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ABSTRACT BOOK

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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).

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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.

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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.

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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.