



Correlation of Thrombosis and Clinicohematological Parameters with JAK2V617F Mutation in Philadelphia-Negative CMPNs: A Study from India

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Abstract

Objective Philadelphia-negative chronic myeloproliferative neoplasms (CMPNs), which include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF), are characterized by the presence of JAK2V617F (exon 14) mutation, and this occurs in 90 to 95% cases of PV and 50 to 60% cases of ET and PMF. Still, this is a matter of debate regarding the correlation of this mutation with thrombosis and clinicohematological parameters in CMPNs. So, we conducted this study to ascertain the association of JAK2V617F mutation with thrombotic complications and clinicohematological parameters of these patients.

Materials and Methods This prospective and retrospective study was conducted during 2018 to 2019 at the Department of Laboratory Sciences and Molecular Medicine of a tertiary care hospital, and 160 CMPN patients were enrolled. Complete hemogram was done and DNA was extracted, followed by real-time qualitative polymerase chain reaction to check for JAK2V617F mutation. This mutation was then correlated with complications, mainly thrombosis, hematological parameters, and clinical parameters such as age and splenomegaly.

Results Among 160 CMPN patients, 60 were females and 100 were males, with male to female ratio of 1:0.6, and age range of 27 to 85 years. Total 91 (56.9%) patients were JAK2V617F positive and the remaining 69 (43.1%) were negative for this mutation. We observed statistically significant correlation of leukocyte count, splenomegaly, and thrombosis in JAK2V617F-mutated patients as compared to unmutated patients.

Conclusion This study emphasizes the importance of JAK2V617F mutation in CMPNs and stresses on its association with clinical, hematological phenotype, and thrombotic complications, which may open new horizons in prognostication and management protocol.

Keywords

- JAK2V617F mutation
- Philadelphia-negative CMPNs
- thrombosis

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Introduction

Chronic myeloproliferative neoplasms (CMPNs) which include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are characterized by clonal proliferation of abnormal hematopoietic stem cells, bone marrow hypercellularity, splenomegaly and extramedullary hematopoiesis, tendency for thrombotic/bleeding complications, and acute leukemia transformation.^{1,2} The main genomic mutation of these three disorders is JAK2V617F (exon 14), and this mutation occurs in almost 90 to 95% cases of PV and 50 to 60% cases of ET and PMF.^{3–5} The other mutations such as calreticulin (CALR) and myeloproliferative leukemia virus oncogene (MPL) are also seen in smaller percentage of CMPN.^{4,5} Thrombohemorrhagic complications are frequently observed in JAK2V617F-mutated patients which is a cause of concern, keeping in view the morbidity and mortality associated with these complications. As per the literature, CMPNs are predisposed to both venous and arterial thromboses with higher prevalence of venous thrombosis in JAK2V617F-positive cases in CMPNs.^{3,6–8}

The other significant risk factors for thrombotic events in CMPN patients are increasing age, raised platelet count, previous history of thrombosis, and increased hematocrit. The presence of JAK2V617F mutations are associated with leukocytosis, thrombocytosis, platelet activation, and these factors may also play a significant role in the pathogenesis of thrombotic events as well as may have impact on increasing the thrombotic risk in these patients.^{3–5} However, this is still a matter of debate regarding associative role of this mutation with thrombosis as there are mixed reviews as per the literature.^{9–11}

Association with hemogram is also documented in the literature, though with variable results. In comparison to few studies which showed raised total leukocyte count (TLC) in JAK2V617F mutation-positive patients as compared with JAK2V617F-negative patients, other studies show no correlation of these factors with JAK2V617F mutational status.¹²

In view of above-mentioned, we conducted this study to ascertain the association of JAK2V617F mutation with thrombotic/bleeding complications and hemogram parameters in 160 Philadelphia (Ph)-negative CMPN patients.

Patients/Materials and Methods

This is a retrospective and prospective study done in 160 Ph-negative CMPN patients over the period of 2 years (2018–2019) at the Department of Laboratory Sciences

and Molecular Medicine of this tertiary care hospital. These patients were followed up for another 15 months till the outbreak of coronavirus disease 2019 pandemic and further follow-up could not be done in view of ongoing pandemic. These CMPN patients (PV, PMF, and ET) were diagnosed as per the World Health Organization's 2016 criteria for myeloproliferative neoplasms.¹³ Three mL of whole blood was collected from the patients in EDTA vacutainer and complete hemogram was done and DNA was extracted followed by real-time qualitative polymerase chain reaction (PCR) using LH 750 real-time PCR machine to check for JAK2V617F mutational status using commercially available kit (03 B BlackBio real-time qualitative PCR kit, Make: India). Detailed history of thrombosis of headache, abdominal pain, and breathlessness was recorded in these patients for correlation with JAK2V617F mutation. The arterial and venous thromboses were diagnosed radiologically by venous Doppler studies and computed tomography angiography. In suspected PV patients, further exon 12 mutation analysis was not done due to nonavailability of this testing in our molecular laboratory. JAK2V617F mutation was then correlated with complications mainly thrombosis, hematological parameters, particularly, TLC, platelet count, and clinical parameters such as age and splenomegaly. Ethical approval for the study protocol was obtained from institutional ethical committee, and written informed consent was taken from all patients. The statistical analysis was performed by using nonparametric statistics for continuous variables and chi-square test for categorical variables.

Results

Among 160 CMPN patients, 60 were females and 100 were males, with male to female ratio of 1.66:1 and age range of 27 to 85 years. Eighty out of 160 (50%) cases were PV, 33/160 (20.6%) were ET cases and 47/160 (29.4%) were PMF cases. Out of these 160 cases, 91 (56.9%) were JAK2V617F positive and the remaining 69 (43.1%) were negative for this mutation. The general clinical profile of the patients including hematological parameters of JAK2 V617F-positive CMPN patients in comparison to JAK2 V617F-negative patients is summarized in ► **Table 1**. Correlation of JAK2V617F mutation with thrombosis in CMPN is summarized in ► **Table 2**.

Arterial and venous thromboses were statistically significant in JAK2V617F-positive patients and hematological parameters such as TLC were also statistically significant when correlated with JAK2V617F mutation ($p < 0.05$). Furthermore, arterial thrombotic events included stroke, limb

Table 1 Clinical, hematological, and biochemical parameters

Clinical parameters	JAK2-positive patients (91/160)	JAK2-negative patients (69/160)	p-Value
Age (y) (median)	58	49	0.001
Splenomegaly	71	20	0.000
TLC (/cumm) (median)	10,500	8,000	0.031
Platelet (/mm ³) (median)	380,000	390,000	0.657

Abbreviation: TLC, total leukocyte count.

Table 2 Correlation JAK2V617F mutation with thrombosis

Molecular studies	Total number of patients <i>n</i> = 160	Patients with thrombosis		Patients with-out thrombosis		<i>p</i> -Value
		<i>N</i>	%	<i>N</i>	%	
JAK2 positive	91	59	64.8	32	35.2	0.0004
JAK2 negative	69	29	42.1	40	57.9	
Total	160	88		72		

ischemia, and cardiovascular events. Venous thrombotic events included portal vein thrombosis, sagittal vein thrombosis, and other unusual site venous thrombotic events.

Discussion

CMPNs are the clonal disorders that exhibit hyperproliferation defects in hematopoietic cells of bone marrow without significant alterations in cellular differentiation and maturation. The term CMPN covers chronic myeloid leukemia (CML), PV, ET, and PMF. Granulocytes, erythrocytes, and platelets are increased primarily in CML, PV, and ET, respectively; however, due to clonal driver mutations such as JAK2V617F and CALR, there may be proliferation of more than one cell lineage in these disorders, for example, panmyelosis is observed in PV, and thrombocytosis can be seen in ET. Increased tyrosine kinase activity caused by JAK2V617F mutation increases the cytokines which trigger the hyperplasia of erythrocytic, granulocytic, and megakaryocytic progenitor cells. Correlation of JAK2V617F with thrombosis and hematological parameters is documented in the literature with variable results.^{9–12}

In our study, we observed raised leukocyte count in 43/91 (47.2%) JAK2V617F-positive patients as compared with 18/69 (26%) JAK2V617F-negative patients, and this difference was found to be statistically significant ($p = 0.031$) (►Table 1). Our results are in concordance with study by Tefferi et al (2011) and Cetin et al (2014) in American and Turkish populations, respectively.^{10,14} However, our results are not in line with Sazawal et al in Indian population.¹² However, the rising trend in leukocyte count was observed, though not statistically significant as per study by Sazawal et al.¹²

In the present study, we observed thrombosis in 59/91 (64.8%) JAK2V617F-positive as compared with 29/69

(42.1%) JAK2V617F-negative patients, and using chi-square test, this difference was found to be statistically significant (chi-square value of 8.247 and the $p = 0.0004082$) (►Table 2). The overall frequency of thrombosis, both arterial and venous, in Ph-negative CMPN patients in our study is 88/160 (55.0%). The analysis of associative role of JAK2V617F mutation with thrombosis in various populations across the world in comparison to the present study is summarized in ►Table 3. The results in our study are in concordance with studies from Switzerland, China, and Japan conducted by Kralovics et al in 2005, Cheung et al in 2006, and Ohyashiki et al in 2007.^{15–17} However, studies from New York and Manchester, United States, by Tefferi and Vainchenker 2011 and Wolanskyj et al in 2006, respectively, showed no significant association of thrombosis with JAK2V617F mutation (►Table 3).^{10,11}

Furthermore, our previous study from Indian population, though from different tertiary care institute in a relatively smaller subset of patients (total 65 Ph-negative CMPN cases), showed positive correlation of thrombosis in JAK2V617F-mutated patients (62.5%) as compared with unmutated group (►Table 3).² Most likely causes of thrombosis in JAK2V617F-mutated CMPN cases can be increasing hemoglobin, TLC, and increasing platelet activation along with hyperresponsiveness through impairment of protooncogene MPL (cMPL) signal transduction and elevated platelet–white blood cell aggregation through excess P selectin expression.² However, in view of mixed opinion as per various studies regarding association of JAK2V617F mutation with thrombosis, further studies in larger subset of patients may give more information and open new horizons in this field. However, no statistically significant correlation of bleeding

Table 3 JAK2V617F mutation association with thrombosis in various populations across the world

Year of study	CMPN patients	Study population	JAK2 association with thrombosis	JAK2 positive vs. JAK2 negative in thrombosis (%)
Singh et al (2018)	65	India	Yes	62.5 vs. 48
Ohyashiki et al (2007)	49	Japan	Yes	29 vs. 6
Cheung et al (2007)	60	China	Yes	62 vs. 26
Tefferi et al (2006)	63	Manchester	No	No difference
Wolanskyj et al (2006)	150	New York	No	No difference
Present study	160	India	Yes	64.8 vs. 42.1

Abbreviation: CMPN, chronic myeloproliferative neoplasm.

was found with JAK2V617F mutational status in CMPN patients in our study.

In our study, we observed splenomegaly in 71/91 (78%) JAK2V617F-positive patients as compared with 20/69 JAK2V617F-negative patients, and this difference was found to be statistically significant ($p=0.000$). These results are concordance with the study by Ayer et al (2017) and Zaho et al (2016) in Turkish and Chinese populations, respectively.^{18,19}

Conclusion

This study highlights the importance of JAK2V617F mutation in Ph-negative CMPNs and stresses on its association with clinicohematological phenotype of the patient (age, spleen size, and hemogram) and thrombotic complications. Further, the correlation of this mutation with thrombosis in CMPN patients may open new horizons in prognostication and management protocol of these patients. However, further studies in larger subset of patients are needed to confirm our findings.

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None.

Conflict of Interest

None declared.

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