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Clinical characteristics of essential thrombocythemia patients depend on the mutation status

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Abstract

The impact of the mutation status on the clinical course and the outcome of essential thrombocythemia (ET) patients has not yet been completely established. A total of 171 patients with diagnosed ET were tested and subsequently grouped, according to their mutation status – Janus Kinase 2 (*JAK2*) – 112 patients, calreticulin (*CALR*) – 36 patients, and thrombopoietin receptor (*MPL*) – 5 patients. Moreover, 18 individuals were triple-negative (with non-mutated *JAK2*, *CALR*, and *MPL*). *CALR*-mutated patients preferentially were male, with higher platelets (PLT) counts (mean PLT = 1 002.3) and lower hemoglobin and hematocrit levels at the diagnosis, compared to the *JAK2* (mean PLT = 933.6), *MPL* (mean PLT = 940.8) and triple-negative patients (mean PLT = 822.6) ($p = 0.0035$). The patients with *CALR* mutated, and the triple-negative ones had a lower risk of arterial and venous thrombosis (3% and 5.6% cases at the time of diagnosis, respectively) than the patients with *JAK2* mutation (7.2%) ($p = 0.9210$). The overall survival rate did not differ statistically between the groups.

Keywords:
essential thrombocythemia, *JAK2, CALR, MPL*

Introduction

Essential thrombocythemia (ET) belongs to myeloproliferative neoplasms, with an incidence of 2.5 cases per 100,000 inhabitants, and is characterized by an increased risk of thrombosis. The molecular events leading to the development of ET are heterogeneous: 50–60% of the patients with ET carry a mutation in the Janus Kinase 2 (*JAK2*) gene [1], 3–7% of the patients' mutation in the thrombopoietin receptor (*MPL*) gene, and about 20% in the calreticulin (*CALR*) gene [2, 3]. Thus, molecular confirmation of clonality that supports unambiguously diagnosis of ET is possible nowadays in 80–90% of the patients. The remaining 10% of patents are grouped into the triple-negative category [4, 5]. Molecular heterogeneity of ET may translate to a different clinical course of the disease. Some early reports suggest indeed that such differences exist. Here we present a cohort of 171 ET patients, treated at our institution, aiming at exploring the impact of the mutation status on the clinical course and features of the disease.

Material and methods

Patients

This study included a cohort of 171 patients, diagnosed and treated for ET at the University Clinical Centre at the Medical University of Gdansk between 2003 and 2014. The median follow-up was 4.7 years. The ET diagnosis was established according to the World Health Organization (WHO) criteria from 2008 [6]. Briefly, post-ET myelofibrosis (pET-MF) was diagnosed consistently with the criteria of the International Working Group of Myelofibrosis Research and Treatment [7]. The transformation into acute leukemia was defined by the WHO criteria [6]. Distribution of the risk for vascular complications was rated as follows: high risk at the age of ≥60 years old and/or previous thrombosis, low risk at the age of <60 years old, and/or no thrombosis history [8]. The prognostic score of thrombosis for essential thrombocythemia (IPSET thrombosis) score was not calculated due to a lack of patients' cardiovascular risk factors data. Thrombotic events were defined as described by Barosi et al. [7]. The study protocol was approved by the Local Bioethics Committee at the Medical University of Gdansk and was carried out following the Declaration of Helsinki. Informed consent, concerning the use of the clinical data for research purposes, was obtained from all the participants.

Molecular analysis

The *JAK2* V617F mutation status was assessed using the amplification refractory mutation system-polymerase chain reaction (*ARMS-PCR*) described by Jones et al. [9]. The patients with a non-mutated *JAK2* gene were further evaluated for *CALR* exon 9 and *MPL* exon 10 mutations using high-resolution melting assay and Sanger sequencing [2, 10].

The patients with *JAK2*, *CALR*, and *MPL* mutations and the triple-negative individuals were compared in terms of their demographic characteristics (sex distribution, age at diagnosis of ET), the clinical and laboratory features at the time of diagnosis, the clinical outcome (fibrotic or leukemic transformation), and the overall survival (OS).

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The patients were treated according to the national recommendations. Cytoreductive therapy, using hydroxyurea was administered to the high-risk patients, which is with the incidence of arterial or venous thrombosis, elderly, and with comorbidities increasing the risk of a thrombotic event. Other treatment approaches included the use of anagrelide, busulfan, and interferon alfa.

Statistical analysis

Normal distributions of the continuous variables were verified by the Kolmogorov-Smirnov test, while their statistical characteristics were presented as arithmetic means, standard deviations (SDs), medians, and ranges. Statistical characteristics of the discrete variables were presented as numbers and percentages. A Student's *t*-test or the Mann–Whitney *U*-test was used for intergroup comparisons of the continuous variables and distributions of the discrete variables. They were compared with the Pearson chi-square test or the Fisher exact test. The power and the direction of the relationships between the presence of mutation types, the demographic, and the clinical characteristics of the ET patients were determined based on the odds ratios (ORs) and their 95% confidence intervals (95% CI), calculated during logistic regression analysis. Survival curves were prepared with the use of the Kaplan-Meier method and compared with the log-rank test. All calculations were carried out using the Statistica10 package (Stat Soft, USA) and the threshold of statistical significance was set at $p \leq 0.05$.

Results

Distribution of the mutation status

The group of 171 ET patients includes 112 (65%) patients with a *JAK2* mutation, 36 (21%) with *CALR* mutation, 5 (3%) with *MPL* mutation, and 18 (11%) triple-negative cases.

Overall, eight types of *CALR* mutations were detected. In the cohort of the 36 *CALR*-mutated patients, we found 14 (39%) individuals with a 52 bp deletion (c.1099-1150del – type 1) and 15 (41%) patients with a 5bp insertion (c.1154-1155insTTGTC – type 2). Seven (20%) patients were the carriers of one out of six other mutation types, with potentially three novel variants.

Demographic characteristics

The median age at diagnosis between patients with *JAK2*, *CALR*, and *MPL* mutations and of the triple-negative ones were 59, 53, 66, and 52, respectively. The groups did not differ in terms of their age distribution, but there was a tendency toward lower age in the *CALR* and the triple-negative groups. There were more females (63%) than males in the whole ET group. The preponderance of females to males was preserved in all mutation subgroups except for *CALR* mutated patients with more males (56%) than females. The difference between the latter group and the *JAK2* mutated group reached statistical significance. The demographic, as well as the clinical characteristics, are summarized in table I.

Laboratory parameters at the time of diagnosis

The laboratory parameters of all groups, according to the mutation type, are presented in table II. At the time of diagnosis, the *JAK2*-mutated patients had mean hemoglobin (Hb) and hematocrit (Hct) levels (14.5 ± 1.5 g/dL and 44 ± 4.3 g/dL, respectively), compared to the other groups, and lower PLT counts when compared to the *CALR* and the triple-negative individuals; however, all Hb and Hct values remained within the normal range limits. There was a trend toward a higher white blood count (WBC) in the *JAK2* group, in comparison to the *CALR* and the triple-negative groups ($p = 0.1467$).

Vascular complication risk and the observed incidence of vascular complication at diagnosis concerning the mutation status

More patients with high risk were observed in the *JAK2* group (59.1%) than in the *CALR* and the triple-negative groups (36.7% and 27.8%, respectively). The difference in the distribution of the vascular complication risk between the *JAK2*, the *CALR*, and the triple-negative groups was statistically significant ($p = 0.0264$). The incidence of arterial or venous thrombotic events at the diagnosis was observed more often in the *JAK2* group than in the other groups (7.2 *vs.* 5.6 and 3.0% in the triple-negative and the *CALR* group respectively), but the difference did not reach statistical significance ($p = 0.9210$). No incidence of arterial or venous thrombotic events was observed in the *MPL* group, possibly due to a low number of individuals (n = 5).

Treatment and the incidence of vascular complications concerning the mutation status and survival

During the follow-up period, arterial and venous thrombotic complications occurred in 7.2% of the patients from the *JAK2* group, whereas in the *CALR*, the *MPL*, and the triple-negative groups no thrombotic complications were observed. The patients with *CALR* or *MPL* mutation as well as the triple-negative individuals had a lower risk of arterial or venous thrombosis than the patients with *JAK2* mutation. Thrombosis-free survival was the highest in the *CALR* group. Bleeding complications were observed only in the *CALR* group (3.0%) during the follow-up. Transformation to pET-MF occurred in 2.9% of all patients during the follow-up, including two patients from the *JAK2* group, two and one individuals from the *CALR* and the *MPL* groups, respectively. No transformation to pET-MF in the triple-negative group was observed. The difference between the groups was statistically significant ($p = 0.0325$).

The OS did not differ statistically among the three groups: the *JAK2*, the *CALR*, and the triple-negative. Due to a low number of *MPL*-mutated patients (n = 5), the analysis was not performed in this group.

Clinical characteristic of the CALR subtypes

We compared clinical and laboratory parameters (Tab. III) between types 1 and 2 *CALR* subgroups. We found no statistical difference in the demographic characteristic (age and sex), the laboratory

Table I. Clinical features of 171 patients with ET, according to the mutation status

	JAK2+ n = 112 (65.5%)	*CALR*+ n = 36 (21%)	*MPL*+ n = 5 (3%)	Triple negative n = 18 (10.5%)	*p*-value
Age at diagnosis					
Mean (SD)	58.1 (15.1)	54.3 (14.9)	66.0 (11.3)	51.6 (11.6)	
95% CI	[55.3; 61.0]	[49.2; 59.4]	[48.0; 84.0]	[45.8; 57.4]	
Range (min–max)	23.0–88.0	24.0–82.0	54.0–78.0	30.0–74.0	
Median	59.0	53.0	66.0	52.0	0.0948
Sex					
Female	74 (66.1%)	16 (44.4%)	3 (60.0%)	14 (77.8%)	
Male	38 (33.9%)	20 (55.6%)	2 (40.0%)	4 (22.2%)	0.0594
Symptoms at diagnosis					
None	97 (87.4%)	30 (90.9%)	5 (100.0%)	16 (88.9%)	
Thrombotic	5 (4.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Cardiovascular	3 (2.7%)	1 (3.0%)	0 (0.0%)	1 (5.6%)	
Bleeding	3 (2.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Others	3 (2.7%)	2 (6.1%)	0 (0.0%)	1 (5.6%)	0.9210
Risk grade					
High	65 (59.1%)	11 (36.7%)	2 (50.0%)	5 (27.8%)	
Low	45 (40.9%)	19 (63.3%)	2 (50.0%)	13 (72.2%)	0.0264
Complications					
None	101 (91.0%)	32 (97.0%)	4(100.0%)	16 (100.0%)	
Thrombotic	7 (6.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Cardiovascular	1 (0.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Bleeding	0 (0.0%)	1 (3.0%)	0 (0.0%)	0 (0.0%)	
Others	2 (1.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.7070
Transformation					
Myelofibrotic	2 (1.8%)	2 (6.9%)	1 (25.0%)	0 (0.0%)	0.0325
Leukemic	16 (14.8%)	2 (6.5%)	1 (25.0%)	3 (17.6%)	0.5438

CALR – calreticulin; ET – essential thrombocythemia; JAK2 – Janus Kinase 2; MPL – thrombopoietin receptor

parameters (Hb, Hct, WBC, and PLT count), the thrombotic incidences, the transformation risk, or in the OS.

Discussion

Discoveries of molecular changes in myeloproliferative neoplasms nowadays, allow confirmation of the diagnosis molecularly in 80–90% of ET subjects. Therefore, not only *JAK2* but *CALR* and *MPL* mutation screening should be performed in every case of a suspected ET [11]. In our study, the molecularly defined ET patients accounted for 89% of the entire cohort. Similar data were presented by other authors [4], though in some studies the percentage of molecularly undefined ET patients was higher [12, 13]. Therefore, it is crucial to perform a precise differential diagnosis, to exclude secondary thrombocytosis in case of a lack of molecular markers. We should also be aware, that even in the case of a confirmed *JAK2*, *CALR*, or *MPL* mutations, other myeloid neoplasms (such as prefibrotic myelofibrosis and refractory anemia with ringed sideroblasts with marked thrombocytosis RARS-T) can mimic ET in their presentation. A trephine biopsy examination (different morphology of megakaryocytes) can help distinguish ET from other myeloid neoplasms [14]. Distribution of the *JAK2*, the *CALR*, the *MPL*, and the triple-negative patients in our cohort was similar to previous reports [4]. However, in the cohorts analyzed by Tefferi et al. [5] and Andrikovics et al. [15], there were more *CALR*-mutated individuals (32 and 33%, respectively *vs*. 21%), and less *JAK2*-mutated patients than in our group. Whereas among the 1150 ET patients, described by Finazzi et al [16], the *CALR* individuals accounted for only 16% of the entire group. In our *CALR* cohort, we found slightly more patients with type 2 mutation than those with type 1 (41 *vs*. 39%). However, this observation is not consistent with other studies [5, 15], which reported the prevalence of a type 1 mutation at 50% of the *CARL*-mutated patients. Similarly to our studies, there was no difference between the subtypes 1 and 2, in terms of the demographic and the clinical characteristics. Further, population-based studies are necessary to confirm the real mutation distribution in ET patients.

Whether the molecular changes could have an impact on the laboratory parameters and the clinical characteristics of ET patients, has remained a matter of debate ever since the *CALR* mutation discovery. Most researches state that *CALR*-mutated ET patients are characterized by younger age, higher PLT count, a lower incidence of thrombotic events, and a higher transformation rate to MF, in

Table II. Laboratory parameters of 171 patients with ET, according to the mutation status

	JAK2+ n = 112	*CALR*+ n = 36	*MPL*+ n = 5	Triple negative	*p*-value
WBC					
Mean (SD)	10.4 (6.3)	8.6 (2.6)	9.2 (5.5)	8.8 (2.7)	
95% CI	[9.2; 11.7]	[7.6; 9.6]	[0.4; 18.0]	[7.4; 10.2]	
Range (min–max)	3.0–63.0	4.4–14.1	5.0–17.3	2.0–13.5	
Median	9.6	8.3	7.2	8.7	0.1467
Hb					
Mean (SD)	14.5 (1.5)	13.7 (1.0)	12.8 (1.2)	13.5 (1.1)	
95% CI	[14.2; 14.8]	[13.3; 14.0]	[10.9; 14.7]	[12.9; 14.0]	
Range (min–max)	9.8–17.7	11.5–15.8	11.2–14.1	11.0–14.9	0.0139
Median	14.7	13.5[2]	13.0	13.6[1]	0.0034
Hct					
Mean (SD)	44.0 (4.3)	41.0 (3.5)	–	39.4 (2.5)	0.0002
95% CI	[42.9; 45.1]	[39.4; 42.5]	–	[37.9; 41.0]	0.0043
Range (min–max)	30.0–52.0	32.0–49.0	–	33.0–42.3	
Median	44.0	41.0	–	39.0	
PLT					
Mean (SD)	933.6 (931.8)	1,002.3 (259.6)	940.8 (232.6)	822.6 (319.8)	0.0035
95% CI	[757.5; 1 109.7]	[911.7; 1 092.9]	[652.0; 1 229.6]	[663.6; 981.6]	0.0406
Range (min–max)	491.0–10,000.0	452.0–1,624.0	563.0–1,135.0	475.0–1,399.0	
Median	787.5	1,024.0	1,006.0	652.0	

CALR – calreticulin; ET – essential thrombocythemia; Hct – hematocrit; Hb – hemoglobin; JAK2 – Janus Kinase 2; PLT – platelets; MPL – thrombopoietin receptor; WBC – white blood count

Table III. Laboratory parameters of 29 patients with types 1 and 2 CALR mutations

	5bp insertion n = 15	52 bp deletion n = 14	*p*-value
WBC			
Mean (SD)	8.2 (2.6)	9.1 (2.8)	
95% CI	[6.6; 9.7]	[7.3; 10.9]	
Range (min–max)	4.4–13.3	5.4–14.1	
Median	7.5	8.6	0.7175
Hb			
Mean (SD)	22.2 (30.9)	14.0 (1.1)	
95% CI	[3.5; 40.9]	[13.3; 14.7]	
Range (min–max)	12.3–125.0	12.4–15.8	
Median	13.5	14.0	0.4265
Hct			
Mean (SD)	42.1 (3.5)	40.8 (2.6)	
95% CI	[39.4; 44.7]	[38.6; 43.0]	
Range (min–max)	37.7–49.0	37.0–45.0	
Median	42.0	40.5	0.5790
PLT			
Mean (SD)	1,036.0 (313.2)	952.1 (233.9)	
95% CI	[855.2; 1,216.8]	[817.0; 1,087.1]	
Range (min–max)	452.0–1,624.0	662.0–1,415.0	
Median	1,052.5	889.0	0.5035

CALR – calreticulin; Hct – hematocrit; Hb – hemoglobin; PLT – platelets; WBC – white blood count

comparison to *JAK2*-mutated patients [4, 5]. Our study showed that *CALR*-mutated patients are characterized by lower Hb and Hct levels, and there is a trend of a lower leucocyte count and a younger age at the diagnosis, compared to the *JAK2*-mutated subjects. We showed, that the *CALR*-mutated individuals are characterized by a higher PLT count in comparison to the *JAK2*-mutated patients. This observation is consistent with other studies already performed and suggests a difference in the biological effects of the mutation status in the *JAK2* and the *CALR* genes [15, 17]. It has been stated that a mutated *JAK2* promotes erythroid and granulocyte lineage, whereas a mutated *CALR* selectively promotes megakaryocytic proliferation [18].

Interestingly, the *CALR*-mutated patients, despite a higher PLT count, might be characterized by a lower rate of thrombotic events. This observation was also confirmed in our study group. Passamonti et al. [19] state that new risk assessment factors should be implemented in the risk stratification of ET patients. Moreover, the novel low-risk factors should include the presence of a *CALR* mutation. However, a study performed by Finazzi et al. [16] on 1150 ET patients showed that a *CALR* mutation does not have any significant impact on the IPSET thrombosis score. A low number of thrombotic events in *CALR*-mutated patients raises a question on the role of the PLT in the thrombotic events in ET individuals. More detailed studies are required regarding the role of the level and the activity of the von Willebrand factor, which could be more relevant for thrombosis occurrence than the PLT count [20].

Despite the clinical differences of the *JAK2*, the *CALR*, and the triple-negative ET patients, we did not show any variation in the OS. The results presented by other authors are inconsistent. Klamfl et al. [2] showed a survival advantage for the *CALR*-mutated patients, but

Tefferi et al. [5] as well as others [15] did not confirm this observation. The reason for inconsistent data could be the inadequate time of the follow-up.

The differences in the clinical courses of the *JAK2*-mutated and the *CALR*-mutated patients, especially in terms of the thrombosis risk and the myelofibrotic transformation rate, provides, in our opinion, a rationale for a different therapeutic approach to the high-risk patients. At present, two randomized trials, comparing hydroxyurea and anagrelide in ET patients have been performed. One randomized trial, comparing aspirin combined with either anagrelide or hydroxyurea, showed a lower incidence of fibrotic transformation and arterial thrombosis in the hydroxyurea treated patients [21]. The second one showed no difference between hydroxyurea and anagrelide treated patients, in terms of the fibrotic and the thrombosis rates [22] However, none of the studies compared the patients concerning the *JAK2* and the *CALR* mutation status. We and others have proved that the mutation status defines the subtypes with substantially different clinical courses. The *JAK2*-mutated patients represent a different phenotype, with a higher risk of thrombosis and a lower risk of myelofibrotic transformation.

Conclusion

In conclusion, based on ours and other available studies, it can be stated that *CALR*-mutated patients display a distinct phenotype, compared to *JAK2*-mutated and *MPL*-mutated ET patients. Consequently, different therapeutic strategies should probably be implemented for these groups of patients, but further studies are necessary.

Authors' contributions

WP, AH – contributed to the design of the study. AL – performed the mutation analysis. WP, AM, MB, AL, MC – analyzed the data. All authors – involved in the data collection, edited and approved the final version of the manuscript.

Conflict of interest

The authors declare no conflicts of interest.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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