Methods: 33 newly-diagnosed patients with PMF were analyzed in this study. Baseline RDW values were obtained in addition to other routine blood analyses (CRP, LDH, complete blood count and iron metabolism parameters) and *JAK2* V617F mutational status. Patients were staged according to IPSS prognostic scoring system, liver and spleen size were assessed by palpation. The Mann Whitney U test, the Pearson correlation and the X^2 test/ the Fisher test were used where appropriate. Survival analyses were performed using methods of Kaplan and Meier, the log-rank test and the Cox regression analysis. All statistical tests were two-sided and P values <0.05 were considered significant.
Results: Median RDW was 19.0% (15.2% - 22.5%). RDW correlated significantly with hemoglobin (p=0.005), CRP (p=0.031), spleen size (p=0.036) and IPSS score (p=0.003). Patients with more pronounced anisocytosis had an inferior overall survival (OS)-very-high RDW (≥19.0%) *vs* high RDW (15.1% - 18.9%) subgroup, HR 5.37, p=0.002. RDW remained significantly associated with OS (p=0.002) in a multivariate model including IPSS score, hemoglobin level and CRP.

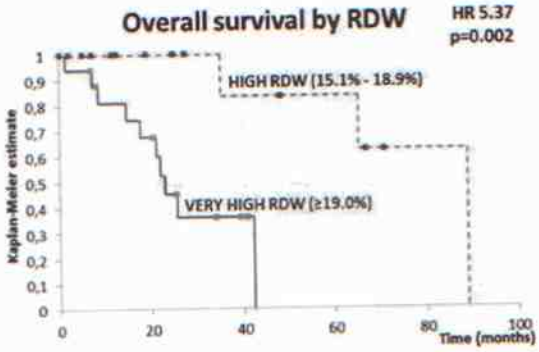

Figure 1.

Summary/Conclusions: PMF pathogenesis surpasses inflammation as only cause of anisocytosis. A higher degree of anisocytosis is associated with more advanced disease features and a decreased overall survival. RDW encompasses standard prognostic score and may help in the rapid detection of patients with an unfavorable prognosis.

PB2036

RISK FACTORS FOR THROMBOTIC COMPLICATIONS IN PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

E Simonovic[1,*], L Macukanovic-Golubovic[2], V Colic[1]
[1]Internal Medicine, General Hospital, Leskovac, [2]Internal Medicine, Clinic of Haematology, Nis, Serbia

Background: Myeloproliferative neoplasms (MPN) are a group of clonal hematopoietic stem cell malignancies, characterized by overgrowth of one or more blood lines with normal or nearly normal maturing of those cells in the bone marrow and extramedullary hematopoietic organs. MPN are acquired prothrombogenic conditions. The mechanism of increased predisposition to thrombosis in myeloproliferative neoplasms is not clear enough. It is thought that the mechanisms that lead to thrombosis in MPN are the following: increased blood cell mass; abnormal platelet function and the phenomenon of spontaneous aggregation. The following factors have been associated with the incidence of thrombosis: the increased level of products formed in the activation of platelets (thromboxane, p-selectin); increased microparticle formation as the part of a membrane with various cell structures of platelet origin; JAK2V617F mutation. In MPN patients an increased activity of coagulation system occurs due to resistance to the anticoagulant function of thrombomodulin.
Aims: The aim of this study is to monitor potential risk factors for the development of thrombotic complications in patients with Philadelphia-negative chronic myeloproliferative neoplasms.
Methods: During the five-year period we monitored the occurrence of thrombotic complications in 139 patients of both sexes, aged between 30 and 87 years, being diagnosed with Ph-myeloproliferative neoplasm. Patients were classified into the following groups: 1. Group with the polycythemia vera (PV) (61); 2. Group with essential thrombocythemia (ET) (28); 3. Group with idiopathic myelofibrosis (IMF) (25); 4. Group with unclassified myeloproliferative neoplasm (MPNs) (25). The following possible risk factors were monitored: age, leukocyte count, platelet count, the presence of JAK2V617F mutation, cardiovascular risk factors (smoking, hypertension, diabetes mellitus, dyslipidemia). We used methods of clinical, laboratory, ultrasound and CT scans.
Results: The highest percentage of thrombotic complications (arterial and venous) was found in the group of patients with ET and MPNs (p <0,01), and then in the group with PV (p <0,05). In all three groups, the incidence of thrombotic complications in patients older than 60 years was higher (p<0,001). The leukocyte count ranged from 2,2-17,1x10^9/L and the platelet count ranged from 10,2 -1856,5x10^9/L. The highest leukocyte count was recorded in the group of patients with PV and MPNs (p <0,001) and the lowest in the group of patients with the IMF (p <0,01). The highest platelet count was found in the group of patients with ET, and the lowest in the group of patients IMF. Thrombotic complications in those groups were more frequent in percentage with patients with leukocytosis, but statistical significance was present only in the group with MPNs. No statistical significance was detected between the platelet count and thromboembolic complications in either group. Thrombotic complications were more frequent in JAK2V617F positive patients, but the statistical significance existed only in the group with PV. Considering cardiovascular risk factors only hypertension was significantly more common in the group with PV and MPNs. The largest number of patients with thrombotic complications had two or more cardiovascular risk factors (p <0,05).
Summary/Conclusions: The patients over 60 years of age, as well as the presence of two or more cardiovascular risk factors are the most important for the incidence of thrombosis. Leukocytosis and JAK2V617F may be considered as potential risk factors for thrombosis in patients with myeloproliferative neoplasms, particularly with PV, ET I MPNs. Further follow-up and a larger number of subjects are needed. The follow-up of patients with unclassified myeloproliferative neoplasms has particularly important, which showed a high prevalence of thrombotic complications, and with the aim of their further differentiation.

PB2037

JAK2, MPL, AND CALR MUTATIONS IN CHINESE HAN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

J Xu*
Clinical Hematology, Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School, Nanjing, China

Background: Essential thrombocythemia (ET) is a chronic Philadelphia chromosome-negative myeloproliferative neoplasm characterized by the overproduction of circulating platelets in the periphery due to the excessive proliferation of megakaryocytes in the bone marrow [1]. The recurrent Janus kinase 2 (JAK2) V617F mutation has been an important molecular marker for myeloproliferative neoplasms (MPNs) since its discovery in 2005 [2]. However, only 50-60% of ET cases are associated with the JAK2 V617F mutation. Among the 40% of patients with ET who lack the JAK2 V617F mutation, 3-5% carry mutations at codon 515 of the gene encoding the thrombopoietin receptor, a myeloproliferative leukemia virus oncogene (MPL) [3]. In 2013, somatic mutations in calreticulin (CALR) were found in 20 to 25% of patients with ET or primary myelofibrosis (PMF) [4,5]. Like JAK2 and MPL mutations, somatic mutations of Calreticulin (CALR) have been identified as a potentially powerful diagnostic tool for patients with ET [6].
Aims: we studied a population of patients with ET and analyzed the frequency of JAK2, CALR, or MPL mutations as well as patients' hematological characteristics.
Methods: The patients and the data were selected retrospectively from the myeloproliferative neoplasm database established for scientific research at the Department of Hematology of Drum Tower Hospital. A total of 110 patients with ET were enrolled (60 females and 50 males with a mean age of 55.7 years, range 13–88 years); they had been diagnosed at the Department of Hematology between 2012 and 2015 according to the WHO Classification of Tumours of Hematopoietic and Lymphoid Tissues (2008)[7]. The clinical and laboratory data were reviewed from medical records.
Results: Among the 110 patients tested, JAK2 V617F was the most common mutation, observed in 62 patients (56.3%), while CALR mutations were detected in 21 patients (19.1%). One patient (0.9%) carried the MPL W515L mutation. A mutation in JAK2 exon 12 was not detected in any patient. Two ET patients had both CALR and JAK2 V617F mutations. The incidence of triple-negative (negative for JAK2/MPL/CALR) patients was 25.5% (28/110).
Summary/Conclusions: In summary, we have described the mutation profile of our Chinese cohort of ET patients. CALR mutations are a useful diagnostic marker for JAK2/MPL-negative ET patients because they are typically mutually exclusive with a JAK2 mutation and are present in a relatively high frequency.

PB2038

WHAT IS THE SECRET OF THE JAK2 MUTATION ALLELE LOAD STABILITY IN SOME PATIENTS WITH MPN?

T Subbotina[1,2,*], I Olkhovskiy[1,3], R Shaikhutdinova[2], E Dunaeva[4], K Mironov[4], A Gorbenko[1], M Stolyar[1,2], E Vasiyliev[1,5], M Mikhalev[1,6], G Shipulin[4]
[1]Krasnoyarsky branch of Hematology Research Center Russian Ministry of Health Ministry of Health, [2]Siberian Federal University, [3]Krasnoyarsk Scientific Center SB RAS, Krasnoyarsk, [4]Federal Budget Institute of Science «Central research institute for Epidemiology», Moscow, [5]Krasnoyarsk regional hospital, [6]City Clinical Hospital № 7, Krasnoyarsk, Russian Federation
Background: It is known that the level of JAK2 mutation allele load in patients with myeloproliferative neoplasms (MPN) in the disease dynamics can be continuously stable and not be associated with clinical symptoms or effects of hydrox-

yurea (HU) (Theocharides A et al, Haematologica, 2008; Besses C et al, Br J Haematol, 2011). Reasons and mechanisms of this kind of «homeostasis» in the level of circulating mutation JAK2 positive cells in some patients are unclear.
Aims: Evaluate the level of JAK2 mutation allele load in MPN patients in the disease dynamics and HU effects.
Methods: This study included 14 JAK2 positive (V617F or exon 12 mutations) MPN patients. The informed consents from these patients were obtained. DNA was extracted from venous blood leukocytes. Quantification of JAK2 V617F and JAK2 exon 12 mutations allele load was performed by pyrosequencing method as described in (Dunaeva E et al Klin Lab Diagn, 2014). JAK2 variance (MUT) was calculated as a measure of relative changes in allele load between the baseline and follow-up sample (Theocharides A et al, Haematologica, 2008).
Results: Following variants were identified according to the level of JAK2 mutation allele load in the disease dynamics and HU effects (Table 1): 1) The level of JAK2 mutation allele load was increased more than twice (№3 and №13). Until now this patients do not have the significant clinical MPN manifestations and do not need HU-treatment; 2) The level of JAK2 mutation allele load was found to remain stable over follow-up time of observation independently of whether patients were already or not under HU treatment at the time of first sampling; 3) The level of JAK2 mutation allele load was reduced after HU treatment. We did not find any dependency between the allele load dynamics and patient's clinical status, disease phenotype, disease duration and venous blood cellular account.

Table 1. Characteristics of the patients included in the study.

№	Age of manifestation (years)	Sex	Disease	HU	% JAK2 mutation baseline	% JAK2 mutation last sample	JAK2 variance (MUT)	Time between two assessments (mo)
JAK2 V617F mutation-positive patients								
1	67	F	ET*	No	26	27	n.a.	6
2	70	F	PV	No	34	32	n.a.	10
3	71	F	PV	No	16	35	+115	18
4	59	F	Post-PV MF	Yes	82	88	n.a.	15
5	51	F	PV	Yes	76	73	n.a.	20
6	44	F	PV	Yes	69	77	+11	12
7	59	F	PV*	Yes	42	42	n.a.	27
8	45	F	ET*	Yes	8	7	n.a.	36
9	75	F	Post-ET MF*	Yes	14	10	-36	18
10	57	F	PV*	Yes	31	22	-29	6
11	59	F	PV	Yes	81	54	-33	36
12	35	M	MF	Yes	27	20	-27	15
JAK2 exon 12 mutation-positive patients								
13	61	M	PV*	No	15	40	+166	24
14	48	M	PV*	No	11	11	n.a.	4

The allelic load was determined at the primary address to the doctor with MPN symptoms

Summary/Conclusions: Our data suggest the JAK2 mutation allele load can remain stable for a long time in some patients. The observed increase of the mutation allele load in untreated patients probably will be to increase to the level of its stabilization at full development of the clinical MPN picture. The differences of the allele load dynamics between individual patients may be associated with the individual features of the intracellular signaling networks and will be the subject of additional studies.

PB2039

PLATELET COUNT AS A RISK FACTOR FOR HEMORRHAGIC COMPLICATIONS IN PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

E Simonovic[1,*], L Macukanovic-Golubovic[2], V Colic[1]
[1]Internal Medicine, General Hospital, Leskovac, [2]Internal Medicine, Clinic of Haematology, Nis, Serbia

Background: Myeloproliferative neoplasms (MPN) are a group of clonal hematopoietic stem cell malignancies, characterized by overgrowth of one or more blood lines with normal or nearly normal maturing of those cells in the bone marrow and extramedullary hematopoietic organs. Hemorrhagic syndrome is a complication that occurs in about a quarter of patients with PV and even 60% of patients with ET. Bleeding can complicate the clinical course of IMF. It has been manifested in the form of petechiae and ecchymoses, or may be life-threatening as uncontrolled esophageal bleeding. It occurs due to ineffective megakaryocytopoiesis, retention of platelets in the enlarged spleen, qualitative platelet disorders, acquired deficiency of factors V and vWF, disseminated intravascular coagulation. The increased platelet count affects the adsorption of the larger von Willebrand multimers onto the platelet membrane, thus acting to eliminate them from circulation and degradation.
Aims: The aim of this study is to monitor the platelet count as a potential risk factor for occurrence of hemorrhagic complications in patients with chronic myeloproliferative neoplasms.
Methods: During the three-year period we monitored the occurrence of hemorrhagic complications and platelet count in 120 patients of both sexes aged between 27 and 86 years, being diagnosed with Ph-myeloproliferative neoplasms. Patients were classified into the following groups: 1. Group with the polycythemia vera (PV) (51); 2. Group with essential thrombocythemia (ET) (24); 3. Group with idiopathic myelofibrosis (IMF) (20); 4. Group with unclassified myeloproliferative neoplasm (MPNs) (25). We used methods of clinical, laboratory, endoscopy, ultrasound and CT scans.
Results: Platelet count ranged from 2,2-2134,5,1x10^9/L. The highest platelet count was recorded in the group of patients with ET and MPNs ($p<0,001$) and the lowest in the group of patients with the IMF ($p<0,01$). There was no statistical significant difference detected between the groups of patients with PV and MPNs with regard to platelet count. The highest percentage of hemorrhagic complications was found in the group of patients with ET and IMF ($p<0,01$) and then in the group with MPNs. Hemorrhagic complications have been more frequent in patients with platelet count below 10x10^9/L ($p<0,05$) and in patients with platelet counts over 1000x10^9/L ($p<0,01$). Life-threatening bleeding complications were the most common in patients with platelet count below 5x10^9/L and in patients with platelet count over 1500x10^9/L. Haemorrhage was the cause of mortality in 15% of patients with MPN.
Summary/Conclusions: The platelet count can be considered as a significant parameter for monitoring the risk of hemorrhagic complications in patients with myeloproliferative neoplasms, particularly with ET and IMF. Further follow-up and a larger number of subjects are needed. The follow-up of patients with unclassified myeloproliferative neoplasms has particularly important, which showed a high prevalence of hemorrhagic complications, and with the aim of their further differentiation.