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RESEARCH

JAK2 V617F, MPL, and CALR Mutations in Essential Thrombocythaemia and Major Thrombotic Complications: A Single-Institute Retrospective Analysis

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Abstract Thrombo-haemorrhagic events are the main cause of morbidity and mortality in essential thrombocythemia. The aim of this study was to estimate the incidence of thrombotic events and the impact of the *JAK2V617F*, *MPL* (*W515L, W515K, W515R, W515A* and *S505N*) and *CALR* (type-1, type-2) mutations on 101 essential thrombocythaemia patients (72 females and 29 males with a mean age of 61 years) diagnosed in a Southern Hungarian regional academic centre. The incidence of major thrombosis was 13.86 %. Sixty percent of the patients carried the *JAK2V617F* mutation. The *MPL* mutations were analysed by sequencing and the *W515L* was the only one we could identify with an incidence of 3.96 %. Type-2 *CALR* mutation could be identified in 3 cases among the patients who had *JAK2/MPL*-unmutated ET. Statistical analyses revealed that the *JAK2V617F* mutation was associated with significantly increased levels of platelet (p=0.042), haemoglobin (p=0.000), red blood cell (p=0.000) and haematocrit (p=0.000) and hepatomegaly (p=0.045) at diagnosis compared to *JAK2V617F* negative counterparts, however there was no significant association between the *JAK2V617F* mutation status (relative risk: 1.297, 95 % CI 0.395–4.258; p=0.668) and subsequent thrombotic complications. The impact of *JAK2V617F*, *MPL W515L* and *CALR* mutations on the clinical findings at the diagnosis of ET was obvious, but their statistically significant role in the prediction of thrombotic events could not be proven in this study. Our results indirectly support the concept that, besides the quantitative and qualitative changes in the platelets, the mechanisms leading to thrombosis are more complex and multifactorial.

Keywords Myeloproliferative neoplasms · Essential thrombocythaemia · *JAK2 V617F* mutation · *MPL* mutations · *CALR* mutations · Thrombosis

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Introduction

Essential thrombocythaemia (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]. ET patients are at a possible risk of progression to myelofibrosis or/and acute myeloid leukaemia, but thrombo-haemorrhagic events are the main causes of morbidity and mortality in ET: the reported incidence ranges between 11 % and 25 % [2–4]. The disease-related haemostatic abnormalities, the pathogenesis of thrombosis seen in ET and the accurate prediction of these events are currently highlighted issues. However, the mechanisms leading to thrombosis are still unknown: besides the quantitative

and qualitative changes in the platelets, the pathogenesis appears to be more complex and multifactorial [5–7, 2, 8–10]. There are well-known risk factors (age >60 and prior thrombosis) but a more accurate risk-guided management requires the consideration of additional factors, such as thrombocytosis, leukocytosis, cardiovascular risk factors and the Janus kinase 2 (*JAK2*) *V617F* mutation status (found in approximately 50–60 % of ET patients). The careful combination of these factors may facilitate the prognosis of future vascular events in patients diagnosed with ET [2, 11–16]. Barbui and co-workers have recently published their recommendations for the assessment of thrombosis risk in ET patients. Their recommendations are based on the results of a multicentric study and suggested to consider the *JAK2 V617F* mutation status and the combination of cardiovascular risk factors additionally to the age and the patients' prior thrombotic events [14].

However, a remarkable proportion (50–40 %) of ET patients are reported to be *JAK2 V617F*–negative cases, yet thrombotic complications can also be observed in this group. The question therefore emerges whether there are putative molecular markers that could be considered as thrombosis risk factors in the *JAK2 V617F*-negative cases. Beside the JAK-STAT pathway, the TPO-c-MPL system - a regulator of megakaryopoiesis and thrombopoiesis - is presumed to have a role in the clinical course of the *JAK2 V617F*-negative ET patients [17, 18]. Myeloproliferative leukemia virus oncogene (*MPL*) mutations are gain-of-function mutations leading to an overexpression of messenger RNA thus to TPO overproduction [19, 18]. The most common *MPL* mutation, the *W515L* has a 1–5 % frequency in ET and has been suggested to have an effect on the transformation of haematopoietic cells via a constitutive activation of the JAK-STAT signaling. However, the predictive potential of this mutation has not yet been fully established [20–22].

In *JAK2 V617F* and *MPL* unmutated ET, calreticulin (*CALR*) somatic mutations has been recently identified, which are the result of frameshift mutations, caused by exon 9 deletions or insertions: type-1 (c.1092_1143del) and type-2 (c.1154_1155insTTGTC) [23–25]. According to the relevant literature a more indolent clinical course (characterized by younger age, lower leukocyte count, higher platelet count, and decreased risk of thrombosis) was observed in the cases of patients with *CALR* mutations than in patients with the *JAK2 V617F* mutation [23–25].

The primary aim of the present retrospective study was to evaluate the incidence of thrombotic events in ET patients diagnosed between 1999 and 2011 in the Southern Hungarian regional clinical centre responsible for the oncohaematology management of approximately 1,3 M inhabitants [26]. Further aims were to estimate the incidence of the *JAK2 V617F*, *MPL W515L*, *MPL W515K*, *MPL W515R*, *MPL W515A*, *MPL S505N*, and *CALR* type-1; type-2 mutations in our retrospective study population and to evaluate their potential in thrombosis prediction and their clinicohaematological associations.

Patients and Methods

Patients and Data Collection

Patients and the data were selected retrospectively from the myeloproliferative neoplasm database established for scientific research at the 2nd Department of Internal Medicine and Cardiology Centre and from the DNA bank of the patients at the Institute of Medical Genetics, both institutes are at the University of Szeged. One hundred and one patients with ET were enrolled (72 females and 29 males with a mean age of 61 years, range 14–95 years) who were diagnosed at the Department of Internal Medicine and Cardiology Centre between 1999 and 2011. Further data were from the patients' clinical medical files and all the haematological anamnestic data of the patients were reviewed. The study was approved by the Regional and Institutional Human Medical Biological Research Ethics Committee. Informed consent was not required. The study was conducted according to the Declaration of Helsinki.

The thrombotic events before and after the clinical diagnosis of ET were retrospectively collected in the case of each patient, with especial focus on arterial, cardiovascular (myocardial infarction, MI), cerebrovascular (ischaemic stroke or a transient ischaemic attack (TIA)) and venous thrombotic events (deep venous thrombosis or pulmonary embolism thrombosis, and cerebral sinus and venous thrombosis). Two patients died during the follow-up period.

The haematological management strategy was based on risk-oriented recommendations: low-risk patients (age< 60 years, without prior thrombotic event) received anti-platelet therapy if it was necessary, while high-risk patients (age >60 years and/or with prior thrombotic event) were given myelosuppressive drugs (e.g.,:hydroxyurea) alone or in combination with anti-platelet therapy if it was necessary [27, 28]. The main demographic and clinicohaematological characteristics of the study population are presented in Table 1.

Laboratory Methods

DNA was isolated from EDTA-stabilized peripheral blood samples and screened for the *JAK2 V617F* mutation with an allele-specific PCR method [29]. The *JAK2 V617F* mutation negative samples were futher characterised for *MPL* mutations (*W515L*, *W515K*, *W515R*, *W515A*, *S505N*) by allele-specific PCR reactions and subsequent agarose gel electrophoresis. A forward primer *(5'-TGGGCCGAAGTCTGACCCTTT-3') (F)* and a reverse primer *(5'-GAAGTGCGAAGCCGTAGGT-3')*

Table 1 Demographic and clinicohaematological characteristics of the study population

Characteristics of the cohort	Data
Males (N, [%])	29 (28.71)
Females (N, [%])	72 (71.28)
Median follow-up (range) (months)	30.4 (0.26–155.4)
Age at diagnosis, median (range) (years)	61 (14–95)
Median white blood cell count at diagnosis (range) (G/L)	9.4 (4.5–34)
Median platelet count at diagnosis (range) (G/L)	664 (78–2240)
Prior major vascular events (number of events)	38
Cerebrovascular events (stroke/TIA) (number of events [%])	11 (10.89)
Cardiovascular events (AMI) (number of events [%])	16 (15.84)
Venous thrombotic events (number of events [%])	11 (10.89)
Major vascular events in the follow-up (number of events)	16
Cerebrovascular events (stroke/TIA) (number of events [%])	10 (9.90)
Cardiovascular events (AMI) (number of events[%])	3 (2.97)
Venous thrombotic events (number of events [%])	3 (2.97)
Conventional risk factors	
Age >60 (N, [%])	52 (51.48)
Prior thrombotic events (number of events [%])	38 (27.72)
JAK2 V617F-positive cases (N, [%])	61 (60.39)
Treatment (N, [%])	
No treatment	16 (15.8)
Antiplatelets	43 (42.6)
Hydroxyurea (alone or in combination with antiplatelets)	42 (41.6)

(R) and an allele-specific forward primer 5'-GGGCCTGCTG CTGCTGAGGCT-3' *(FW515L)* for *MPL W515L* and 5'-TGGGCCGAAGTCTGACCCTTT-3' for *MPL W515R* analysis have been used, respectively. PCR reactions were performed and the *MPL W515L* and *W515K* mutations were assessed as it was described previously [30].

Allele specific primers 5'-TGGGCCTGCTGCTGCTGA GTC-3' for *MPL W515R*, 5'-GGGCCTGCTGCTGCTGAG GGC-3' for *MPL W515A* and 5'-GCATCTAGTGCTGGGC CTCCA-3' for *MPL S505N* were designed to detect futher mutations of exon 10 of the *MPL* gene.

Parallel with the allele-specific PCR method, samples were sequenced by an automated single capillary genetic analyser (ABI PRISM 310, Applied Biosystems, Life Technologies).

To assess the *CALR* mutation status we performed fragment analysis with FAM labeled primers (forward: 5'-*AGTT TGGCAACGAGACGTG*-3', reverse: 5'-*GAGTCTCACAGA GACATTATTTGG*-3') on 22 patients who had *JAK V617F* negative essential thrombocythaemia. To further characterize the types of mutations we performed bidirectional Sanger sequencing using the BigDye 3.1 Terminator Cycle Sequencing Kit (Applied Biosystems).

Statistical Analysis

Clinical and genetic data were collected and subjected to statistical analysis with Statsoft Statistica v 9.1 (Statsoft) and SPSS 17 software (IBM).

To compare the overall effects of series of variables such as 1.) all thrombotic events after the clinical diagnosis of ET and subtypes of arterial thrombosis; 2.) cardiovascular MI; 3.) cerebrovascular TIA or stroke; 4.) venous thrombotic events (deep venous thrombosis or pulmonary embolism thrombosis, and cerebral sinus and venous thrombosis); 5.) age; 6.) main clinical characteristics; 7.) median white blood cell count; 8.) median platelet count; 9.) median haemoglobin count; 10.) median red blood cell count; 11.) hepatomegaly; 12.) splenomegaly and 13.) hepatosplenomegaly, Mann-Whitney test was performed in the *JAK2 V617F* mutation-positive and –negative subgroups.

To estimate the probability of thrombotic events in the presence of the *JAK2 V617F* mutation, and the conventional risk factors: age >60, and the presence of a prior thrombotic event multivariate binary logistic regression analysis was performed.

The probability of thrombosis-free survival was assessed (for the overall cohort) in four subgroups: the *JAK2 V617F* mutation-positive (from now on referred as *JAK2 V617F*(+)), low-risk patients, the *JAK2 V617F*(+) high-risk patients, the *JAK2 V617F* mutation-negative (from now on referred as *JAK2 V617F*(-)) low-risk patients, and the *JAK2 V617F*(−) high-risk patients; the Kaplan–Meier method was applied followed by the log-rank test [31]. The high-risk patiens were those who were aged >60 years, and/or with a prior thrombotic event, while the patients in the low-risk category <60 years without any prior thrombotic event [28]. This risk-based stratification of the patients allowed us to consider the potential influence of the applied therapy on the subsequent thrombotic events.

Results

The retrospective analysis of the thrombotic events in the cohort revealed 38 prior vascular events in 28 (27.7 %) patients: cerebrovascular events (stroke/TIA) in 11 cases (10.8 %), cardiovascular events (MI) in 16 (15.8 %) cases and venous thrombotic events in 11 (10.8 %) cases. During the

haematological follow-up after the diagnosis of ET, 16 events were observed in 14 (13.9 %) patients: 10 (9.9 %) cerebrovascular events (stroke/TIA), 3 (3.0 %) cardiovascular events (MI) and 3 (3.0 %) venous thrombotic events.

The univariate statistical comparison of the *JAK2 V617F* negative and positive patients revealed no statistically significant association with all thrombotic events (p=0.651) or the separately analysed cardiovascular events (MI) (p=0.849), cerebrovascular events (TIA or stroke) (p=0.558) or venous thrombotic events (deep venous thrombosis, pulmonary embolism thromboses, and cerebral sinus and venous thrombosis) (p=0.849). However, at the time of the ET diagnosis with age <60 years (p=0.060) several laboratory findings such as median platelet count (759.25 vs. 685.58 G/L; p=0.042), haemoglobin level (145.19 vs. 128.78 g/L; p=0.000), red blood cell count (5.08 vs. 4.23 T/L; p=0.000) and haematocrit (43.73 vs. 38.38 %; p=0.000) were significantly indicative for a higher risk of thrombotic events in the *JAK2 V617F*(+) group compared to the *JAK2 V617F*(−) one. At the same time the median white blood cell count was not significantly higher in the subgroup of the *JAK2 V617F*(+) patients (10.75 G/L) as compared with the subgroup without the mutation (10.69 G/L) (p=0.401).

A significantly higher number of hepatomegaly cases were observed in the *JAK2 V617F*(+) group (n=9; 14.8 %) than in the *JAK2 V617F*(−) group (n=1; 2.5 %) (p=0.045). The number of the observed splenomegaly (in the *JAK2 V617F*(+) group vs. *JAK2 V617F*(−) group (p=0.973)), and hepatosplenomegaly (in the *JAK2 V617F*(+) group vs. *JAK2 V617F*(−) group) (p=0.383) did not differ significantly at the haematological diagnosis. The results of the Mann-Whitney test are shown in Table 2.

Multivariate binary logistic regression analysis on the subsequent thrombotic events after the diagnosis of ET revealed a significant partial effect of prior thrombotic events at a significance of 10 % (relative risk: 2.876, 95 % CI 0.847–9.774; p= 0.090). However, the same analyses could not prove either a significant association between the *JAK2 V617F* mutation status (relative risk: 1.297, 95 % CI 0.395–4.258; p=0.668) nor the age over 60 years (relative risk: 0.981, 95 % CI 0.316–3.048; p=0.974) and the probability of subsequent thrombotic complications. Antiplatelet and myelosuppressive drugs administrated to low and high risk patients may masked the role of the investigated factors and may have prevented the recognition. Therefore subsequent statistical analyses were carried out on four subgroups of the patients: the *JAK2 V617F*(+) low-risk subgroup, the *JAK2 V617F*(+) high-risk subgroup, the *JAK2 V617F*(−) low-risk subgroup and the *JAK2 V617F*(−) high-risk subgroup in order to estimate the impact of the presence of the *JAK2 V617F* mutation on the probability of thrombosis-free survival during the follow-up period. Categorizing the patients into high-risk and low-risk subgroups allowed us to take into account the potential influence of the therapy on the subsequent events in these subgroups. The low-risk patients were <60 years without prior thrombotic events, who had received anti-platelet therapy when necessary. The high-risk patients were >60 years, and/or had suffered prior thrombotic event, and were on myelosuppressive drugs e.g.,: hydroxyurea alone or in combination with anti-platelet therapy [27, 28]. The results of the Kaplan–Meier curves and the log rank test (Mantel-Cox) revealed only non-significant differences, p=0.548 (Fig. 1).

Table 2 Results of the Mann-Whitney tests for the comparison of the patients in the two subgroups based on the presence or the absence of the JAK2 V617F mutation

VARIABLES		Mann-Whitney p-value
JAK2 V617F(+) patients versus *JAK2 V617F(−) patients*		
Thrombotic events after diagnosis	All	0.651
	Cardiovascular events	0.849
	Cerebrovascular events	0.558
	Venous thrombotic events	0.849
Age		0.060*
Clinical characteristics and laboratory findings at ET diagnosis	White blood cell count	0.401
	Platelet count	0.042**
	Haemoglobin count	0.000**
	Red blood cell count	0.000**
	Haematocrit level	0.000**
	Hepatomegaly	0.045**
	Splenomegaly	0.973
	Hepatosplenomegaly	0.383

A significant difference at a level of 10 % is denoted by *, and those at 5 % by **

To answer the question whether MPL mutations may serve as a prognostic factors in the prediction of future thrombotic events in the group of *JAK2 V617F*(−) ET patients, we analysed the *MPL W515L/K/R/A*, and *S505N* mutations. In the case of 4 *JAK2 V617F*(−) ET patients, DNA was not available for further analyses. 36 *JAK2 V617F*(−) negative ET patients were identified (11 males; 25 females), with a mean age of 55.52 years (range: 14–95 years). The median follow-up period was 72 months (range: 12–156 months). The demographic and clinicohaematological characteristics of these *JAK2 V617F*(−) ET patients are summarized in Table 3. The allele specific PCR and the subsequent sequence analyses revealed only one type of *MPL* mutation, the *W515L* could be detected in 4 samples, and the other examined *MPL* mutations were not detected in the *JAK2 V617F*(−) patients. This ratio of *MPL W515L* mutation positivity is in line with the literature data [22, 21, 32, 3].

The main clinical characteristics of the *JAK2 V617F*(−) patients with or without the *MPL W515L* mutations at the

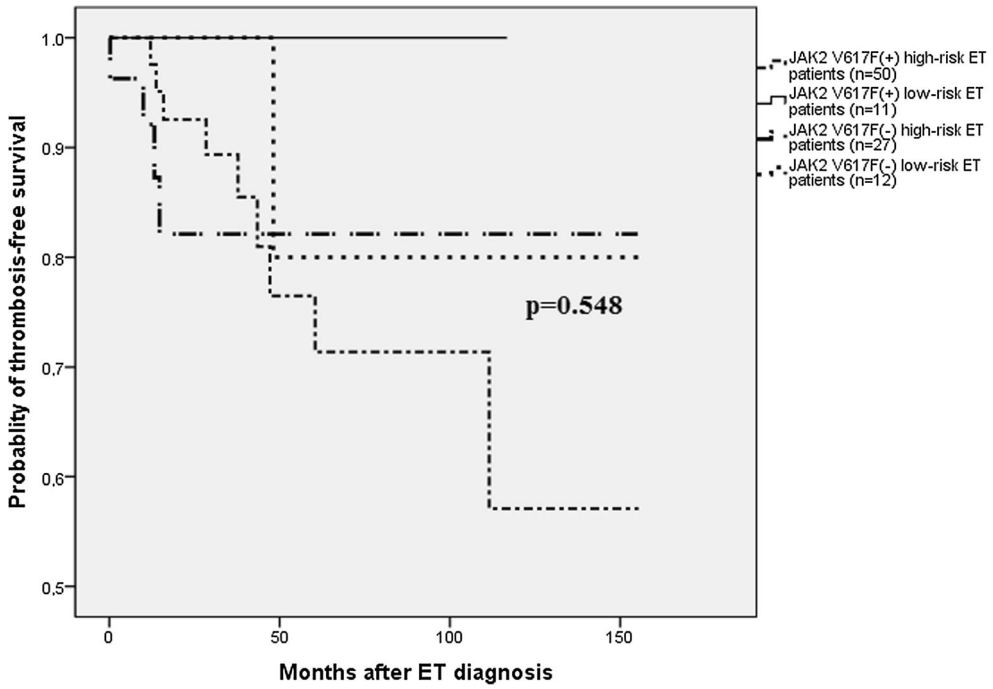

Fig. 1 The probability of thrombosis-free survival in the haematological follow-up period in the *JAK2 V617F*(+) low-risk, *JAK2 V617F*(+) high-risk, *JAK2 V617F*(−) low- risk and *JAK2 V617F*(−) high–risk, subgroups of ET patients

time of the ET diagnosis and the thrombotic events diagnosed in the follow-up period are compared in Table 3. The difference in the numbers of the *MPL W515L* mutation positive (from now on referred as *MPL W515L* (+)) versus *MPL W515L* mutation negative (from now on referred as *MPL W515L* (−)) patients does not allow a meaningful statistical analysis, but the results clearly demonstrate that the *MPL W515L* mutation was predominantly observed in female patients, and in patients with older age (median age 70 years). *MPL W515L*(+) positive patients exhibited a higher median platelet count at diagnosis (845.50 G/L) than the *JAK2 V617F*(−), *MPL W515L*(−) patients (585.00 G/L).

In the group of *JAK2 V617F*(−) and *MPL W515L*(−) ET patients, we analysed the *CALR* type-1; type-2 mutations. The allele specific PCR and the subsequent sequence analyses revealed only type-2 of *CALR* mutation (c.1154_1155insTTGTC), which could be detected in 3 samples. The detected type-2-*CALR* mutation was predominantly observed in patients with relatively younger age (median age 49 years) with higher median platelet count at diagnosis (951 G/L), with lower median leukocyte count (7.4 G/L) and lower number of thrombotic events were observed compared with the *JAK2 V617F*(−) *MPL W515L*(−) or *JAK2 V617F*(−) *MPL W515L*(+) positive patients (Table 3).

Discussion

In ET patients, the main causes of morbidity and mortality are thrombo-haemorrhagic complications, the reported incidence ranges between 11 % and 25 % [2–4]. In a cohort of 101 ET patients diagnosed between 1999 and 2011 in our regional academic centre, the incidence of major thrombotic events among them was 13.86 %.

Similarly to the literature data, we found a 60 % incidence of the *JAK2 V617F* mutation among our ET patients, however we were not able to demonstrate the association between the *JAK2 V617F* mutation and the thrombotic events in our study group [14]. Although the *JAK2 V617F* mutation was associated with significantly increased levels at the diagnosis of the platelet, haemoglobin, red blood cell and haematocrit counts and hepatomegaly but its direct effect on the sum of thrombotic events in the follow-up period and separately on cardiovascular events cerebrovascular events or venous thrombotic events did not prove its suggested predictive potential. However, these results indirectly support the concept that the *JAK2V617F* mutation contributes to intrinsic changes in both megakaryocyte and platelet biology beyond the increase in cell numbers [33–35]. In the comparison of the *JAK2 V617F*(+) low-risk, *JAK2 V617F*(+) high-risk, *JAK2 V617F*(−) low-risk and *JAK2 V617F*(−) high-risk subgroups only a non-significant tendency could be observed in the thrombosis free-survival of the patients. The results of another single centre analyses also revealed that the *JAK2 V617F*(−) cases did not differ significantly from the *JAK2 V617F*(+) cases in the incidence of thrombosis [36, 37]. We suggest that the number of involved patients might be determinative in view of clear evaluation of the predictive value of the *JAK2 V617F* mutation in thrombosis [14, 4, 38].

Thrombotic events can also be observed in a remarkable proportion (50–40 %) of *JAK2 V617F*(−) ET patients. Therefore *MPL* mutations were also determined as putative

Table 3 Demographic and clinicohaematological characteristics of *JAK2 V617F*(−) patients

Characteristics	Data		
	JAK2 V617F(−) MPL W515L (−) patients	JAK2 V617F (−) MPL W515L (+) patients	JAK2 V617F (−) MPL W515L (−) CALR type-2 (+) patients
Male (N, [%])	10 (9.90)	1 (0.99)	1 (0.99)
Female (N, [%])	22 (21.78)	3 (2.97)	2 (1.98)
Prior vascular events (number of events)	14	3	1
Cerebrovascular events (stroke/TIA) (number of events [%])	3 (2.97)	1 (0.99)	1 (0.99)
Cardiovascular events (AMI) (number of events [%])	8 (7.92)	no events	no events
Venous thrombotic events (number of events [%])	3 (2.97)	2 (1.98)	no events
Major vascular events in follow-up	6 (5.94)	1 (0.99)	no events
Cerebrovascular events (stroke/TIA) (number of events [%])	4 (3.96)	1 (0.99)	no events
Cardiovascular events (AMI) (number of events [%])	1 (0.99)	no events	no events
Venous thrombotic events(number of events [%])	1 (0.99)	no events	no events
Main clinical characteristics at diagnosis (number of events)			
Median age at diagnosis (range) (years)	56.50 (14–95)	70.00 (23–82)	49.00 (38–67)
Median WBC at diagnosis (range) (G/L)	8.68 (6.9–28.6)	9.90 (4.53–34)	7.4 (7.4–11.2)
Median PLT at diagnosis (range) (G/L)	585 (195–1655)	845.50 (78–2240)	951(662–1042)
Median haemoglobin count (range) (g/L)	128.50 (101–162)	125.00 (94–157)	120 (113–153)
Median red blood cell count (range) (T/L)	4.34 (3.35–5.43)	4.45 (2.28–4.78)	3.78 (3.66–5.14)
Median haematocrit level (range) (%)	38.85 (38–47.0)	32.5 (26.0–42.0)	34.8 (34–45.6)
Hepatomegaly cases	1 (0.99)	none	none
Splenomegaly cases	4 (3.96)	1 (0.99)	1 (0.99)
Hepatosplenomegaly cases	1 (0.99)	none	none

(given percentanges refer to the total of all 101 patients)

molecular markers to consider their predictive potential as a thrombosis risk factor in the *JAK2 V617F*(−) cases. The genetic analyses in our cohort revealed a 3.96 % incidence of the *MPL W515L* mutation, which was in the line with the incidence of 1–5 % reported in the literature [22, 21, 32, 3]. Beer et al. reported that *MPL* mutation positivity does not have a prognostic value for thrombosis, major haemorrhage, myelofibrotic transformation or survival among *JAK2 V617F*(−) patients [32]. In our cohort, the *MPL W515L* mutation was observed predominantly in female patients, older age, higher platelet counts as compared with the *JAK2 V617F*(−) *MPL W515L*(−) patients and the predictive potential of the mutation for subsequent thrombosis could not be evaluated. Due to the low incidence of the *MPL W515L* mutation, in ET patients only large prospective multicentre analyses could answer the question whether this mutation in *JAK2 V617F*(−) cases could have a significant predictive potential for thrombosis.

It has been recently reported that *CALR* mutations (which frequencies in ET are estimated between 15 and 32 %) correlated with younger age, higher platelet count, lower hemoglobin level, lower leukocyte count, and lower thrombosis risk. [24, 39, 40]. Our data correspond to that published in the literature: the presence of type-2-*CALR* mutation in our cohort was also associated with a relatively younger age (median age 49 years), with higher median platelet count at diagnosis (951 G/L), with lower median leukocyte count (7.4 G/L) and lower number of thrombotic events.

Conclusions

The impact of *JAK2 V617F*, *MPL W515L* and type-2-*CALR* mutations on the clinical findings at the diagnosis of ET was obvious, but their statistically significant role in the prediction of subsequent thrombotic events could not be proven in this study. Our results indirectly support the concept that, besides the quantitative and qualitative changes in the platelets, the mechanisms leading to thrombosis are more complex, with multifactorial contributions.

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Competing Interests No conflict of interest is reported by the authors.

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