of *JAK2* V617F by droplet digital PCR as standard of care. The British Committee for Standards in Haematology recommends that suspected MPN cases have investigation of *JAK2* exon 12, *CALR* and *MPL* genes if *JAK2* V617F is negative.
Aims: The aim of the project was to improve the MPN service by substituting sequential analysis of individual target regions within the *JAK2*, *CALR* and *MPL* genes with a single assay, and to increase the number of genes available for analysis.
Methods: A commercial next generation sequencing (NGS) gene panel (Oxford Gene Technology, SureSeq Myeloid Panel), coupled with the Illumina MiSeq platform was validated and implemented. The gene panel utilises hybridization based enrichment technology and consists of 25 MPN–related genes. During the validation stage the following were enriched and analysed: 29 positive control samples with 30 known pathogenic variants, 30 negative control samples without known pathogenic variants in the *JAK2, CALR and MPL* genes, and 24 MPN samples of unknown mutational status. Thus so far over 200 clinical samples have been analysed and reported since the service was introduced in October 2016.
Results: The panel has successfully identified: a large range of known pathogenic variants at high sensitivity (*JAK2* V617F variant allele frequency 1%, *CALR* Type I frameshift variant allele frequency 3%), a potential alternative driver mutation in a known low level *JAK2* V617F positive patient, a rare *MPL* exon 4 pathogenic variant and also the detection of low level *CALR* pathogenic variants, which would not have been detected by Sanger sequencing analysis. In one patient the panel identified the presence of two different *JAK2* exon 14 pathogenic variants in cis (*JAK2* V617F and *JAK2* C618R). The *JAK2* C618R prevented the hybridization of the probe binding site of the *JAK2* V617F ddPCR assay which had led to a false negative result by ddPCR. The validation procedure also explored coverage and limits of sensitivity, potential chemistry specific artefacts and identified common polymorphisms for all 25 genes.
Summary/Conclusions: The panel has replaced the current sequential analysis of *CALR, MPL* and *JAK2* exon 12 in *JAK2* V617F negative patients and reduced turn-around-times with increased accuracy and sensitivity compared to Sanger sequencing and fragment analysis. Our current clinical service operates on a two tier system whereby clinicians can request analysis of the full 25 gene panel or a 4 gene subset (*JAK2, CALR, MPL, CBL* as an *in silico* analysis).

PB2037

IN JAK2V617F POSITIVE MYELOPROLIFERATIVE NEOPLASMS, BLEEDING RISK CORRELATES WITH ALLELE BURDEN
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Background: Myeloproliferative neoplasms (MPN) are characterized by the presence of JAK2V617F mutation that is almost invariably associated with polycythemia vera (PV), but also occurs in the majority of patients with essential thrombocythemia (ET) or primary myelofibrosis (PMF). JAK2V617F-positive patients display different laboratory and clinical features from JAK2-wild type, but no clear correlation was found between the JAK2V617F allele burden and natural history of the disease. The most common causes of morbidity and mortality in MPN are thrombotic and hemorrhagic complications, albeit bleedings are less frequent than thrombosis and mostly represented by minor hemorrhages (ecchymosis, epistaxis, menorrhagia and gingival hemorrhage). The impact of different allele burden on bleeding risk is uncertain.
Aims: Aim of our study is to explore whether there is an association between JAK2V617F allele burden and hemorrhagic complications in a large cohort of MPN diagnosed and followed in a single center.
Methods: We selected 253 MPN (121 ET= 47.8%, 124 PV=49% and 8 PMF=3.2%) carrying JAK2V617F mutation. The median follow-up of patients was 8.8 years (0.1 – 37.3 y). Complete medical history and anti-thrombotic drugs use were recorded. Hemorrhagic complications were classified as "major" or "minor" in agreement with ISTH criteria. The patients were categorized into four groups according to the amount of JAK2 mutant allele, (1st quartile 1-25%, 2nd quartile 26-50%, 3rd quartile 51-75% and 4th quartile 76-100%). Nominal variables were compared with X^2 test or Fisher's exact where indicated. Survival has been evaluated only for groups with different prevalence of events during follow-up and were calculated with the Kaplan Meier method and compared with the Log Rank test.
Results: Three patients (1.2%) bleed at diagnosis (1 major and 2 minor hemorrhages) while 27 (11.8%) suffered for hemorrhages during follow-up (10 major and 17 minor). Prevalence of hemorrhages results higher in 4th quartile compared both to 2nd (p=0.003) and to 1st (p<0.001) quartiles. Hemorrhages-free survival is confirmed lower in 4th quartile compared both to 2nd (p= 0.004) and to 1st (p<0.001). The incidence rate of hemorrhages are respectively 0.7/100 pats /y for 1st quartile, 0.65/100 pats /y for 2nd quartile, 1.26/100 pats /y for 3rd quartile and 3.23/100 pats /y for 4th quartile with a IRR of 5 and of 4.6 for the 4th quartile respectively *versus* 2nd and 1st one. No statistically significant difference has been demonstrated in the use of anti-thrombotic drugs among patients of the different quartiles.
Summary/Conclusions: Risk factors for hemorrhage in MPN are not well defined, and there is no risk estimation model for this outcome. Acquired von Willebrand disease, entity of platelet increased count and aspirin use have been implicated in bleeding occurrence. Previous reports fail to demonstrate a correlation between JAK2 mutation and bleeding risk. In contrast, in our cohort we found a significantly higher incidence of bleeding manifestations during follow-up in patients with higher allele burden. Interestingly no differences were seen in administration of anti-thrombotic drugs among quartiles, suggesting an independent role of JAK2 allele burden in the different distribution of hemorrhagic events.

PB2038

JAK2 ALLELE BURDEN IN PATIENTS WITH PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE NEOPLASMS
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Background: The JAK2V617F allele burden (JAK-AB) plays a central role in chronic myeloproliferative neoplasms (cMPNs); its presence has also been advocated in the differential diagnosis of cMPNs and as independent risk factor for venous thromboembolic complications. New treatment with Ruxolitinib may decrease JAK-AB but at the present, it is not clear the clinical advantage of such reduction
Aims: Primary aim of the current study was to evaluate at diagnosis the JAK-AB in patients with Philadelphia negative cMPNs, in order to evaluate any association with standard demographic, clinical and laboratory parameters with particular reference to thrombotic risk**.**
Methods: Peripheral blood samples from patients with Ph-negative cMPNs were collected, DNA from leucocytes was analysed for Jak-2 (V617F) gene mutation with amplification-refractory mutation system (ARMS) PCR, subsequently a real-time quantitative polymerase chain reaction (qRT-PCR) for JAK2V617F allele burden measurement was applied. A multivariate analysis was than performed to evaluate any association of AB with demographic and clinical data.
Results: One hundred and twelve patients with Philadelphia negative cMPNs were investigated: 52 females with a median age at diagnosis of 69 years (age range: 18-95 years), 60 males with a median age of 68 years (age range: 18-82 years). Thirty-four patients had Essential Thrombocythemia (ET), fifty-two had Polycythaemia Vera (PV) and twenty-six had primary myelofibrosis (PMF). JAK2-AB of patients with an age of <69 years and ≥69 years, was respectively evaluated. Patients older than 69 years showed a significantly higher JAK2-AB . JAK-AB was significantly reduced in ET, when compared to PV and PMF. No correlation was found between median values of allele burden and IPSS and DIPSS scores. In patients with PV (n=52), a significant correlation was observed between allele burden and WHO2008 scoring system. No significant correlation was found between allele burden and thrombotic risk according to IPSET-t and IPSET-ET for PV and ET, respectively. Patients with a previous history of thrombosis had the highest JAK2-AB. In PMF, a positive correlation between JAK-AB and grading of fibrosis was found only for the highest grades (PMFIII and IV). JAK-AB had a positive correlation with splenomegaly in PMF.
Summary/Conclusions: Our report cannot confirm any correlation between allele burden and thrombotic risk, according to currently adopted scoring systems. A previous history of thrombosis is however associated with the highest AB in all cases.

PB2039

COMPARISON OF CLINICAL AND LABORATORY DATA, INCLUDING JAK-2 46/1 HAPLOTYPE, BETWEEN PATIENTS WITH IDIOPATHIC ERYTHROCYTOSIS AND POLYCYTHEMIA VERA.
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Background: Idiopathic erythrocytosis (IE) is a relatively rare finding characterized by an increased red blood cell mass without an identifiable cause. Diagnosis of IE is based on the exclusion of primary and secondary erythrocytosis including JAK2-wild-type polycythemia Vera (PV).
Aims: In the current study, we report clinical features and laboratory data able to discriminate IE from PV, at diagnosis
Methods: We have here analyzed clinical and laboratory parameters, including Jak-2 46/1 haplotype, from patients with a confirmed diagnosis of IE and PV, followed from January 2010 to December 2016. Data were statistically analyzed, nominal variables were compared with X^2 test and continuous variables with the Mann-Whitney test.
Results: Overall, 40 patients with IE and 93 patients with PV were included in the current analysis (Table 1). Splenomegaly and itch were reported only in one patient with IE. History of thrombosis and cardiovascular events was positive in one case with IE. Jak-2 (V617F) and exon 12 mutations were negative in all patients with IE, while Jak-2 46/1 haplotype was found at heterozygous state in 18 patients and at homozygous state in 2 patients with IE.

Table 1.

	PV	IE	p
Patients N.	93	40	
MALE N. (%)	59 (63,44%	38 (95%)	0.0001
FEMALE N (%)	34 (36.56%)	2 (5 %)	0.0001
MEDIAN AGE AT DIAGNOSIS, YEARS	66 (23 - 95)	58 (17 - 83)	0.007
SPLENOMEGALY N° (%)	38 (40,86%)	1 (2,5%)	0.000001
ITCH	36 (38,70%)	1 (2,5 %)	0.00000 1
MEDIAN WBC COUNT X10⁹	9,3 (4,535,2)	8.1 (4,2-14,3)	0.03
MEDIAN HB g/dl	17,5 (15,1-21,0)	17,4 (16,1-19.1)	0.9
MEDIAN HT %	53,3 (48.4 - 64.3)	52,4 (48.2 - 55.3)	0.1
MEDIAN PLTS COUNT X 10⁹	435.0 (270-1013)	216 (178.339)	0.001
V617 F OF JAK2 POSITIVE N. (%)	86 (92,47)	0	
JAK2 EXON 12 MUTATION N. (%)	2 (2,15%)	0	
HAPLOTYPE 46/1 OF JAK2, ETHEROZIGOUS	42 (45,16%)	18 (45,0%)	0,98
HAPLOTYPE 46/1 OF JAK 2, HOMOZIGOUS	25 (26,88%)	2 (5 %)	0.008
PATIENTS WITH CARDIOVASCULAR EVENTS OR THROMBOSIS N %	32 (34,4%)	1 (2,5%)	0.000008

Summary/Conclusions: In the current study, we highlight peculiar clinical and laboratory findings of IE, in comparison with Polycythemia Vera. As shown by available studies, Hb and HCT level do not easily discriminate between the two categories of patients while gene panels may be useful to improve diagnostic accuracy of IE. We have here first observed the presence of Jak-2 46/1 haplotype in approximately half patients with IE, even in absence of JAk-2 mutations; the homozygous status was statistically different among PV and IE patients. The role of such association deserves further specific studies.

PB2040

LABORATORY RESPONSIVENESS OF LOW-DOSE ASPIRIN IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Background: The essential thrombocythemia (ET) is a myeloid neoplasm characterized by platelet hyperreactivity and thrombosis. The daily low-dose aspirin (ASA) is a cornerstone in the prevention of the thrombotic events. In the ET an accelerated platelet turnover translates in a renewal of the drug target shortening the duration of cyclooxygenase (COX-1) inhibition and may dictate new dosing strategies particularly in ASA "low-responders" patients.
Aims: Therefore, we evaluated platelet count, β-thromboglobulin (β-TG) and platelet factor 4 (PF4), as markers of platelet activation, the platelet function activity (PFA), as indicator of ASA platelet sensitivity.
Methods: We studied 60 patients (20 men, 40 women; mean age 51 years, range 32-70) with ET according to WHO criteria. The mean duration of disease was 11 years. All patients were on ASA 100 mg once daily. Of the 60 patients, 45 were on anagrelide hydrochloride (daily dose 1.5 mg) (10 men, 35 women), 15 were on hydroxyurea (daily dose 2 mg) (10 men 5 women). None had inherited or acquired thrombotic risk factors. Sixty subjects served as controls. Platelets were measured by automated analyzer. β-TG and PF4 were determined by ELISA. ASA platelet sensitivity was measured by Platelet Function Analyzer (PFA-100).
Results: The mean platelet count was 455±200x109/L. All patients had normal β-TG and PF4 ((12±5 IU/ml and 4±1 IU/ml) and prolonged C/EPI closure time (T, unit: s, n.v. 84-160 s) (249±40 s).
Summary/Conclusions: These findings suggest that in ET patients the daily low-dose ASA represents an optimal dosing strategy and that PFA test may be an useful tool to distinguish between the ASA "normal-responder" and "low-responder" ET patient.

PB2041

CLINICAL AND EXPERIMENTAL CHARACTERISTICS OF MYELOID/ LYMPHOID NEOPLASMS DISPLAYING PDGFRA OR PDGFRB REARRANGEMENT

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Background: According to the 2016 revision to the WHO classification of myeloid neoplasms and acute leukemia, the cases with rearrangement of tyrosine kinase (TK) genes PDGFRA, PDGFRB are classified in Myeloid/lymphoid neoplasms with eosinophilia and rearrangement of PDGFRA, PDGFRB, or FGFR1, or with PCM1-JAK2. It is a rare event that patients presented rearrangements with these genes. And in the past decade, the dose of TKI to cases with PDGFRA and B abnormal was inconclusive.
Aims: The goal of the study was to assess the clinical and experimental characteristics and observe the response of Imatinib(IM) therapy of Myeloid/lymphoid neoplasms with PDGFRA or B abnormal.
Methods: Cytogenetic examination of bone marrow cells obtained from patients was performed by 24h culture method. R banding technical was used for karyotype analysis. PDGFRA and B gene rearrangement were detected by FISH using triple-color of 4q12 and dual color break-apart PDGFRB probes. The fusion genes of rearrangements of PDGFRA and B genes were detected by RT-PCR. Immunophenotype analysis was carried out by flow cytometry. Most of all cases were treated with IM and followed up.
Results: The diagnoses included 27 cases of MPN, 1 case of AML-M2 and 1 case of non-hodgkin lymphoma. 21 cases were PDGFRA rearrangement, the other 8 were PDGFRB abnormal, 7 of 8 were EP fused gene, one of which concurrent with DEK-CAN fused gene, and the eighth had MYO18A-PDGFRB. 7 cases of the 8 PDGFRB rearrangement had a primary abnormality with t(5;12)(q33;p13) and the other one had a secondary abnormality of AML-M2. PDGFRA and B genes rearrangement detected by FISH and multiple-RT-PCR were positive. The immunophenotypical analysis showed myeloid or lymphoid. These cases achieve rapid and durable remissions on IM.
Summary/Conclusions: In summary, patients with significantly anemia and eosinophilia should be screened for the presence of PDGFRA and B rearrangements. The dual-colour FISH is a simple approach and should be added into the diagnostic work-up because these patients respond to imatinib therapy, and sustained responses have been observed. The OS of PDGFRA and B abnormal was similar with a previous report in a western population and another Chinese hematology center.

PB2042

PLATELET AGGREGATION STUDY OF ESSENTIAL THROMBOCYTHEMIA TREATED WITH ANAGRELIDE

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Background: Essential thrombocythemia (ET) is a myeloproliferative neoplasm characterized by thrombocytosis and abnormal megakaryocyte proliferation. Patients with elevated platelet count are considered to be a high-risk group for thromboembolic and/or hemorrhagic complications. In Japan, anagrelide treatment was recently approved for the 1st line as a cell reduction therapy on ET. Even now, there are few study whether the risk of thrombosis has decreased after anagrelide treatment. Moreover, the platelet count problem uncertainty remains what is the best practice to follow when the platelet count in platelet-rich plasma (PRP) exceeds about 600 x10^9/L, in the recent recommendations for the standardization of light transmission aggregometry by the platelet physiology subcommittee of Scientific and Standardization Committee /International Society of Thrombosis and Hemostasis.
Aims: The aim of this study was to characterize the platelet aggregation (PA) in patients with ET. We would also clarify whether there were any changes of hemostatic side effect and platelet aggregability before and after treatment with anagrelide.
Methods: This study has been conducted with blood sample obtained from six healthy subjects, compared to 18 consecutive patients with ET. None of the patients was taking anticoagulants or cytoreductive agents. We also studied six anagrelide-treated patients with ET. Whole blood aggregometry (WBA) and LTA using PRP were performed. ADP-induced PA or collagen-induced PA used natural count PRP and platelet count adjusted PRP with platelet-poor plasma. Data were compared in the groups using the Tukey-Kramer test. This study was approved by the Ethical committee of our hospital. All study procedures were performed in accordance with the Declaration of Helsinki.
Results: The result of WBA was not obtained, because the filter was obstructed by giant platelets. In the natural PRP, even over 900x10^9/L, the platelet aggregability was markedly increased compared with the control (ADP-induced PA: p=0.023, collagen-induced PA: p=0.001), but, was not significantly different (ADP-induced PA: p=0.703, collagen-induced PA: p=0.986) in the count adjusted PRP. These results were not confirmed in cases with platelet counts of less than 600x10^9/L. There was no decrease in platelet aggregation before and after treatment with anagrelide (ADP-induced PA: p=0.3403, collagen-induced PA: p=0.514).
Summary/Conclusions: In the ET patients with platelet counts more than 900x10^9/L, the platelet aggregation by LTA with natural count PRP was remarkably accelerated and this data seemed to reflect the disease state. Although treatment with anagrelide showed cyto-reductive effect without any hemorrhagic complication in patients with ET, it did not fully reduce platelet aggregability.

PB2043

A SINGLE CENTRE EXPERIENCE OF MASTOCYTOSIS

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Background: Mastocytosis considered as a subcategory of myeloid neoplasms based on World Health Organization (WHO) 2016 classification, is characterized by expansion and accumulation of abnormal clonal mast cells in