locations (G1: 79,27% single and 20,73% multiple, G2: 75% and 25%).
For SA, taking into account the clinical improvement and complete healing, interesting results were highlighted comparing T1 *vs* T2 (29,4% *vs* 56,81%, 23,5% *vs* 31,81%), T1+T2 *vs* T3+T4+T5 (49,18% *vs* 87,82%, 29,50% *vs* 81,74%) and T3 *vs* T4+T5 (50% *vs* 92,23%, 50% *vs* 85,44%).
Results comparing an early approach (stage I) *vs* therapies in stage II and III lled almost twice as much to stage 0 (80% *vs* 57,14%)
CONCLUSIONS: In SB no differences between non surgical and surgical approach were highlighted.
In both groups the use of laser significantly improves the outcome of treatments.
Surgical laser approach in Stage III led to complete healing in all cases.
Early surgical approach leads in almost every cases to a complete healing.
Further studies are needed to test if the suspension of the administration of drugs could lead to higher percentage of improvement and/or healing in patients treated through Zoledronic acid combined with antiresorptive-antiangiogenic drugs or antiresorptive-antiangiogenic drugs only.

Oral manifestations in immunodeficient patients

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BACKGROUND: The oral cavity is an anatomical structure characterized by the juxtaposition of soft and hard tissues, and is continuously threatened by the external environment and foreign materials. Diseases and disorders caused by oral microorganisms are very common, particularly dental caries, periodontitis and halitosis. Oral diseases can also arise in the setting of a systemic compromission due to immunodeficiency, whose clinical importance is becoming more and more outstanding. In such conditions, oral manifestations may be more frequent, or even more severe. The aim of this study is to highlight oral and more specifically orthodontic manifestations shared by individuals with immunodeficiencies, so that precautions that will improve patients' quality of life, especially in regard to dental aspects, may be established in the future.
METHODS: A group of subjects (11 males and 9 females, mean age 12-20 years) was selected from patients with immunodeficiencies who referred to the Immunohaematology Department of San Raffaele Scientific Institute in Milan, Italy, between 2010 and 2015. All these subjects underwent the following iter: laboratory tests, microbiological, haematological and immunological evaluation, brain MRI, brain CT, orthopantomography and oral examination, hand–wrist radiography BACKGROUND:ed at evaluating the effects of pathologies of the immune system on patients' growth.
RESULTS: Medical signs and symptoms which are characteristic of peculiar immunodeficiency syndromes, such as recurrent infections, eczema, thrombocytopenia, anaemia, petechiae, ecchymosis, mucosal bleeding and major bleeding, were usually observed. The oral examination revealed gingivitis, periodontitis, aphthous lesions, gingival bleeding, petechiae in the oral mucosa, severe oral infections (caries, pulpitis, abscesses). As for orthodontic aspects, we found a higher incidence of alterations in the physiological eruptive sequence, i.e. inclusions and transpositions, probably caused by untreated inflammatory and infectious processes. Infections affecting permanent teeth may also result in malocclusion, which possibly paves the way for future skeletal problems. In addition to that, immunodeficiency may compromise orthodontic treatments and interfere with their purpose of aligning teeth and solving skeletal issues.
CONCLUSIONS: Since immunodeficiencies comprise a wide spectrum of symptoms and complications, it is mandatory to increase awareness of this entity. Although little attention was dedicated to this matter in the literature, a multidisciplinary approach for the treatment of these patients should always include the dentist, whose role is to intercept pathologies of the oral cavity and ultimately improve their quality of life.

Early onset ONJ in patient treated with denosumab and bevacizumab: case report

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BACKGROUND:
Osteonecrosis of the jaw (ONJ) is a no longer serious complication related to many drugs, notoriously aminobisphosphonate (BP) but also antiresorptives and anti-angiogenetics. Several cases of denosumab-related ONJ have been reported and the overall incidence is similar to that for BP-related ONJ. It is known that, concomitant administration of two or more of these drugs increase considerably the risk of onset and severity of ONJ.
We describe a case report of early onset ONJ in oncologic patient treated with denosumab and bevacizumab, with negative anamnesis for BP administration.
METHODS: In March 2015, a 58-year-old female patient was referred to our department for pain and swelling of upper left maxilla.
Patient reported the following anamnestic data: in 2010, for the diagnosis of breast cancer, she was underwent to right quadrantectomy surgery and radiant treatment; she was treated with monthly subcutaneous injections of 120 mg denosumab (eight doses from April 2014 to November 2014) and bevacizumab (five doses from August 2014 to November 2014); she had no history head and neck radiotherapy and BP administration. One trigger (local risk factor for ONJ) has been recognized: extractions of maxillary left second premolar and first molar have been performed few months before.
Intraoral examination showed a painful area of bone exposure in the left posterior maxilla and erythematous soft tissue with purulent discharge and swelling was detected.
After OPT and CBCT scans, bone necrosis was classified as stage II, according to Bedogni *et al*.
Systemic antibiotic (ampicillina/sulbactam intramuscularly twice daily for 8 days and metronidazole (off-label use) 250 mg orally twice daily for 8 days), local antiseptics (chlorhexidine 0.2% mouth rinses and 0.5% chlorhexidine gel) were administered. The patient was referred to Oral and Maxillofacial surgery for surgical management.
CONCLUSIONS: It is widely described in literature that early-onset ONJ is more hazardous in oncologic patients,

particularly when taking BP plus another ONJ-related drug. It is our opinion that it is necessary to give attention for prevention protocols also to the patients in therapy with all drugs related to ONJ.

Overexpression of Nicotinamide n-Methyltransferase in HSC-2 OSCC cell line: effect on apoptosis and cell proliferation.

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BACKGROUND: The oral squamous cell carcinoma (OSCC) is the most common head and neck malignancy, representing up to 90% of oral cavity cancers. In the last decades, despite progress in therapeutic strategies of OSCC, the 5-year survival rate showed no significant improvement, remaining slightly below 50%.

Nicotinamide N-Methyltransferase (NNMT) is a drug-metabolizing enzyme that is overexpressed in several tumors, including OSCC. In particular, NNMT overexpression in OSCC inversely correlates with lymph node metastasis, pT, pathological staging and histological grading. In addition to the potential role of NNMT as a prognostic factor, the measurement of salivary NNMT could serve as biomarker for early diagnosis of OSCC. In this study, in order to further explore the biological function of NNMT in OSCC cell metabolism, we investigated the effects of plasmid-mediated overexpression of NNMT in OSCC cell line.

METHODS: Human oral cancer cell line HSC-2 was transfected with the pcDNA3-NNMT plasmid. Control cells were transfected with the empty vector (pcDNA3) or treated with transfection reagent only (mock). Real-Time PCR, Western blot, and HPLC assay were used to evaluate NNMT expression, both at mRNA and protein levels. The assessment of cell proliferation was performed with MTT colorimetric assay. Furthermore, the effect of NNMT upregulation on β-catenin, survivin, and Ki-67 expression was also investigated. Data were analyzed using GraphPad Prism software. Differences between groups were determined using the Kruskal-Wallis test.

RESULTS: Compared with mock and pcDNA3-treated, cells transfected with pcDNA3-NNMT displayed significantly increased NNMT expression levels. Real-Time PCR showed a significant NNMT upregulation, that was confirmed at protein level by Western blot analysis. Furthermore, NNMT specific activity was significantly higher in transfected cells compared with controls. The results of MTT colorimetric assay showed that pcDNA3-NNMT plasmid was able to increase cell growth of HSC-2 cells compared with controls.

In order to explore the potential involvement of NNMT in cellular pathways, such as apoptosis, cell proliferation and cell signaling, we examined whether NNMT overexpression was able to affect the expression of β-catenin, survivin, and Ki-67. The results seem to indicate a statistically significant upregulation of survivin ΔEx3 isoform in pcDNA3-NNMT plasmid transfected cells, while the expression of 3B and 2α survivin isoforms was not detectable.

CONCLUSION: Our results show that NNMT overexpression in OSCC cell line significantly increases cell growth. The effect on the antiapoptotic survivin ΔEx3 isoform seems to suggest a possible involvement of NNMT in the proliferation and tumorigenic capacity of OSCC cells.

Prognostic value of mithocondrial DNA analysis in patients with secondary oral squamous cell carcinoma

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BACKGROUND: In head and neck oncology a novel classification of the secondary tumors was recently proposed on the basis of the clonal analysis of the tumors and the genetically altered mucosal field. Second neoplastic lesions can be classified as: Second Primary Tumors (SPTs) independent from the index tumor at the molecular level, Local Recurrences (LRs) or metastases that are instead related to the primary tumor and ''second field tumors" (SFTs), derived from the same genetically altered mucosal field as the primary tumor. The distinction between LR, SPT and SFT is not a simply problem of classification but may influence prognosis and the choice of treatment. mtDNA (D-loop) sequence analysis was proposed in previous studies as a reliable method for establishing the clonal relationship between two neoplastic manifestations. In the present study mtDNA D-loop analysis was applied in a group of consecutive patients experiencing a second loco-regional neoplastic manifestation after surgical resection of a primary Oral Squamous Cell Carcinoma (OSCC). The purpose was to evaluate differences in terms of survival rate between LRs, SPTs and SFTs.

METHODS: The study population consisted of 24 patients who experienced a second neoplastic lesion after a surgical resection of a primary OSCC. 21/24 (87,5%) were limited to the oral cavity whereas 3/24 (12,5%) presented a neck nodal metastasis (LNM) as second event. mtDNA D-loop analysis was performed by deep sequencing and phylogenetic clusterization in all index OSCCs, in all secondary events and in respective normal mucosa. Disease-free survival endpoints was defined as the duration between appearance of second neoplastic lesion and dead of disease or last follow-up visit.

RESULTS: mtDNA analysis showed 7/24 second neoplastic events (31,1%) phylogenetically related to index OSCC, and 17/24 cases (68,9%) phylogenetically independent. The genetic distinction of secondary tumours in LR, SPT and SFT was acquired on the basis of the phylogenetic relationship between normal mucosa of index OSCC and normal mucosa of secondary OSCC. All 7 clonal paired tumours showed respective normal mucosa phylogenetically related, suggesting a genetic diagnosis of LR. Among non clonal patients 3 out of 17 presented respective normal mucosa phylogenetically related, suggesting a genetic diagnosis of SPT whereas in remaining 14 out of 17 non clonal paired lesions also the respective normal mucosa resulted phylogenetically distant entities suggesting the presence of an altered mucosal field and a genetic diagnosis of SFT. The presence of an altered mucosal field in non clonal patients resulted a variable significantly related with a better survival rate ($p<.05$), indeed 2/17 (11,8%) SFTs events failed as compared to 5/7 LR. (71,4%) and 3/3 SPTs (100%).