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Original article

EVALUATION OF THE CORRELATION BETWEEN JAK2V617F, LEUCOCYTOSIS AND THROMBOGENIC RISK IN PATIENTS WITH POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA.

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SUMMARY

Thrombotic events (TE) are the most common, debilitating and fatal complications in Polycythemia Vera (PV) and Essential Thrombocythemia (ET). Approximately 20% of patients have experienced TE at the time of diagnosis. The subsequent cumulative incidence of non-fatal thrombosis is 3.8 incidents/100 patients / year [1, 2]. The risk stratification divides patients by age and a history of thrombosis. There is controversy in the literature about the impact of JAK2 V617F allel burden and changes in peripheral blood counts on the TE frequency.

Aim: The aim of this study is to evaluate the influence of JAK2 V617F allel burden and changes in leukocyte count on the development of thrombotic events in patients with PV and ET.

Methods: We performed a retrospective analysis of the data of 96 patients with PV and 43 patients with ET diagnosed according to the WHO criteria. JAK2 mutational burden was determined by RealTime-PCR. The statistical analysis was performed with SPSS 19.0 software.

Results: In the group of PV patients, thrombotic events were recorded in 46 patients. We did not find a correlation between higher levels of JAK2 V617F allel burden and frequency of TE. Clinically, patients with a higher mutational burden had more pronounced splenomegaly. There was no correlation between leukocytosis at the diagnosis and the number of TE, but there was a significant correlation between leukocytosis and the presence of splenomegaly.

In the group of ET patients (43) we established TE in 13 of them, and 54% were carriers of JAK2 V617F. There was no relationship between the frequency of TE and the increased leukocyte count. Unlike PV patients, the presence of JAK2 V617F ≥50% was associated with pronounced leukocytosis in the group with ET patients.

Conclusions: The JAK2 V617F allel burden and leukocytosis level are prothrombogenic, but are not a-predetermining factor for thrombosis. The current risk stratification criteria for PV and ET, although common, reflect the complexity of thrombotic complications. Clotting mechanisms are different not only in both diseases but also in different parts of the vascular system. More studies are needed involving the functional state of the vascular system to help differentiate individual arterial and venous thrombotic index for each disease.

Key words: JAK2 V617F mutation, thrombosis, myeloproliferative neoplasm, polycythaemia vera (PV), essential thrombocythaemia (ET), leukocytosis, splenomegaly, risk stratification,

INTRODUCTION

Thrombotic complications play a crucial role in the disease and survival of PV and ET. In the known ECLAP study, cardiovascular mortality was found in 41% of all cases (1.5 deaths /100 patients / year) mainly due to myocardial infarction (MI), acute ischemic stroke (AIS), pulmonary embolism (PE), splanchnic vein thrombosis (SVT). [1] Their pathogenesis is multifactorial. [2]. Changes in the number and characteristics of affected cell populations probably depend on the degree of allel burden of the characteristic driver mutation - JAK2 V617F and basically alter the blood hemorheology [3-6]. They modify each of the factors in the Virchow Triad and act prothrombogenically. In PV patients the presence of allel burden of JAK2 V617F ≥50% should predispose to a higher incidence of TE compared to patients with burden of JAK2 V617F <50% on proven mechanisms [7-11]. For ET, the presence of JAK2 V617F should also in-

crease thrombogenic risk [12-14]. So far, the results obtained in several studies, however, are contradictory [3-6, 8, 10, 11]. On the other hand, hemorheology and coagulation disorders include various types of compensatory mechanisms balancing the maintenance of homeostasis [15]. Numerous well-structured studies have not found a link between changes in standard hematologic indicators and thrombotic process [2-11, 14, 16]. At the same time, the main findings of the CYTO-PV study prove that achieving a target hematocrit of less than 45% resulted in significant reductions in cardiovascular mortality rates and thrombosis [17].

AIM

The aim of this study is to evaluate the influence of JAK2 V617F allel burden and changes in leukocyte count on the development of thrombotic events in patients with PV and ET.

MATERIALS AND METHODS

We collected and analyzed the data from 96 patients with PV and 43 with ET retrospectively. The diagnosis was based on the WHO criteria for the disease [18]. In all patients, complex studies including standard haematological laboratory values, molecular analysis for JAK2 V617F, abdominal ultrasound assessment (to evaluate spleen size), and bone marrow histology were performed. The patient characteristics at the onset of the disease are summarized in tables 1 and 2.

Table 1. PV Characteristics of the Patients at Baseline

Characteristic	No patient	values
Age at recruitment-yrs/ Median / Range	96	58.6/20-80
Distribution <60 years / ≥60 years	96	48/52
Sex / Male / Female	96	46/50
Platelet, count, x 10^9/l < 1000 / ≥1000	96	87/9
Platelet, count, x 10^9/l / Median / Range	96	538/100-1300
Hemoglobin, g/l / Median /Range	96	195/155-237
Hematocrit, l/l/ Median / Range	96	0,58/0,46-0,78
White-cell, count, x10^9/l /Median/ Range	96	12,7/5,0-43,0
Red-cell, count, x10^{12}/l/ Median / Range	96	7,09/5,0-9,9
LDH/Median/ Range	96	633/239-4021
$JAK2^{WT}$	96	11
$JAK2^{V617F}$alel burden<50%	96	45
$JAK2^{V617F}$allel burden ≥50%	96	40
Palpabre splenomegaly	96	63
Splenomegaly, size, mm	63	143x63

Table 2. ET Characteristics of the Patients at Baseline

Characteristic	No patient	values
Age at recruitment-yrs/ Median / Range	43	57/23-81/
Distribution <60 years / ≥60 years	43	20/23
Sex / Male /Female	43	19/24
Platelet, count, x 10^9/l < 1000 / ≥1000	43	18 /25
PLT, count, x 10^9/l / Median / Range	43	1207
Hemoglobin, g/l / Median / Range	43	140/81-174
Hematocrit, l/l/Median / Range	43	0,42/0,27-0,56
White-cell, count, x10^9/l /Median / Range	43	12,1/4,1-54,2
Red-cell, count, x10^{12}/l /Median / Range	43	4,89/2,9-7,3
LDHU/l / Median/ Range	43	547/228-1642
$JAK2^{WT}$	43	25
$JAK2^{V617F}$allel burden <50%	43	15
$JAK2^{V617F}$allel burden ≥50%	43	3
Splenomegaly, size mm / patient %	43	134/22

JAK2 V617F mutational burden was determined in DNA isolated from 200 µl EDTA anticoagulated whole blood by RealTime-PCR with Ipsogen JAK2 MutaScreen Kit, QIAGEN, Germany, according to manufacturer's instructions on QuantStudio Dx instrument (Applied Biosystems). The results were classified as negative (allel burden < 2%), low positive (allel burden 2–50%) and high positive (allel burden ≥50%).

For a significance level where a zero hypothesis was rejected p <0.05 was selected. The following statistical methods were applied: descriptive analysis; ANOVA to compare the average levels of more than two variables; correlation analysis to detect a relationship between 2 magnitudes; Chi-Square tests to check proportional differences. The statistical analysis was performed with SPSS 19.0 software.

RESULTS

Demographic data:

The average age of 58.6 years (20 - 80 yrs) has been established in the group of patients with PV (96). A small prevalence of the female sex (women: men 52%: 48%) was determined. In the group of patients with ET (43) the average age of 57 years (23-81) was established, the female sex has had prevalence (women: men –53%: 47%).

Polycythemia vera:

The impact of the mutational burden of JAK2 V617F and changes in haematological markers for TE development was investigated in all 96 patients. From all of the patients, 11 were Wild Type (11%), 45 were with JAK2 V617F mutational burden <50% (47%) and 40 were with JAK2 V617F mutational burden ≥50% (42%) (JAK2 exon 12 assay not conducted). We registered a total of 46 TEs. There was no significant difference in the incidence of TE between the group with JAK2 V617F <50% and those with JAK2 V617F e"50%, but we found a higher incidence of single TE in women (who are more often homozygotes) and almost twice the repeat incidence of TE in men. (Figure 1). More pronounced allel burden showed no influence on the degree of leukocytosis, and patients in all three groups had similar values (figure 2). No difference in leukocyte count was found in patients with and without TE. We have not found a correlation between leukocytosis and other important parameters such as number of TE, type of TE (arterial, venous), form of TE (MI, AIS, PE, SVT). The exception is a significant correlation (ANOVA p = 0.024) between leukocytosis (Leu=13.08±4.4x10^9/l) in patients with splenomegaly and normal leukocyte count in patients without splenomegaly (Leu=11.17±3,7x10^9/l). However, the allel burden of JAK2 V617F correlates with the degree of splenomegaly. For example, splenomegaly ≥150 mm is not detected in any patient with JAK WT, but is detected in 25% of patients with JAK2 V617F allel burden <50% and in 62.5% of patients with JAK2 V617F allel burden ≥50% (Pearson p<0.03) (figure 3).

Fig. 1. Frequency of TE and allel burden at PV

We did not find correlation between the incidence of thrombotic events and mutational burden of JAK2^{V617F} (Pearson=0,096)

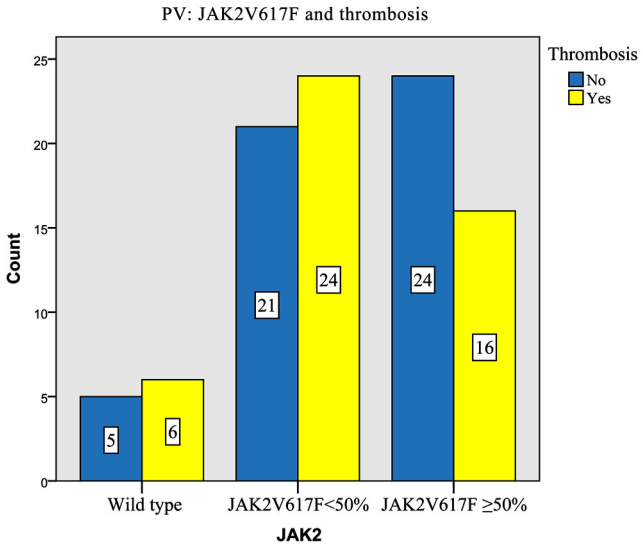


Fig. 2. Leukocyte count and mutant burden of JAK2V617F at PV

Mutation load does not affect leukocyte count

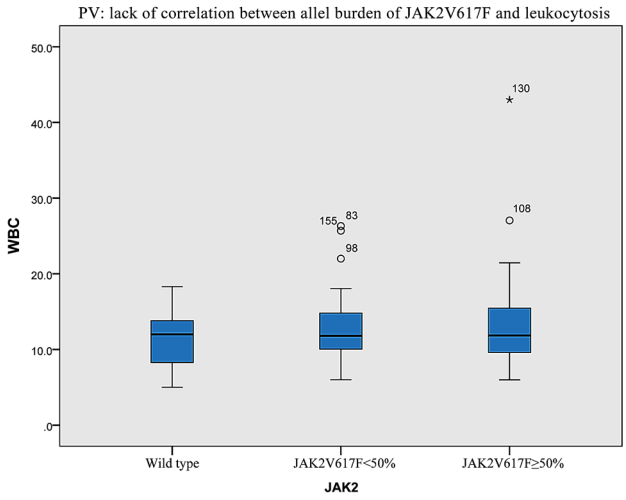
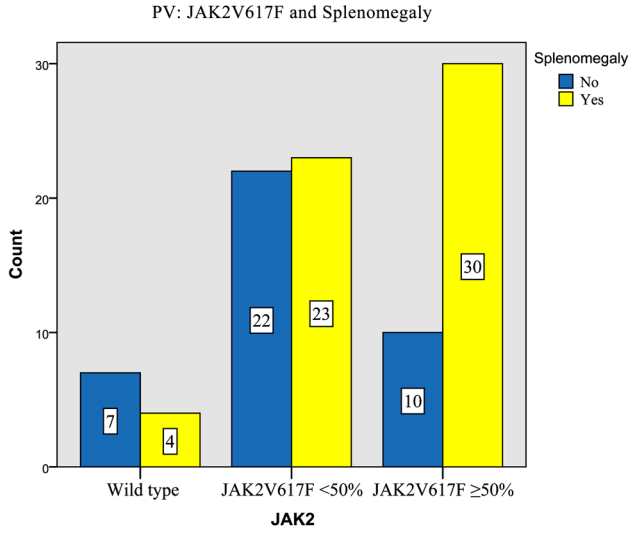


Fig. 3. JAK2V617F mutation burden and sizes of the spleen.

A higher mutational load determines a higher incidence of splenomegaly



Essential thrombocythemia

From all of the 43 patients with ET eighteen (42%) were carriers of JAK2 V617F, as in 3 of them (7%) allel burden of JAK2 V617F was ≥50%. We registered 16 TE in 13 patients, and 7 of them (54%) were carriers of JAK2 V617F. In the analysis of patients without TE, a lack of mutation was found in 19 (63%). There was a tendency for a higher incidence of TE in JAK2 V617F positive ET patients than those who do not carry the mutation (figure 4). Unlike PV patients, the allel burden of JAK2 V617F ≥50% ET patients was associated with marked leukocytosis (Leu=15.65x10^9/l) compared to JAK2 WT (Leu=10.16x10^9/l), and this difference was statistically significant (ANOVA, p = 0.004) (figure 5). However, in this group of patients, we did not establish a link between leukocytosis and the incidence or number of TE.

Fig. 4. Frequency of TE and presence of JAK2V617F at ET

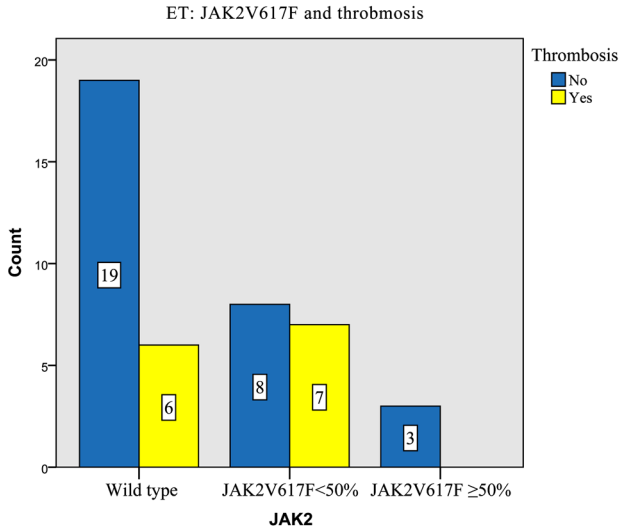
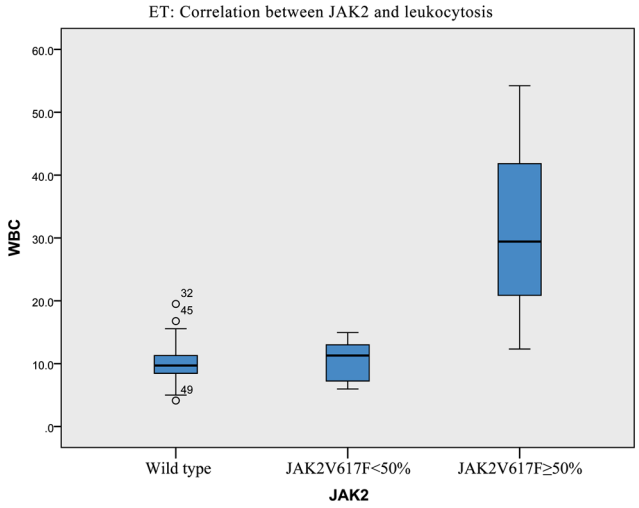


Fig. 5. Leukocyte count and carrier of JAK2V617F at ET.

The higher mutational load of JAK2V617F in ET leads to more pronounced leukocytosis



DISCUSSION

From the analysis, we did not find a correlation between the JAK2 V617F allel burden, the leukocyte count or some of the other standard hematological parameters at PV. A correlation was observed only between the degree of allel burden and the extent of splenomegaly, which corresponds to the data of Vannucchi et al. [8, 9]. In the ET group, we found a tendency for a higher incidence of TE and an increased leukocyte count in the presence of JAK2 V617F mutation. These results do not support the findings in several major studies [3, 10, 11] which conclude that leukocytosis and the higher mutational burden of JAK2 V617F are associated with more TEs. According to another large study of Passamonti et al. [19], the allelic burden of JAK2 V617F ≥50% is a risk factor for post-PV MF transformation, but neither it nor leukocytosis affects the frequency of TE. One likely explanation of these controversial results is the lack of correspondence between the "homozygous JAK2 V617F carrier" and the variable number of clonal circulating leukocytes. In both diseases, none of the other standard blood parameters showed any significance to the development of thrombotic complications. In PV, the increased red-cell mass leads to increased viscosity and impaired hemorheology, practically in some cases they exceed proven critical values. There is no doubt that these changes include multiple compensatory mechanisms (increased blood deposition, opening of AV shunts, increased erythrocyte deformability, vasodilatation) that manage to balance pathological changes. But these mechanisms have not yet been studied in details. Interesting is the fact that when passing through the various compartments of the vascular system, the blood dynamically changes its properties, which cannot be evaluated by a single static indicator such as hematocrit. In the development of a thrombotic process there is a critical combination over a prolonged period of time of various factors dynamic: local blood flow and eventual

turbulence of the blood, atherosclerotic change in vessel patency, vasodilatation and static: changes in cellular composition, vascular endothelium, clotting factors. The conditions of thrombosis are dynamically changing and are fundamentally different in the arterial and venous side of the vascular system. The main fatal and debilitating TEs in PV and ET are MI, AIS, PE and SVT. We believe that it would be appropriate to conduct studies establishing a functional evaluation of the vascular system in assessing the risk of TE.

CONCLUSIONS

JAK2 V617F allel burden and leukocytosis level are prothrombogenic, but not predetermining factors for thrombosis. Although the currently used risk stratification criteria for PV and ET are too general they reflect the complex character of thrombotic complications. Furthermore, there is a difference in clotting mechanisms not only between the both diseases but also in the particular parts of the vascular system. Additional studies are needed to investigate the functional state of the vascular system in order to determine a specific arterial and venous thrombotic index for each nosological unit.

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