, since several genes involved in OS map on chromosome 21. In order to evaluate thyroid function and its relationships with BMD and OS, we enrolled 111 patients (43 males) aged 21-71 in a cross-sectional cohort study. Mean age was 38 ± 12 year, mean BMI was 28.88 ± 7.12 kg/m^2. 48 patients (43%) were under thyroid replacement therapy with levotiroxine, 29 of them euthyroid (TSH between 0.3 and 2.8 uUI/ml) due to therapy. We measured TSH, fT4 and total antioxidant capacity (TAC) in fasting blood plasma sample collected at 9:00 am. BMD was assessed at lumbar spine and femoral neck by dual-energy X-ray absorptiometry scan (DEXA scan) performed by a Hologic Discovery. Total antioxidant capacity (TAC) was evaluated with a colorimetric method, using the system metamyoglobin-H_2O_2 and the chromogen ABTS; the latency time (LAG, sec) in the appearance of ABTS radical species is proportional to antioxidant content of the system. Mean TSH was higher and basically out of range in females (mean ± SD TSH = 3.04 ± 2.22 uIU/ml) than in males (2.49 ± 1.37 uIU/ml, *P* = 0.02), without age related differences. Unexpectedly, no links between TSH and BMD were found, without significant differences between non osteopenic and osteopenic patients. It was possible to study correlations between TSH and TAC in 33 patients. 16 of them were hypothyroid and a linear correlation between TAC and lumbar spine BMD was found (r^2 = 0.33, p<00.5). Nevertheless, mean TAC was lower in patients under thyroid replacement therapy (LAG = 65.83 ± 19.40 sec) than in the others (LAG = 76.15 ± 16.96 sec). Our data suggest that the role of thyroid disorders in the pathogenesis of low BMD in people with DS is controversial, spoiled by replacement therapy and associated with a higher oxidative stress in hypothyroid patients. In fact, in hypothyroid patients, BMD was inversely proportional to LAG phase rather than TSH value. Further study should assess whether antioxidant supplementationcould be necessary to prevent osteopenia in hypothyroid patients with DS.

PP268 - A SUSPECTED PRIMARY HYPERPARATHYROIDISM RECURRENCE

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Background: primary hyperparathyroidism (PHPT) is characterized by high serum calcium levels, in presence of high PTH levels. It is caused by single adenoma in 80%, hyperplasia or multiple adenomas in 20% and cancer in 0.5% of cases. **Case report:** a 40 year old woman occurred to our observation with suspected PHPT recurrence. The previous year, an incidental finding of hypercalcemia during pregnancy was detected. A week after childbirth, after a fainting episode, she was carried to the first aid where high serum calcium level (19 mg/dl) was confirmed. A diagnosis of PHPT was done on the basis of high PTH level (1790 pg/ml) and hypercalcemia. Neck ultrasound showed a 22 mm hypoechoic area behind the thyroid right lobe and a smaller hypoechoic area behind the left lobe. Parathyroid scintigraphy (PS) showed a tracer confluence at the lower part of the right thyroid lobe. A right paratiroidectomy was performed with intraoperative PTH decline >50%; the histologic diagnosis was parathyroid adenoma. During the following 15 days a low dose calcium therapy was prescribed for limb paresthesias. One month after surgery she come to our observation complaining of widespread osteo-articular pain; laboratory tests showed increased PTH (450 pg/ml), normal calcium and phosphorus levels, low 24 hour urinary calcium. Genetic analysis to rule out MEN and other iper-PTH familiar syndromes was negative. Vitamin D deficiency was discovered (18 ng/ml) and Bone Mineral Density (BMD) showed a marked osteopenia. Thyroid ultrasound showed a 13 mm hypoechoic area behind the thyroid left lobe with a FNAB-PTH wash >3447 pg/ml. PS was negative. Vitamin D supplementation has led to progressive reduction of PTH plasma level until normalization and progressive reduction of the left lobe area; also vitamin D, serum and urinary levels of calcium and phosphorus come back to the norm. BMD has normalized and osteo-articular pain disappeared. The diagnosis of hyperparathyroidism secondary to vitamin D deficiency, concomitant hungry bone syndrome and left parathyroid compensatory hyperplasia was performed. **Discussion:** vitamin D should be measured in all patients with PHPT because its insufficiency is common in these patients. Vitamin D deficient patients have increased risk of fractures with respect to vitamin D-replete patients. Replacement therapy increases levels of serum vitamin D and reduces serum PTH, but some authors argue that may worsen hypercalcemia and hypercalciuria. **Conclusion:** it's still controversial whether vitamin D supplementation may worsen PHPT, so it's important to mesure serum calcium and 24 hours urinary calcium before and during vitamin D therapy in patients with PHPT and vitamin D insufficiency.

PP269 - THE ENVIRONMENTAL POLLUTANT CADMIUM MODIFIES HUMAN OSTEOBLASTS HOMEOSTASIS IN VITRO BY ALTERATION OF WNT/BETA CATENIN PATHWAY AND CASPASE-3 ACTIVATION

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Background: It is well known that the pollutant Cadmium (Cd) is widespread in the environment and can cause significant alterations in human health. Interestingly bone tissue seems to be a crucial target of Cd contamination. In fact, Cd appears to alter bone cell homeostasis. Thus, aim of this study was to further evaluate the effect of Cd on osteoblasts homeostasis, investigating whether Cd might modify osteoblastic cellular proliferation and differentation by a Wnt/β-catenin dependent mechanism or by a process of programmed cellular death (apoptosis) through cytoskeletal alterations and caspases activation.

Methods: To this aim, human osteoblastic SAOS-2 cell line were cultured in McCoy's 5A medium and cells were treated with 10mM cadmium chloride (Cd) for different times.

Results: Cd (10mM) induced in osteoblastic cells a nuclear traslocation of β-catenin and an increased expression of Wnt/β-catenin target genes after 6 hrs of exposure. At the same time a longer exposure to the same Cd concentration induced osteoblastic cell apoptosis. To further characterize the intracellular events we evaluated the effect of Cd exposure on actin filaments and other proteins associated to cytoskeletal actin, characterized by the presence of LIM domains. Interestingly, Cd exposure (15, 24 hrs) reduced LIM proteins expression and induced actin filaments destruction. We also evaluated caspase-3 expression and observed a significant caspase-3 activation after 24hrs of Cd exposure.

Conclusion: Our study shows for the first time that osteoblasts exposed to Cd for short intervals of time stimulated cell proliferation through a Wnt/β-catenin dependent mechanism, likely as a compensatory mechanism in response to cell injury induced by Cd. However, longer exposure to the same Cd concentration induced cells apoptosis through cytoskeleton disruption-mediated mechanism and caspase activation.

PP270 - A CASE OF PRIMARY HYPERPARATHYROIDISM IN PREGNANCY TREATED WITH CINACALCET

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Background. The efficacy and safety of various modes of medical treatment for primary hyperparathyroidism (PHPT) in pregnancy is largely unknown. This report describes a case of PHPT in pregnancy that was treated with the cinacalcet. Case history. We report the case of a woman with PHPT diagnosed at the age of 34 yrs. The patient was symptomatic for kidney stones and biochemistry evaluation revealed a serum calcium (S-Ca) level of 12.6 mg/dl and parathyroid hormone (PTH) of 109 ng/L. All instrumental examinations (neck ultrasound, Sestamibi scan and SPECT-TC) found no pathological parathyroid tissue. MEN1 mutation was negative. Cinacalcet and colecalciferol were started, but poorly tolerated. The patient became pregnant 17 months later, while on therapy. Given the unknown teratogenic effects of cinacalcet, the calciomimetic was stopped. During pregnancy, she was admitted 2-3 times a week for i.v. saline infusions. In the 24th week of pregnancy cinacalcet (15-30 mg/day) was restarted, but nausea and hyperemesis ensued. Surgery was proposed in the 2nd trimester of pregnancy, but was refused and postponed to the postpartum period. In the 32nd week of pregnancy, a cesarean section was carried out as planned. A blood test 8 weeks after the cesarean section revealed an elevated PTH level, as noted in the pre-pregnancy period.

Time	Treatment	S-Ca (mg/dl)	PTH (ng/L)
1st trimester	SF 0.9% 1000ml i.v.x2/week	12.1 - 15.1	48
2nd trimester	SF 0.9% 1000ml i.v. x3/week	11.2 - 13.3	44 - 62
3rd trimester	Cinacalcet 15-30 mg/day	11.1 - 12.5	-
post-partum	no therapy	11.6 - 12.0	46 - 124

The neonate. Ultrasound scans revealed a normal fetus. Immediately after delivery, the infant (2 kg) was transferred to the neonatal intensive care unit (Ca++ 1.12mmol/l). Oral calcium gluconate 10% (100 mg/kg) and vitamin D (alfacalcidol 2gtt/day) in milk feeding were started. After 6 weeks, calcium supplementation was stopped (Ca++ 1.13mmol/l). Conclusion. Only 3 PHPT cases of women on cinacalcet therapy in pregnancy have been published in the medical literature. In the present case, hydration was useful in controlling S-Ca, and cinacalcet therapy helped to control S-Ca, unless it is dangerously high. In the present case, cinacalcet tolerance in pregnancy was very poor, which precluded the administration of higher doses. It remains unclear whether PTH levels in pregnancy were reduced owing to a possible role of placental PTH-rP.

PP271 - CASE REPORT: A RARE CASE OF PARATHYROID CARCINOMA WITH MANDIBULAR BROWN TUMOR.

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Parathyroid carcinoma is a rare endocrine malignancy (0.005% of all cancer) and accounts for less than 1% of cases of primary hyperparathyroidism. No gender preponderance has been demonstrated and the mean age at the time of diagnosis is about 40 years. Upon physical examination, a palpable neck mass is evident in 40-70% of cases and about 60-65% of patients present with calcium level greater than 14 mg/dl. Brown tumor lesions occur in less than 0.1% of patients affected by hyperparathyroidism. Few cases of mandibular brown tumor lesions associated with parathyroid carcinoma have been described in the literature worldwide.

Here we present the case of a 44 years old man admitted at our institution because of hypercalcemia (more than 15 mg/dl). Previous history (2009) of surgical removal of the right mandible because of a lesion classified as cementoma was referred. He discovered hypercalcemia in middle September 2014 during routine tests performed in anticipation of right mandibular prothesis replacement surgery made necessary due to the evidence of an abscess.

At the time of our evaluation parathormone levels were elevated (470 pg/ml, n.v. 10-60) and routine tests confirmed hypercalcemia over 15 mg/dl. Neck ultrasound discovered the presence of a hypoechoic nodule of 20x19x33 mm with microcalcifications in the middle-basal position of the right thyroid lobe. CT scan confirmed the presence of the lesion which was described as an extrathyroidal lesion with intense uptake at technetium 99m sestamibi scanning.

The patient was sent to surgery. At histology a parathyroid carcinoma was diagnosed with solid-trabecular patterns, vascular invasion, mitotic figures, atypical nuclei aspects with a ki67 index of 10%. The patient developed severe postoperative hypocalcemia requiring calcitriol and calcium carbonate supplementation. The revision of CT scan performed in 2009 and of histological slides of the mandibular lesion revealed the presence of a mandibular brown tumor.

PP272 - UNUSUAL ASSOCIATION BETWEEN TYPE I OSTEOGENESIS IMPERFECTA AND HYPOGONADOTROPIC HYPOGONADISM IN MALE PATIENT

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CASE PRESENTATION: A 30 year old man was referred at Metabolic Bone Disease outpatients clinic of Department of Endocrinology with already diagnosed hypogonadotropic hypogonadism under treatment since 3 years with testosterone enanthate. He referred an history of multiple minimal trauma fractures of arm and leg long bones (8) since he was 6 years old and he presented pain and walking impairment due to recent left third metatarsal bone fracture; his physical examination was normal except for blue sclerae. He referred intense fatigue. Blood tests showed: serum calcium 9.9 mg/dL (9-11), phosphorus 4.5 m/dL (4 to 5.7), alkaline phosphatase 190 U/L (<187); Parathyroid Hormone 63 pg ml (10-75); 25OH vitamin D 25 ng mL (> 30); pituitary hormones were all in normal range except for FSH 0,6 mU/ml (5-18); LH 0,7 mU/ml (5-18). MOC DEXA showed lumbar Z-score and T-score = - 1,4 SD. A strong suspicion for type 1 Osteogenesis Imperfecta (OI) was confirmed by DNA molecular analysis that showed the presence of mutation c.1081C / T (p.Arg361 *; Arg183Stop) in heterozygosis for exon 17 of the COL1A1 gene. The audiometric examination showed a bilateral normal hearing. Patient was prescribed neridronate intravenous therapy (2 mg/Kg/3 months) plus 800 IU/day cholecalciferol supplementation, in association with monthly administration of 250 mg testosterone enanthate. Twelve months after treatment with neridronate and vitamin D no new fractures were experienced and patient's fatigue decreased. The SF-12 self-administered questionnaire prescribed before and after 12 months of treatment showed a significative improvement in the perception of Health Related Quality of Life (HRQoL) from 30 to 60.

CONCLUSION: COL1A1 gene mutation assessed in our patient is suggestive for type I Osteogenesis Imperfecta; it results in a "quantitative" defect in the biosynthesis of type I collagen reducing the synthesis of structurally normal proalpha 1chains. In our patient the concomitant hypogonadotropic hypogonadism delayed diagnosis of OI, since hypogonadism *per se* leads to bone fragility and fatigue. This case report suggests that OI suspicion is mandatory in all cases of multiple and precocious fractures hystory cases, even in the presence of other known conditions predisposing to bone fragility.

PP273 - ANTHROPOMETRIC, METABOLIC , BONE PARAMETERS AND MEDITERRANEAN DIET IN CAMPANIA REGION

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Introduction: Mediterranean diet (MD) has shown benefits in patients with cardiovascular disease and in the prevention and treatment of related conditions, such as diabetes, hypertension and metabolic syndrome while it is unclear its association with the bone health.

Aim: To investigate the association between adherence to MD and bone and metabolic parameters.

Methods: 418 healthy people (105 male and 313 female, medium age 50±14 years) were recruited during the "CAMPUS SALUTE ONLUS" 2013-2014 events. To all the participants a validated 14-item questionnaire for the assessment of adherence to MD (PREDIMED) was administered during a face-to-face interview by a certified nutritionist. PREDIMED score was calculated as follows: 0–5, low adherence; score 6–9, average adherence; score ≥10, high adherence to MD. In all subjects we assessed anthropometric parameters, BMI, blood pressure, glycemia, T score and BMD by calcaneal ultrasonography (QUS).

Results: 31% of people have normal BMI (<25 Kg/cm2), 40% were overweight (25-30 Kg/cm2) and 19% obese (>30 Kg/cm2) .

According to the PREDIMED score 8,8% of people have poor adherence, 55,5% medium adherence and 27,4% high adherence to MD. Based on the results of QUS analysis 46,3% of people have normal T-score (T-score >-1,5), 46% have a T-score diagnostic for osteopenia (-1,5 <T-score<-2,5) and 7,7% have a T-score diagnostic for osteoporosis (T-score<-2,5).

Higher MD Mediterranean adherence was inversely correlated to weight (r=-0,2, p<0,001), waist circumference (WC) (r=-0,2, p<0,001), BMI (r=-0,1, p<0,001), glycemia (r=-0,1, p<0,001) and directly correlated to T-score(r=0,2, p<0,001 and BMD (r=0,2, p<0,001). In particular, BMI and WC were directly correlated to consumption of red meat (BMI r=0,5, p<0,001; WC r=0,4, p<0,001) butter (BMI r=0,1, p<0,018; WC r=0,1, p<0,006) and inversely correlated to fish consumption (BMI r=-0,1, p<0,001; WC -r=0,1, p<0,03). T-score and BMD measured by QUS were directly correlated to extra virgin olive oil (T-score r=0,2, p<0,001; BMD r=0,2, p<0,001), vegetables (T-score r=0,3, p<0,001; BMD r=0,3, p<0,001) and fruits consumption (T-score r=0,4, p<0,001; BMD r=0,3, p<0,001)and inversely correlated to dried fruit consumption (T-score r=-0,1, p<0,03; BMD r=-0,1, p<0,03).

Conclusions: Our results indicate that MD has a protective effect on bone health and metabolic parameters.

PP274 - IS THE RISK OF PRIMARY HYPERPARATHYROIDISM INCREASED IN PATIENTS WITH UNTREATED BREAST CANCER?

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Previous studies reported an increased frequency of primary hyperparathyroidism (PHP) in patients with treated breast cancer (BC): the reason of this association is still unknown. All these studies were performed in BC patients after proven with surgery, during or after antitumor treatments including radiotherapy, chemotherapy or hormonal therapy.

In order to rule out the possible effect of these treatments in the development of PHP we evaluated 186 women with BC and 233 women with thyroid cancer (TC, n=122) or benign thyroid diseases (BTD, n=111). In all patients serum calcium, albumin, PTH and 25-OHvitD were measured before any treatment.

Serum calcium concentrations were significantly higher in BC than in TC and BTD groups (median values 9.5

mg/dl, 9.3 mg/dl and 9.3 mg/dl, respectively), but, according to a logistic regression model, calcium was not

significantly different between the 3 groups when age was taken into account. In all patients, serum alcium was in the normal range, indicating that no case of overt PHP was present. Five patients (1 in BC, 2 in TC and 2 in BDT groups) had serum calcium close to the upper limit of normal range, high PTH and low 25-OHvitD, indicating a possible PHP with hypercalcemia masked by concomitant 25-OHvitD deficiency.

In conclusions in untreated BC group, no patient had overt PHP and 1/186 (0.5%) presented a possible PHP masked by 25-OHvitD deficiency, a PHP frequency much lower than that observed in treated BC patients. These data suggest that the treatments of BC may be responsible for the increased frequency of PHP reported in previous studies.

PP275 - PARATHYROID CARCINOMA IN SECONDARY HYPERPARATHYROIDISM: A CASE REPORT

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Background: Parathyroid carcinoma is one of the rarest known malignancies that occur sporadically or as a part of a genetic syndrome. It accounts for approximately 1% of patients with primary hyperparathyroidism. However, cases of parathyroid cancer arising in patients with secondary and tertiary hyperparathyroidism from chronic renal failure have been reported. We reported a case of parathyroid carcinoma in a woman with secondary hyperparathyroidism.

Case: A 69-year-old caucasian female suffering from severe chronic renal failure (GFR: 14 ml/min) with secondary hyperparathyroidism and anemia, osteoporosis, diabetes and hypertension was refered to our hospital because of weight loss, nausea, vomiting, asthenia, adynamia, muscle weakness and progressive osteoarticular pains.

Physical examination: BMI 22.2 kg/m2, PAO 120/80 mmHg, palpable mass in the left neck. Biochemical: PTH 4590 pg/ml, serum calcium/phosphorus 8.6/4.4 mg/ml, albumin 3.8 mg/dl, urinary calcium/phosphorus: 29/394 mg/24 h, FA 537 U/L.

Ultrasonography of the neck revealed a large extrathyroidal hypervascular mass on the left (3 x 2 cm). SestaMIBI scan of the neck revealed marked increased radiotracer activity in the region of the left thyroid lobe. The patient underwent to hemithyroidectomy of left lobe and parathyroidectomy of lower and upper left parathyroids. Histology revealed parathyroid carcinoma of the lower parathyroid. At follow-up of 2 years with periodically ultrasonography of the neck, TC total body, PTH and serum calcium levels the patient was free of recurrence.

Conclusions: Although the parathyroid carcinoma is very rare in patients with secondary hyperparathyroidism you have to consider it when in the presence of very high levels of PTH the serum calcium levels are normal.

PP276 - BONE METABOLISM IN CHILDREN WITH DIFFERENT FORMS OF EPIDERMOLYSIS BULLOSA: A PROSPECTIVE STUDY ON DETERMINANTS OF LOW BONE MASS.

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Background: Low bone mass has been identified as a possible complication of generalized forms of Epidermolysis Bullosa (EB), although predominant determinants of this disorder remain poorly defined. Aim of the present study was to assess bone metabolism in children with generalized and localized forms of EB, to estimate the prevalence of low bone mass in the different subtypes of EB and to identify the possible determinants of low bone mineral density.

Methods: Prospective, observational study on 20 children (11 M, mean age (SD) 11.7±3.9 years) with EB (4 with Dominant Dystrophic EB, 2 with Kindler Syndrome, 3 with EB Simplex, one with Junctional EB and 10 with Recessive Dystrophic EB). Each patient underwent clinical history, physical examination, laboratory studies, X-ray of left hand and wrist for bone age and dual energy X-ray absorptiometry scans of lumbar spine. Primary outcomes were areal bone mineral density (aBMD Z-scores) and bone mineral apparent density.

Results: Mean aBMD Z-score was -1.82±2.33 (range -7.6/1.7), being pathologically reduced (<-2 SD) in 8 patients (40%) whereas aBMD Z-scores bone age were low in 7 patients (35%, mean±SD -1.45±2.26). Extent of blistering (B=-0.07±0.15, P=0.0004) and 25-hydroxyvitamin D serum levels (B=0.103±0.032, P=0.005) were the most important elements influencing aBMD, with skin involvement being inversely associated with mineralization (0.7 decrease in aBMD Z-score for every 10% increase in extent of blistering). A significant correlation between aBMD Z-score and IGF-1 SD, c-reactive protein and sodium serum levels was also found. Immobility was another important factor interfering with peak bone mass achievement.

Conclusions: Low bone mineral density can be considered a systemic complication of EB, with extent of blistering and 25-hydroxyvitamin D levels being its major determinants. Thus, complete evaluation of bone metabolism should be performed in EB patients. Longitudinal study on present cohort is in progress to verify in particular the impact of colecalciferol supplementation.

PP277 - FAMILIAL HYPOCALCIURIC HYPERCALCEMIA (FHH) ASSOCIATED WITH A NOVEL HOMOZYGOUS LOSS-OF-FUNCTION MUTATION, E671D, OF THE CALCIUM-SENSING RECEPTOR GENE

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Familial Hypocalciuric Hypercalcemia (FHH) is an autosomal inherited dominant disease characterized by mild to moderate asymptomatic hypercalcemia, relative hypocalciuria, and inappropriately normal PTH levels. Fractional urinary excretion of calcium is important to distinguish patients with FHH from others forms of primary hyperparathyroidism (PHPT). The calcium/creatinine clearance ratio is <0.01 in FHH and higher in PHPT. Heterozygous and homozygous/compound heterozygous mutations of calcium sensing receptor gene (*CASR*), a G-protein-coupled receptor, are responsible for FHH type 1 and neonatal severe hyperparathyroidism (NSHPT), respectively. NSHPT is a much more serious disorder characterized by marked calcemia, hypotonia, skeletal demineralization, and can occur in the offspring of patients with FHH. In this study we report the case of 61-years-old woman referred to our Department for suspected PHPT. Biochemical analysis revealed high serum calcium (13 mg/dl) and ionized calcium (1,9 mmol/l) levels, PTH moderately elevated (78 pg/ml) and calcium/creatinine clearance ratio <0.01, suggestive of FHH. Serum calcium screening of her relatives showed normal values in the mother, brother and sons, while the father and the half-sister had mild hypercalcemia (10,3 and 10,9 mg/dl, respectively). Of note, her parents were consanguineous. Germline proband and parents' DNA was extracted from peripheral blood cells and the entire coding region and splice junctions of the *CASR* gene were directly sequenced. The proband was homozygous for the change c.2013G>C, that resulted in a novel missense mutation, E671D. The same mutation, in a heterozygous state, was present in the her parents and in her half-sister and absent in 100 chromosomes of healthy control subjects. In conclusion, we identified a novel homozygous mutation, located in the first extracellular loop of *CASR*, in an adult woman with hypercalcemia and hypocalciuria. The proband was born in good health and has had a normal neurological growth, so we can assume that the mutation E671D could have a mild effect. So far, at least three similar cases with homozygous presentation and a FHH phenotype have been described in the literature and functional studies of the mutations showed a mild functional inactivation in-vitro, demonstrating that not all *CASR* mutations found in a homozygous state necessarily cause neurodevelopmental defects due to an elevated hypercalcemia in infancy and childhood, but can resemble a classical FHH phenotype.

PP278 - THE SODIUM LOAD LEADS TO AN INCREASE OF BONE RESORPTION AND DECREASE OF BONE APPOSITION IN POSTMENOPAUSAL FEMALES

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Background. In vitro studies suggested that vasopressin (AVP) exerts a negative role on bone turnover by increasing bone resorption and decreasing bone formation. No data are available on the effect on bone tissue of the sodium load, which is a well-known stimulus for the AVP secretion.

This study was aimed to assess the changes of bone turnover markers after a sodium load.

Patients and Methods. Six postmenopausal female patients (age 65±12.5 years) with hypertension underwent a saline infusion test (infusion of 2 liters of 0.9% saline in 4 hours, SIT), for suspected primary hyperaldosteronism, which was not confirmed after SIT in any subject. We measured, at baseline, at the end of the SIT and 1 and 2 hours after the end of the SIT, sodium (sNA), ionized calcium (iCa), parathyroid hormone (PTH), osteocalcin (OC), C-terminal telopeptides of type I collagen (β-CTX), osmolarity (pOsm) in blood and sodium (uNA), calcium (uCa) and osmolarity (uOsm) in spot urine.

Results. As compared with baseline levels, at the end of the SIT, sNa (144.2±0.3 vs 146.8±0.6 mEq/L, p=0.03), β-CTX (412.1±18.9 vs 544.9±28.0 ng/mL, p=0.01), PTH (45.7±4.3 vs 60.1±5.0 pg/mL, p=0.005) significantly increased, while iCa (1.21±0.1 vs 1.16±0.1 mmol/L, p=0.04), OC (25.8±0.9 vs 20.9±1.7 ng/mL, p=0.01), UCa (9.9±2.1 vs 5.4±1.0 mg/dL, p=0.05) and uOsm (534.6±91.4 vs 284.6±41 mOsm/L, p=0.04) decreased and pOsm (294.2±3.3 vs 295.6±4.3 mOsm/L, p=0.46) remained stable.

At 1 hour after SIT uNa (148.8±8.9 mEq/L, p=0.04) and β-CTX levels (544.3±26.2 ng/mL. p=0.03) remained elevated, pOsm increased (304.2±2.6 mOsm/L, p=0.01), OC remained inhibited (19.8±1.4 ng/mL, p=0.02) and urOsm tended to increase (477.4±47 mOsm/L, p=0.42) as compared to baseline levels.

At 2 hour after SIT pOsm (307.4±0.7 mOsm/L, p=0.02), PTH (53.4±5.5 pg/mL, P=0.05) and sNa remained elevated (147±0.7 mEq/L, p=0.02), β-CTX returned to baseline levels (433.7±31.9 ng/mL, p=ns), while urOsm remained comparable to that 1 hour after SIT (440.8±63.5 mOsm/L, P=0.79) .

Conclusions. The acute sodium load induces an uncoupling of bone turnover with an increase of bone resorption and a decrease of bone apposition and it is associated with increased PTH levels. The possible role of AVP remains to be clarified.

PP279 - MEASURING BMD AT FOREARM AIDS IN DIAGNOSING OSTEOPOROSIS AND IDENTIFIES MORE PATIENTS FOR SURGERY IN ASYMPTOMATIC PRIMARY HYPERPARATHYROIDISM

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Reduction in bone mineral density (BMD) is a common feature in primaryhyperparathyroidism (PHPT), involving mostly cortical site. In the management of asymptomatic PHPT (aPHPT), guidelines indicate measuring BMD at lumbar spine, hip and forearm and surgery is recommended for patients with a T-score of -2.5 or less at one of these sites. However, BMD at forearm is not always performed. Our aim was to evaluate the impact of measuring forearm BMD in the clinical and therapeutical management of aPHPT. We retrospectively reviewed a prospective database of 116 patients with aPHPT at our institution between 1996 and 2013. The study cohort was identified by examining those patients who at the time of diagnosis had a dual x-ray absorptiometry (DXA) scan at all three sites. In all patients we measured PTH, total serum and ionized calcium, urinary calcium excretion, vitamin D and creatinine levels. Out of 116 patients with aPHPT we identified 13 (group A, 11,2%) who had a T score lower than - 2.5 at forearm only, of which 6 (5.2%) possessed the criteria for surgery identified on the basis of forearm BMD only. Group B were the remaining 103 patients. Group A was older than group B (71±7.6 vs 62.7±11.8 yrs, $p< 0.016$) while no significant difference was found in the biochemical measurements or in the BMD values at either of the other sites. In conclusion, in our series of aPHPT, in 11.2 % of patients, DXA on three sites revealed osteoporosis at forearm, but not at other sites. Among these patients, half were identified for surgery based on BMD at forearm. Except for age, these patients did not show any clinical, biochemical or BMD difference from the remaining patients. Preoperative forearm DXA increases the number of patients who meet the criteria for surgery based on BMD alone.

PP280 - CINACALCET IN PRIMARY AND TERTIARY HYPERPARATHYROIDISM: OUTCOMES AND SIDE EFFECTS

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Aim: We aimed to describe the clinical features of patients treated with Cinacalcet for primary and tertiary hyperparathyroidism followed in our centre, with a focus on the treatment patterns employed, adverse drug reactions and outcomes in serum calcium and PTH levels.

Methods: Patients treated with Cinacalcet were retrospectively selected among all those affected by primary or tertiary hyperparathyroidism followed in the Endocrinology Unit, at Maggiore della Carità University Hospital in Novara; age, gender, clinical and biochemical features at baseline and 12 months after starting medical therapy were collected.

Results: Among 30 patients with primary or tertiary hyperparathyroidism, 15 patients (12 female, 3 male, mean age at diagnosis 66.6 ± 15.1 years), were selected: 12 were affected by primary hyperparathyroidism, 3 by tertiary hyperparathyroidism. Moreover, 2 patients were affected by Multiple Endocrine Neoplasia (MEN) type 1 and 1 patient had a parathyroid carcinoma. At diagnosis, most of the patients suffered from osteoporosis (n=8, 53.3%), followed by kidney stones (n=3, 20.0%) and hypertension (n=2, 13.3%); two patients were asymptomatic. In most patients, Cinacalcet was prescribed because they refused surgery (n=7, 46.7%), surgery could not be performed in 4 cases (26.7%) because of no evidence of disease at imaging, and contraindicated in other 4 cases (26.7%) because of co-morbidities or previous neck surgery. The mean calcium level at diagnosis was 12.2 ± 0.8 mg/dl and PTH level was 206.9 ± 118.7 pg/ml (normal range: 10-65 pg/ml). At 12 months follow-up, the mean calcium level was 10.3 ± 0.9 mg/dl and PTH level was 183.0 ± 127.6 pg/ml. The starting Cinacalcet dose was 30 mg once a day in all cases. Most patients (n=9, 60.0%) continued with the same dosage, while in 2 cases (13.3%) an increase till 60 mg per day was needed. Side effects were seen in 4 cases: one patient (6.7%) discontinued Cinacalcet because of nausea and vomiting, while other 3 patients (20.0%) complained anorexia, needing a dose reduction.

Conclusion: Despite the small sample of our study, our data agree with Literature. In fact Cinacalcet normalizes Calcium level in most patients, reduces PTH level and appear to be well tolerated. However, regarding the side effects, even if anorexia is usually considered a less common event, in our population appeared to be the most common side effect, leading to a dosage decrease.

PP281 - FRACTURE RISK ASSESSMENT IN PATIENT WITH ADDISON'S DISEASE

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Glucocorticoids excess cause osteoporosis and bone fractures. Less known is the effect of cortisol when administered as replacement doses like in patients with Addison's disease (AD).

Aim of this study was to asses bone fractures risk in AD. For this purpose 87 patients (28 males and 59 females; mean age 44,63 ± 12,37 yars) were compared with 85 healthy age-sex- and BMI-matched subjects. The duration of disease ranged from 0.5 to 55 years (Mean ± SD: 11,15 ± 10,16). Urinary and salivary cortisol , PRA in the upright position, urinary and salivary cortisol, serum sodium, potassium, calcium, phosphorus urinary calcium 24/h, bone alkaline phosphatase, creatinine, parathyroid hormone, serum CrossLaps, 25 (OH) Vitamin D, 1,25 (OH) $_2$Vitamin D were determined. Total body bone mineral density (BMD) and body fat/lean composition, lumbar spine (L1-L4), femoral neck and total femur were measured by an Hologic Discovery W device. A DXA spinal morphometry was performed to assess vertebral fractures.

Results: AD patients had higher daily urinary cortisol excretion and salivary cortisol (p=0,048 and p=0,0006 respectively), suggesting that the cortisol replacement was higher than the physiological dose. Nineteen patients (11,78%) showed at least one vertebral morphometric fracture, while only eight in the healthy subjects (4,48%), Odds ratio=2,65 (95% IC 1.05-6.67; p=0.036). The densitometric and laboratory variables related to bone metabolism were substantially similar in both groups. AD women had an early menopause age. A negative correlation was found between hydrocortisone daily dose and all the densitometric parameters. The cumulative dose of glucocorticoids and the disease duration were higher in the AD with fractures.

Conclusions: AD patients showed a higher risk of vertebral morphometric fractures than healthy subjects. Such risk does not seem to be related to a lower BMD or to common alterations of mineral metabolism. The Longer duration of the disease and the consequent increased intake of glucocorticoids in fractured AD may be the most responsible for the increased bone fragility. We cannot however exclude the possibility that other pathophysiological mechanisms, related to adrenal insufficiency and autoimmune disease in itself, may be involved in the genesis of the fractures.

PP282 - SCLEROSTIN INFLUENCES THE MENOPAUSAL RELATIONSHIP BETWEEN BONE AND BODY COMPOSITION IN WOMEN WITH SEVERE OBESITY

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A crosstalk between bone and adipose tissue exists and has been related to several mechanisms, which include their common stromal origin, multifold hormone regulation, and bone-active adipokines. Epidemiology and case-control studies pinpointed a disadvantageous effect of obesity on cortical bone, bone strength and risk of nonvertebral fragility fractures after menopause. Sclerostin, an established regulator of bone mineralization, predicts bone loss and postmenopausal hip fracture risk, yet its role in obesity remains largely unknown. To investigate the early changes of bone mass in obese women at menopause and their potential relationship with sclerostin levels, this cross-sectional study analyzed bone turnover markers, glucose metabolism, sclerostin levels, bone-active hormones, body composition and bone mineral density (BMD) by dual X-ray absorptiometry (DXA) in 28 premenopausal (age 44.7±3.9 yrs; BMI 46±4.2 kg/m^2) and 28 postmenopausal obese otherwise healthy women (age 55.5±3.8 yrs; BMI, 46.1±4.8 kg/m^2).

Menopause did not modify glucose homeostasis, leptin, vitamin D and PTH levels, and fat body mass. Compared to their counterpart, postmenopausal women showed higher levels of bone turnover markers CTX and NTX ($p<0.005$), increased adiponectin concentrations ($p<0.05$), and lower values of fat-free body mass ($p<0.05$). DXA showed no difference in lumbar spine BMD between groups. Oppositely, postmenopausal women displayed significant decrements of BMD at the total hip ($p<0.0005$), femoral neck ($p<0.0001$), and total skeleton ($p<0.005$) compared to the premenopausal stage. In bivariate correlation analysis, skeletal BMD was directly correlated with fat-free body mass ($p<0.001$) and BMI ($p<0.05$), and negatively to fat body mass ($p<0.05$). There was no association between BMD and glucose homeostasis, mineral status, adipokines, vitamin D or PTH levels. In our severely obese sample, sclerostin levels did not change across menopause but were able to independently predict lumbar spine BMD in multivariate regression analysis ($p<0.001$). At odds with this result, hip ($p<0.05$) and skeletal BMD ($p<0.001$) were best predicted by menopause by negative association and fat-free body mass by positive association.

Our preliminary findings suggest that severe obesity protects lumbar spine BMD immediately after menopause, when bone loss seems to predominate at the hip. In this complex setting, sclerostin levels elicit a favorable effect at the lumbar spine, which possibly results from a complex interaction between hormonal milieu, body composition and metabolic homeostasis.

PP283 - A PROSPECTIVE STUDY ON JUVENILE PRIMARY HYPERPRATHYROIDISM POPULATION

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Primary hyperparathyroidism (PHPT) is a common disorder in adults but is uncommon in young people and features of Juvenile PHPT (J-PHPT) are debated in literature. The aim of the study was to evaluate the characteristics of PHPT in juvenile sporadic (S) and familial (F) patients.

It's a monocentric prospective study at a referral center in 154 patients with ≤40 years. Patients were evaluated at diagnosis and at the last follow-up visit (median follow-up 2 years), comparing clinical presentation, biochemical, densitometric, histological parameters, percentage of cure after parathyroidectomy (PTx) between S and F.

One hundred-twelve patients had SJ-PHPT, 31 patients had Multiple Endocrine Neoplasia type 1 (MEN1) syndrome and 11 Familiar Isolated Hyperparathyroidism (FIHP). Symptomatic nephrolithiasis was observed in 44% of SJ-PHPT and in 48.4% of F-J-PHPT and aspecific neuropsychic symptoms in 95% of all patients.

Ninety SJ-PHPT and 27 FJ-PHPT underwent PTx. The histology showed in SJ-PHPT and FJ-PHPT respectively: a single adenoma in 86 and 7 patients, hyperplasia in 2 and 19 patients , carcinoma in one SJ-PHPT and exploration without excision of parathyroid tissue was observed in 1 S-JPHPT and 1 F-JPHPT.

Ionized serum calcium and PTH significantly decreased after PTx in both group. The persistence/recurrence rate of disease was 15% in sporadic cases and 52% in familial cases. There were not statistically significant ($p<0.001$) differences in biochemical and densitometric markers between sporadic and familial group.

In both groups males showed a more statistically significant ($p<0.001$) severe PHPT for both biochemical and densitometric markers. When the overall patients were stratified for age ≤25 and > 25 years, younger patients appeared to have a significantly ($p<0.001$) more severe disease.

In conclusion, J-PHPT is generally sintomatic and more severe in males; it has a higher rate of persistence/recurrence disease, even in sporadic patients; S and F patients show similar features.

PP284 - SUBCLINICAL PRIMARY ALDOSTERONISM: A NEW CAUSE OF SECONDARY OSTEOPOROSIS

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Background: The involvement of mineralcorticoids on bone metabolism has been reported (Runyan AL, Am J Med Sci 2005). Indeed, patients with Primary Aldosteronism (PA) have an high prevalence of osteoporosis (OP) and fractures (Fx) (Salcuni AS, JBMR 2012). Aim of our study was to evaluate the prevalence of subclinical PA in patients admitted to our Metabolic Bone Disease Outpatient Clinic.

Patients and Methods: Since November 2012 to January 2015, 2032 subjects were admitted to our Metabolic Bone Disease Outpatient Clinic. Among them, 1769 were excluded because were hypokaliemic or assumed drugs known to affect bone or mineralcorticoids metabolism, while 263 subjects (249 female, 14 male) take part in the study. In all subjects the causes of secondary osteoporosis were excluded (Eller-Vainicher C, EJE 2013) and Bone Mineral Density (BMD) and Vertebral morphometry was performed by Dual X-Ray Absorptiometry. Moreover all subjects were screened for PA with aldosterone-to-renin ratio and in those who screened positive, confirmatory tests were performed.

Results: Among 263 subjects, 179 were osteoporotics and 84 were not. Subclinical PA was diagnosed in 8 out of 179 osteoporotic patients (4.5%) and 1 out of non osteoporotic subjects (1.2%). PA was observed in 3 out of 61 fractured patients (4.9%) and in 6 out of 202 not fractured subjects (3%). In the 9 patients with subclinical PA there was a significant higher urinary calcium excretion (318 ± 120 vs 198 ± 110 mg/die, $p< 0.05$) and serum PTH levels (84 ± 35 vs 58 ± 26 pg/mL, $p< 0.05$). In all subjects there was a significant direct association between serum aldosterone and urinary calcium excretion ($r= 0.14$, $p=0.03$) or PTH ($r= 0.17$, $p= 0.008$).

Conclusions: Our data suggest an association between PA and OP. Although a causal effect cannot be derived from a cross sectional study, our prospective study together with data already published suggest that PA should be considered among the rare causes of secondary OP.

PP285 - CHANGES IN FEMORAL SHAFT CORTICAL THICKNESS AND BMD WITH SHORT- AND LONG-TERM ALENDRONATE USE: THE FRACTURE INTERVENTION TRIAL (FIT) AND THE FIT EXTENSION (FLEX)

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Initial case reports of atypical femur fracture frequently mentioned “generalized thickened cortices” in association with AFF and it remains a minor criteria in the ASBMR AFF definition. Whether bisphosphonates cause cortical thickening indicting weaker bone in some or all patients is unknown. We aimed to longitudinally evaluate changes in cortical thickness over 10 years of treatment using DXA scans from the Fracture Intervention Trial(FIT) and the FIT Long-Term Extension Trial(FLEX).

We included data from 644 participants from FIT and 321 from FLEX. Hip BMD was measured using the same Hologic QDR 2000 densitometers for both the FIT and FLEX trials. Cortical thickness of the hip DXA was analyzed using HSA software at the femoral shaft and 2 other regions.

The pattern of change in cortical thickness was similar at the femoral shaft, intertrochanter and femoral neck increasing in those on alendronate in FIT by 4- 6%. During FLEX, women on alendronate remained at a similar level of cortical thickness (with no further increases) while those on placebo decreased in cortical thickness. The pattern of change in BMD (from HSA or DXA) was similar to that seen for cortical thickness.

We saw no evidence that there was a subset of women who had larger changes alendronate than expected based on a Gaussian (normal) distribution. Changes in cortical thickness and BMD were highly correlated.

In conclusion, short-term use of alendronate is associated increased cortical thickness. However, continuing for 5 more years did not further increase thickness. No subset of women with either short or extended use of alendronate exhibited greater than expected increases in cortical thickness at any hip regions. Our results do not support a link between long-term bisphosphonate use and extraordinary increases in femoral shaft cortical thickness.

PP286 - DENOSUMAB EFFECT ON TRABECULAR BONE SCORE (TBS) IN SEVERE OSTEOPOROSIS: TWO YEARS FOLLOW-UP

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Il denosumab è un anticorpo monoclonale umano diretto contro il RANK ligando, ad azione antiriassorbitiva, essendo in grado di ridurre numero ed attività degli osteoclasti.

Scopo dello studio è stato quello di valutare l'effetto della terapia con denosumab sui marcatori di rimodellamento osseo, sulla BMD e sul trabecular bone score (TBS), in donne affette da osteoporosi severa. A tal fine, sono state valutate, per un periodo di 24 mesi, 40 donne in postmenopausa (età media 77±5 anni), ad alto rischio di frattura. Il 71% delle pazienti presentava precedenti fratture vertebrali multiple, il 29% oltre alla frattura vertebrale (singola o multipla) aveva anche frattura femorale. Il 65% delle pazienti assumeva supplementi di calcio ed il 97% di vitamina D. Sono stati valutati parametri biochimici di turnover osseo (CTX-telopeptide C terminale del procollagene tipo I e bALP- fosfatasi alcalina ossea) al basale ed ogni 6 mesi. La BMD vertebrale, con rispettiva TBS, e la BMD femorale sono state valutate a cadenza semestrale. La valutazione morfometrica con DMX è stata eseguita al basale, mentre solo nuove fratture cliniche sono state valutate al follow-up. Sia i CTX sia la bALP si riducevano significativamente dopo 24 mesi di trattamento (CTX da 383± 324 a 53±83 pg/ml, $p<0.0001$; bALP da 21.1±29.7 a 5.3±1.6 mcg/l, $p<0.005$), nonostante livelli sovrapponibili ed ottimali di vitamina D. Dopo 24 mesi di terapia con denosumab si osservava un miglioramento significativo della BMD vertebrale ($p<0.001$), del collo femorale ($p<0.05$) e dell'intero femorale ($p<0.05$). L'analisi del TBS ha mostrato un costante e progressivo incremento di tale parametro: 1.016±0.15 al basale, 1.017±0.14 a 6 mesi, 1.029±0.14 a 12 mesi, 1.083±0.13 a 18 mesi e 1.101±0.10 a 24 mesi, con un incremento percentuale dopo 24 mesi di terapia pari al 8%. In conclusione, la terapia con denosumab si associa a marcata inibizione dei marcatori di turnover osseo e ad un significativo miglioramento non solo della BMD, ma anche della qualità dell'osso, valutata tramite TBS.

PP287 - POLYMORPHISMS IN GLUCOCORTICOID RECEPTOR GENE NOT INFLUENCE THE DEVELOPMENT OF DIABETES AFTER KIDNEY TRANSPLANTATION (NODAT)

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INTRODUCTION: New-onset diabetes after transplantation (NODAT) is a recognized metabolic complication (from 20% to 50%) and it is associated with increased risks of graft rejection, infection, cardiovascular disease and death. Transplant-specific risk factors for NODAT, such as corticosteroids and calcineurin inhibitors, play a dominant role in its pathogenesis. Furthermore polymorphisms in glucocorticoid receptor gene (GRP) are common in the human population and play a role in regulation of glucocorticoid sensitivity. **OBJECTIVE**: Determine the prevalence, genetic and clinical risk factors for NODAT. **PATIENTS AND METHODS**: We studied 120 kidney allograft recipients (Male 67, Female 53, mean age 55.6 ± 11.9 year, mean of follow-up 5,0±2,42 year) regularly followed at Transplantation Center, Division of Nephrology, Ospedali Riuniti Ancona. The presence of arterial hypertension, dyslipidaemia, BMI and glycemic state (impaired of fasting plasma glucose -FPG- and on glucose tolerance –IGT-) were assessed in all patients. Patients were genotyped for two GRP (BclI, A3669G) using Real-Time PCR System and Taqman allelic discrimination assays. **RESULTS**: Patients with FPG/IGT at the last follow-up (55%) had a BMI significantly increased compared with patients without NODAT (71.8*vs*64.5 kg *p<0.05*;25.2*vs*23.3 kg/m^2 *p<0.05*). There were no significant differences in gender, mean daily steroids doses and genetic polymorphism in GR between patients with NODAT and healthy control. Patients with Bcl1 polymorphism had higher prevalence of FPG/IGT (58.8%*vs*46.4%) without statistical significance; prevalence of A3669G polymorphism is similar in two groups. **CONCLUSIONS**: BMI, obesity and age are a risk factors for NODAT; in contrast gender and daily steroids doses and are not correlates with its development. Our study excludes a role of genetic polymorphism in GR in the development of this frequent and important complication