Original Article

Mutation Status Defines Subtypes of Essential Thrombocythemia and Relation to Polycythemia Vera in Iraqi Patients

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Abstract

Background: Polycythemia vera (PV) and essential thrombocythemia (ET) are a part of the BCR-ABL1-negative myeloproliferative neoplasms (MPNs) that harbor mutation in Janus kinase 2 (JAK2), CALR, or MPL gene. **Objectives:** The objective of this study was to investigate the impact of JAK2 and CALR mutations on the clinical course and hematological phenotype of ET patients and to evaluate the biological and clinical features of ET and PV sharing the same type of mutation in JAK2V617F. **Materials and Methods:** This was a cross-sectional study that included 94 patients diagnosed with MPN, of them 47 had PV and 47 had ET. JAK2V617F mutation was assessed using either allele-specific PCR or JAK-2 quantitative real-time PCR kit. JAK2-negative patients were further assessed for the existence of CALR mutations using SNP biotechnology MPN screening kit. **Results:** JAK2 mutation was identified in 29 ET patients, whereas CALR mutations were confirmed in 18 patients. JAK2-mutated ET patients were significantly older than those with CALR mutations. Seventy-six were reported to have a mutation in JAKV617F, of them 47 were diagnosed as PV and 29 as ET. JAK2V617F-mutated PV patients had significantly higher levels of hemoglobin, hematocrit, and WBC than JAK2-mutated ET patients. On the other hand, JAK2-mutated PV patients exhibited lower platelet count than ET harboring the same mutation. **Conclusion:** JAK2-mutated ET represents a distinct clinical entity that has a hematological and clinical phenotype ranging between JAK2-mutated PV and CALR-mutated ET. The analysis of the mutational status is essential in discriminating subtypes of MPN and confirming the diagnosis in ET and PV patients.

Keywords: CALR, essential thrombocythemia, Janus kinase 2, MPL, myeloproliferative neoplasms, polycythemia vera

Introduction

The World Health Organization (WHO) classified the Philadelphia-negative myeloproliferative neoplasms (MPNs) into polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF).[1] These disorders are characterized by excessive production of fully functional and mature blood cells. The Philadelphia-negative MPNs exhibited interrelated clinical characteristics, a mutual molecular background, and common disease-related complications such as thrombosis and the tendency for transformation into acute leukemia.[2]

All MPNs arise from the expansion of a distinct hematopoietic stem cell, harboring somatic mutations that ultimately give rise to all blood elements.[3] The Janus kinase 2 (JAK2) V617F point mutation is the most frequent type of driver mutations, detected in nearly 75% of Philadelphia-negative MPN patients.[4] Approximately 95% of PV and 60% of ET or PMF patients harbor a mutation in JAK2V617F.[5] JAK2V617F mutation results when the valine is replaced by phenylalanine in codon 617. JAK2V617F mutation can influence the risk of thrombosis in MPN patients and is considered an important risk factor for thrombotic events in ET patients.[6] The remaining 5% of PV patients harbor deletion or insertion mutations in JAK2 exon 12.[7] The shared mutation type and the overlapping clinical

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characteristics of PV and ET patients, especially at their early stages, represent a challenge to most clinicians.

According to the WHO, hemoglobin and hematocrit values are important markers for distinguishing the two conditions despite that their sensitivity and specificity were not verified. Furthermore, the red cell mass cannot be predicted by depending on the red cell values. Many studies clarified that PV and ET patients harboring mutation in JAK2V617F represent a biological continuum that is under the effect of genetic and physiological modifiers that determined the degree of erythrocytosis in those patients.[8] On the other hand, the JAK2V617F mutant allele burden might be another factor that contributes to the different phenotypes associated with the same type of mutation.[9]

Somatic mutations in MPL exon 10 are present in 5% of ET or PMF patients, with the most frequent being WPLW515 L/K,[10,11] whereas most ET or PMF patients, who are not carrying a mutation in JAK2V617F or MPL, harbor frameshift mutations in the CALR gene.[12]

CALR is a gene encode for calreticulin, a multifunctional Ca-binding protein located in the endoplasmic reticulum. The somatic frameshift mutations in the CALR gene are responsible for about 25%35% of PMF cases and 15%24% of ET cases.[13] The heterogeneity in the molecular background of ET may reflect a distinct clinical course of the disease. JAK2-mutated ET seems to have more aggressive clinical course than CALR-mutated ET.[14] Furthermore, many studies had revealed that CALR-mutated ET is frequently associated with lower hemoglobin and leukocyte counts, high platelets, and low risk of thrombosis.[15] Accordingly, these findings highlight the importance of including the JAK2, MPL, and CALR mutations as diagnostic and prognostic markers for MPN.

In the current study, we investigated the impact of JAK2 and CALR mutations on the clinical characteristics and hematological phenotype of ET patients. In addition, we compared the biological and clinical features of ET and PV sharing the same type of mutation in JAK2V617F.

Materials and Methods

Patients and samples

This cross-sectional study included a cohort of 94 patients, who are diagnosed as ET or PV in the medical city, Baghdad Hospital, Department of Hematology, and the National Center for Research and Treatment of Hematology/Mustansiriyah University between December 2021 and May 2022. Among the 94 MPN patients included in the study, 47 patients were diagnosed with PV and 47 patients were diagnosed with ET. Verbal consent was obtained from all eligible patients. Our inclusion criteria were in accordance with the 2016 WHO criteria for the diagnosis of Philadelphia-negative MPN (PV or ET).[1]

Blood samples were obtained between 8:00 am and 12:00 pm. Clinical and hematological variables, such as hemoglobin, hematocrit, platelet count, leukocyte count, and the presence of hepatomegaly, splenomegaly, and hepatosplenomegaly, and thrombotic events were recorded at the time of diagnosis by reviewing the medical records. Genomic DNA was extracted from peripheral blood samples, using the QIAamp DNA Micro Kit (QIAGEN, Germany) or ReliaPrep Blood gDNA Miniprep System (Promega, USA) according to the manufacturer's instruction. The concentration of DNA was assessed using a spectrophotometer (Qubit 4 fluorometer from Thermo Fisher Scientific).

All MPN-suspected patients were first evaluated for the presence of JAK2V617F mutation using either allele-specific PCR as described in the previously published protocol[16] or JAK-2 quantitative-real time PCR kit (SNP, Turkey) according to the manufacturer's instruction. Patients with nonmutated JAK2 were further assessed for the existence of CALR exon 9 (Type 1, Type 2, and other mutations) using SNP biotechnology MPN screening kit (Turkey) according to the instruction protocol.

Statistical analysis

Statistical analysis of data was conducted using the Minitab 19 software. The normality of the numerical variables was checked using Anderson–Darling test.

Numerical variables were summarized as median and range, whereas categorical variables as count and relative frequency (%) of each categorical group. Comparisons of quantitative variables between groups of patients were carried out using an unpaired two-sample *t*-test or the nonparametric Mann–Whitney test. On the other hand, qualitative variables were compared using the Fisher's exact test. $P < 0.05$ was considered statistically significant.

Results

Clinical and laboratory characteristics of patients with Janus kinase 2 and CALR mutations

Mutations in JAK2 and CALR genes were analyzed in 47 newly diagnosed ET patients to study the impact of these mutations on their demographic and clinical characteristics, as shown in Tables 1 and 2. Among the studied group, 29 patients had a mutation in JAK2V617F, whereas CALR mutations were detected in 18 patients. No gender preference or difference in body mass index (BMI) was observed between the studied groups. Meanwhile, JAK2-mutated ET patients were significantly older than those with CALR mutations. Besides, we noticed that a large proportion of JAK2- and CALR-mutated ET patients had never smoked, and no one with JAK2 mutation is currently smoking versus two in the CALR-mutated group. No significant difference was found in the percentages of nonsmokers, smokers, and former smokers in both groups [Table 1].

In addition, the leukocyte counts were either normal or marginally elevated in most ET patients. No difference was noted in the total leukocyte counts between JAK2-mutated ET, and ET patients harboring CALR mutations. No statistical

significance was observed in the median levels of absolute neutrophils, basophils, and monocyte counts in ET patients harboring mutation in JAK2 versus CALR. Both JAK2 and CALR mutated ET were characterized by modest-to-severe thrombocytosis. Although the median platelet counts in JAK2 mutated ET was higher than the median platelets count in CALR mutated ET, the result was statistically insignificant [Table 2].

Next, we examined the clinical features of ET patients based on their mutation status. Only two patients had hepatomegaly at diagnosis, and both patients were carrying a mutation in JAK2V617F. No hepatomegaly was detected in CALR-mutated ET. On the other hand, six ET patients had splenomegaly at onset, 17.2% of them carried a mutation in the JAK2 gene, and 5.6% carried a CALR mutation. No significant differences in the prevalence of hepatomegaly, splenomegaly, and hepatosplenomegaly were found between the JAK2- and CALR-mutated ET patients [Table 2].

We further examined the frequency of thrombotic events among ET patients [Figure 1]. We found that 5 of the ET patients presented with thrombotic events at diagnosis, of them 4 (13.8%) harbored JAK2V617F mutation and 1 (5.6%) had a mutation in the CALR gene. Our result showed that the thrombotic events at diagnosis were more prevalent in JAK2 than CALR-mutated ET patients, although the results were not statistically significant.

Clinical and laboratory characteristics of patients with polycythemia vera and essential thrombocythemia carrying JAK2V617F mutation

Since both PV and ET patients were carrying the same type of mutation in the JAK2V617F gene, but they had different phenotypes, we compared their blood indices. In JAK26171F-mutated PV patients, about two-third were male (63.8%), while in JAK2V617-mutated ET patients, there was no sex preference [Table 3]. When comparing the proportions of males in PV and ET, no significant difference was seen in gender distribution between the two groups.

About 76 patients had a mutation in JAKV617F, as reported in Table 3. The median age of the entire cohort was 62. Although JAK2-mutated PV patients were older than JAK2-mutated ET patients, no significant difference was observed in age between the studied groups.

No significant difference in BMI was found between PV and ET patients. Most PV and ET patients never smoked, and only 8.3% of patients with PV are currently smoking versus no

Table 1: Baseline characteristics of patients with essential thrombocythemia stratified depending on the type of the mutations

	All ET (*n*=47), *n* (%)	ET JAK2V617F (*n*=29), *n* (%)	ET CALR (*n*=18), *n* (%)	*P*
Age (years)	52 (14-90)	58 (32-90)	45 (14-74)	<0.002
BMI (kg/m^2)	27.2 (20.28-42.34)	27.34 (20.28-42.34)	26.85 (20.8-35.6)	NS
Gender				
Male	22 (46.8)	14 (48.3)	8 (44.4)	NS
Female	25 (53.2)	15 (51.7)	10 (55.6)	NS
Smoking status				
Never	39 (83)	25 (86.2)	14 (77.8)	NS
Current	2 (4.2)	0	2 (11.1)	NS
Former	6 (12.8)	4 (13.7)	2 (11.1)	NS

Data were expressed as median and range, or as relative frequencies. BMI: Body mass index, ET: Essential thrombocythemia, NS: Not significant, CALR: calreticulin, JAK2: Janus kinase 2

Table 2: Comparison between the hematologic and clinical characteristics of patients with essential thrombocythemia stratified according to the type of the mutations

	All ET (*n*=47)	ET JAK2V617F (*n*=29)	ET CALR (*n*=18)	*P*
Hemoglobin g/dL	12.53 (9.99-15.2)	12.71 (10.21-15.2)	11.84 (9.99-14.6)	NS
Hematocrit (%)	39.8 (30.8-49.3)	40.88 (32.47-49.3)	36.2 (30.8-45.3)	NS
Leukocyte×10^3/µL	10.09 (5.07-22.29)	10.4 (5.07-22.29)	9.88 (5.86-16.19)	NS
Absolute neutrophils ×10^3/µL	6.93 (2.39-16.16)	7.76 (3.39-16.16)	6.18 (2.39-12.0)	NS
Eosinophils ×10^3/µL	0.22 (0.04-1.49)	0.21 (0.04-1.49)	0.25 (0.1-0.5)	NS
Basophils ×10^3/µL	0.10 (0.22-1.18)	0.12 (0.22-0.69)	0.12 (0.22-0.69)	NS
Monocytes ×10^3/µL	0.47 (0.04-1.51)	0.45 (0.04-1.51)	0.48 (0.14-1.36)	NS
Platelets ×10^9/L	684 (168-1500)	873 (435-1500)	805 (509-1928)	NS
Hepatomegaly, *n* (%)	2 (4.2)	2 (6.9)	0	NS
Splenomegaly, *n* (%)	6 (12.7)	5 (17.2)	1 (5.6)	NS
Hepatosplenomegaly, *n* (%)	1 (2.1)	1 (3.4)	0	NS

Data were expressed as median and range. ET: Essential thrombocythemia, NS: Not significant, CALR: Calreticulin, JAK2: Janus kinase 2

active smokers in the JAK2-mutated ET group. No remarkable difference was observed in the number of smokers, former smokers, and nonsmokers between PV and ET patients.

The laboratory features of the MPN patients carrying a mutation in JAK2V617F are listed in Table 4. When performing a comparison between JAK2V617F-mutated PV and ET, PV patients had significantly higher levels of hemoglobin and hematocrit than ET patients. The study cohort (JAK2V617F MPN) is characterized by leukocytosis and thrombocytosis. Leukocytosis was reported in both PV and ET patients harboring JAK2V617F mutation, but it was significantly higher in PV patients. The median absolute neutrophil count was significantly lower in ET when compared to PV patients with JAK2V617F mutation. No significant difference was found in median levels of eosinophils, basophils, and monocytes between JAK2-mutated PV and JAK2-mutated ET. Similarly, the platelet count in JAK2-mutated PV was significantly lower when compared to the JAK2-mutated ET [Table 4].

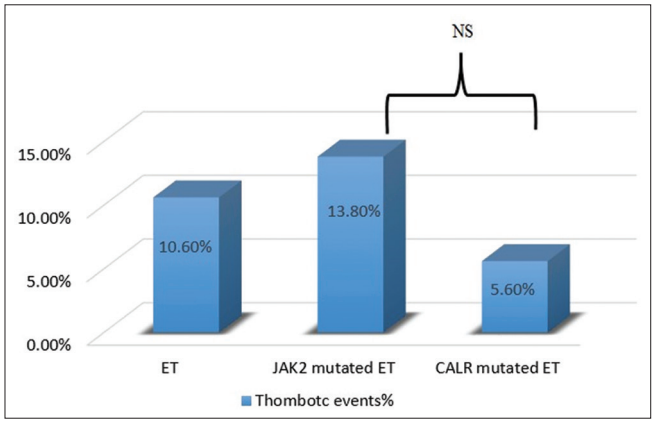

Figure 1: Thrombotic events at diagnosis in ET, JAK2-mutated ET, and CALR-mutated ET

For hepatomegaly and hepatosplenomegaly, no significant differences were reached between the JAK2-mutated PV and ET. Conversely, in JAK2-mutated PV, there was a trend toward a higher rate of splenomegaly than did the JAK2-mutated ET patents, but the results did not reach statistical significance [Table 4].

When comparing the frequency of thrombotic events in the study cohort [Figure 2], 9 JAK2V617F-mutated PV patients were found to have thrombotic events at diagnosis compared to only 4 JAK2V617F-mutated ET patients. Although the

Table 3: The different parameters of patients with polycythemia vera and essential thrombocythemia carrying Janus kinase 2 V617F mutation

	All MPN JAK2V617F (*n*=76)	PV JAK2V617F (*n*=47)	ET JAK2V617F (*n*=29)	*P*
Age (years)	62 (24-90)	64.5 (24-83)	58 (32-90)	NS
Gender, *n* (%)				
Male	44 (57.9)	30 (63.8)	14 (48.3)	NS
Female	32 (42.1)	17 (36.2)	15 (51.7)	NS
BMI (kg/m^2)	27.4 (17.26-42.34)	27.46 (17.26-32.65)	27.34 (20.28-42.34)	NS
Smoking status, *n* (%)				
Never	62 (81.6)	37 (79.2)	25 (86.2)	NS
Current	4 (5.3)	4 (8.3)	0	NS
Former	10 (13.2)	6 (12.5)	4 (13.8)	NS

Data were expressed as median and range or as relative frequencies. BMI: Body mass index, MPN: Myeloproliferative neoplasm, PV: Polycythemia vera, ET: Essential thrombocythemia, NS: Not significant, JAK2: Janus kinase 2

Table 4: Comparison between the hematologic and clinical characteristics of patients with polycythemia vera and essential thrombocythemia carrying Janus kinase 2 V617F mutation

	All MPN JAK2 V617F (*n*=76)	PV JAK2V617F (*n*=47)	ET JAK2V617F (*n*=29)	*P*
Hemoglobin g/dL	16.6 (10.21-23)	17.7 (16.2-23)	12.71 (10.21-15.2)	<0.001
Hematocrit (%)	48 (25.8-72)	54.65 (45.8-72)	40.88 (32.47-49.3)	<0.001
Leukocyte ×10^3/μL	12.97 (5.07-47.1)	13.7 (7.55-47.1)	10.4 (5.07-22.29)	<0.004
Absolute neutrophils ×10^3/μL	8.98 (3.39-32.9)	10.39 (3.69-32.9)	7.76 (3.39-16.16)	<0.02
Eosinophils ×10^3/μL	0.26 (0.01-1.49)	0.287 (0.01-1.09)	0.21 (0.04-1.49)	NS
Basophils ×10^3/μL	0.13 (0.02-0.74)	0.142 (0.02-0.76)	0.12 (0.22-0.69)	NS
Monocytes ×10^3/μL	0.57 (0.01-8.42)	0.66 (0.01-8.42)	0.45 (0.04-1.51)	NS
Platelets ×10^9/L	684 (168-1500)	545.9 (168-1500)	873 (435-1500)	<0.001
Hepatomegaly, *n* (%)	8 (10.6)	6 (12.7)	2 (6.9)	NS
Splenomegaly, *n* (%)	22 (28.9)	17 (36.2)	5 (17.2)	NS
Hepatosplenomegaly, *n* (%)	5 (6.5)	4 (8.5)	1 (3.4)	NS

Data were expressed in median and range. MPN: Myeloproliferative neoplasm, PV: Polycythemia vera, ET: Essential thrombocythemia. NS: Not significant, JAK2: Janus kinase 2

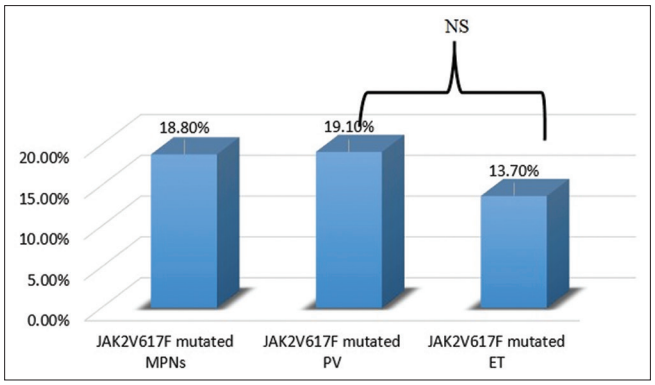

Figure 2: Thrombotic events in JAK2V617F-mutated MPNs, JAKV617F-mutated PV, and JAK2V617F-mutated ET

thrombotic events were more prevalent in PV patients, the results were statistically not significant.

Discussion

Throughout the past years, the discovery and characterization of driver mutations in the genes implicated in the initiation of MPNs enable affirmation of the diagnosis molecularly in nearly 80% of ET patients and the vast majority of PV patients. Consequently, screening for mutations in JAK2, CALR, and MPL is required for the diagnosis of MPNs.[1]

In the current study, the mutation status was identified in 47 patients with ET and 47 patients with PV. Since many MPN disorders share the same mutation status and mimic the clinical presentation of ET and PV, it is crucial to perform bone marrow examination concurrently with precise clinical evaluation to distinguish between the different MPN types.[13] Mutation status distribution among our cohort was comparable to earlier reports.[17,18] In addition, CALR gene mutation was not detected in PV patients recommending the benefit of CALR mutation molecular analysis for differentiating subtypes of MPN.[19]

Since the discovery of CALR mutation, many researchers have debated that the mutational status has an impact on the laboratory and clinical characteristics of ET patients. Similar to previous reports, ET patients harboring CALR mutation were younger than JAK2V617F-mutated ET.[6] However, in the current study, we had noticed that the two groups did not differ in terms of their hemoglobin, hematocrit, and leukocyte count, but there was a tendency toward lower values in CALR-mutated ET. Besides, the platelet count was similar in both groups. Our results were slightly different from most researchers who affirmed that CALR-mutated ET patients had lower hemoglobin and hematocrit values and higher platelet counts.[6,14] This discrepancy could be explained by the limited number of samples used in this study. The high platelet counts detected in CALR-mutated ET patients reflect the role of the CALR gene in the development of megakaryocytes in the bone marrow. Despite the considerable number of CALR-mutated ET patients who had platelet counts more than 1000×10^9/L, the thrombotic risk had a tendency toward higher values in JAK2-mutated ET patients. Our findings were in agreement with a large study published by Carobbio *et al*., 2011, who involved 891 patients with ET.[20] Carobbio *et al*. showed that JAK2 mutation is a strong independent risk factor for thrombosis.

The absence of JAK2 mutation and markedly elevated platelet count are the main features of CALR-mutated ET. Consequently, the pathogenesis of vascular events associated with ET might not be due to platelet count *per se*. Instead, increased granulocytes concurrently with platelet activation could be a more important factor that contributes to the vascular event pathogenesis in ET patients rather than platelet count alone.[21]

ET patients harboring CALR mutations were found to have a lower incidence of splenomegaly than JAK2-mutated ET, although our results did not reach statistical significance. Our results are in general line with Nunes *et al*., 2015, as they found that CALR-mutated MPN patients had a lower incidence of splenomegaly than patients carrying JAK2 mutation.[22] In addition, some investigators had reported that PV and ET patients harboring a mutation in JAK2V617F represent a biological continuum, because of the shared mutation type and the relatively higher levels of hemoglobin, hematocrit, and leukocyte counts with increased risk of thrombosis in JAK2-mutated ET.[8]

To further support this hypothesis, a direct comparison was made between PV and ET patients harboring JAK2V617F mutation. In keeping with other published reports, a higher but not significant proportion of thrombotic events were documented in JAK2V617F-mutated PV when compared to JAK2V617F-mutated ET.[23] However, many studies have reported different results, Carobbio *et al*., 2009, reported a significantly higher rate of thrombosis in JAK2-mutated PV.[24] The different results may be attributed to the limited number of subjects included in our study. Furthermore, this study depended only on the mutational status with no measurement of JAK2 allele burden. Several studies had documented that the amount of mutant allele burden has an influence on the rate of thrombotic events in JAK2-mutated ET and PV.[25]

Conclusion

In general, our study verifies that JAK2-mutated ET categorizes as a distinctive clinical entity that has a hematological and clinical phenotype in-between JAK2-mutated PV and CALR-mutated ET. In addition, our data were in line with the importance of mutational status analysis to distinguish subtypes of MPN. JAK2 and CALR mutations are important markers that consolidate the precise diagnosis and prognosis in ET and PV patients.

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Conflicts of interest

There are no conflicts of interest.

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