

Clinicopathological Spectrum of BCR-ABL-Negative Myeloproliferative Neoplasms with Correlation with Janus-Associated Kinase 2 Mutation

Abstract

Background: Non chronic myelogenous leukemia (non-CML)/BCR-ABL-negative myeloproliferative neoplasms (MPNs) include essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF) (apart from chronic neutrophilic leukemia and chronic eosinophilic leukemia, which are rare). They are uncommon clonal disorders of adults, with an incidence ranging from 0.5 to 3/100,000 persons, BCR-ABL negative, and characterized by the activation of Janus-associated kinase 2 (JAK2). Very few studies have been reported from India. **Aims and Objectives:** The aims and objectives of this study were to analyze the clinicopathological spectrum and to determine the frequency of JAK2 mutation in patients of non-CML/BCR-ABL negative MPNs. **Materials and Methods:** Clinical and morphological features and frequency of JAK2 mutation in patients with PV, ET, and PMF were studied at a tertiary care hospital. The material was retrieved from the hematopathology records and reviewed. **Results:** JAK2V617F mutation was found in 10 of 14 cases (71%) of MPNs, 100% in PV, 50% in ET, and 71% of idiopathic myelofibrosis. The presence of JAK2V617F mutation was associated with a higher hemoglobin level ($P < 0.05$), a higher TLC ($P < 0.05$), and higher age ($P < 0.05$). Results showed that there are morphologic differences, and megakaryocytic morphology represents a useful clue for the differential diagnosis of these three BCR-ABL-negative MPN subtypes. **Conclusion:** The JAK2 V617F mutation was detected in 71% of patients with MPN disorders. Peripheral blood mutation screening for JAK2 V617F should be incorporated into the initial evaluation of patients suspected to have MPNs. Differences in megakaryocytic morphology provide the histomorphological hallmark of BCR-ABL-negative MPN subtypes.

Keywords: Janus-associated kinase 2 mutation, marrow morphology, myeloproliferative neoplasm

Introduction

Non chronic myelogenous leukemia (non-CML) or BCR-ABL-negative myeloproliferative neoplasms (MPNs) represent a range of clonal hematological stem cell disorders with overlapping clinicopathological features. The most common BCR-ABL-negative MPNs include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF).^[1,2] They are distinguished from one another on the basis of effects of cell lineages and fibrosis in the bone marrow compartment.^[3] These are uncommon hematological malignancies with worldwide incidence of 2–3/100,000, 1.5–2 per 100,000, and 0.5–1.5/100,000, respectively.^[4]

At present, the mutation which is found most commonly in classical BCR-ABL-negative

MPNs is Janus-associated kinase 2 (JAK2) V617F5. In 2005, the JAK2V617F mutation^[5-7] was shown in 95% of patients with PV and in just more than half of those with ET and PMF.^[5,8] In 2008, the WHO diagnostic criteria for PV, ET, and PMF were revised by incorporating molecular markers (JAK2 and MPL mutations) in the diagnostic criteria and also underscoring the role of histology in differentiating clonal from nonclonal myeloproliferation.^[1]

Atypical megakaryocytes are the histomorphological hallmark of all BCR-ABL-negative MPNs. Since there are very few studies published from India, this study aims to look for the frequency of JAK2 V617F mutations along with clinical and morphologic features in an attempt to identify histologic findings, helping to differentiate these distinct clinicopathological entities.

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Roopa Dixith,
A Sara,
Navatha Vangala,
Shantveer G Uppin,
Megha S Uppin,
AMVR Narendra¹,
Tara Roshni Paul

Departments of Pathology and
¹Hematology, Nizam's Institute
of Medical Sciences, Hyderabad,
Telangana, India

Address for correspondence:

Dr. Tara Roshni Paul,
Department of Pathology,
Nizam's Institute of Medical
Sciences, Punjagutta,
Hyderabad - 500 082,
Telangana, India.
E-mail: troshnip@yahoo.co.in

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Materials and Methods

This was a retrospective and prospective study in which all the non-CML, BCR-ABL-negative MPN patients presenting to a tertiary care hospital from January 2015 to May 2016 were included. CML and other myelodysplastic/MPNs were excluded from the study. All cases diagnosed to have reactive causes of polycythemia, leukocytosis, and thrombocytosis were excluded along with cases where secondary myelofibrosis was diagnosed. Both newly diagnosed cases ($n = 46$) and previously diagnosed cases on follow-up ($n = 8$) were included in this study.

Janus-associated kinase 2 mutation analysis

Screening of JAK2 mutation was done by PCR in 14/54 patients of MPNs. Although the other patients were requested to submit samples for JAK2 mutation analysis, it was not done due to financial constraints. Comparison of hematological parameters between JAK2-positive and JAK2-negative cases was done using independent samples' *t*-test/Mann-Whitney U-test where appropriate. $P < 0.05$ was considered statistically significant.

Morphologic analysis

All slides were reviewed and classified according to the current 2008 WHO criteria.^[1] Clinical details and laboratory parameters were extracted from the medical records. Bone marrow morphologic variables that were evaluated included were as follows: absolute cellularity (percentage), age-adjusted cellularity (classified as decreased, normal, or increased), myeloid/erythroid ratio, and percentage of blasts in the biopsy. We defined megakaryocytes as "PV like" or "PMF-like" or "ET-like" according to the description given in the 2008 WHO classification of the typical megakaryocytes, characterizing these three myeloproliferative neoplasms.

Results

A total of 54 non-CML MPN cases were retrieved during this period and reviewed. Out of which, 28 cases (51.85%)

were diagnosed as PMF, 14 cases (25.92%) as ET, and 12 cases (22.22%) as PV. Of the 54 cases, 33 (61%) of them were male and 21 (38%) were female. Their ages ranged from 25 to 81 years, with a mean age of 56 years. Most of the patients of PMF, ET, and PV presented with varying degrees of splenomegaly. Thrombotic events were seen in nine patients (16.98%), and bleeding episodes were observed in three patients (5%) of PV and ET. The mean Hb of PMF was low 8.7 ± 2.9 gm/dl, compared to ET (14.7 ± 2.8 g/dl) and PV (16.7 ± 2.42 g/dl). The median WBC count was high in all the three MPN subtypes. The median platelet count was high in ET, and in PMF and PV, it was within the normal range [Table 1].

Janus-associated kinase 2 mutation analysis

JAK2 mutation analysis was available in 14 of 54 cases (25%), of which 10 (71.42%) were JAK2 positive. In PMF, five out of seven cases (71%) were JAK2 positive; in ET, two out of four cases (50%) were positive; and in PV, all three cases (100%) were positive [Table 1]. The overall presence of JAK2 mutation was associated with higher hemoglobin level ($P < 0.05$), a higher white blood cell count ($P < 0.05$), and higher age ($P < 0.05$). No association was found for platelet count [Table 2].

Primary myelofibrosis

There were 28 patients of PMF, diagnosed as per the revised WHO criteria 2008, of which 18 (64.28%) were male and 10 (35.5%) were female. Their ages ranged from 28 to 81 years, with a mean age of 53 years. Majority, i.e., 23 cases (82%), of them presented with moderate anemia, splenomegaly, and fever. Five cases (18%) had pancytopenia on evaluation. The mean Hb was 8.7 ± 2.9 , median WBC count was $11.7 \times 10^9/l$, and median platelets was 1.5 lakh/mm.^[3] JAK2 mutation analysis was available in 7 cases, out of which 5 (71%) were positive. Among the 28 cases, 3 (10%) were in the prefibrotic/early stage (2 males and 1 female); 21 (75%) were in fibrotic stage with 12 males and 9 females. Four cases (14%) showed blast transformation to acute myeloid leukemia (AML), which was confirmed by cytochemistry and flow cytometry [Table 3].

Table 1: Clinical and laboratory features of patients ($n=54$) with non chronic myeloid leukemia myeloproliferative neoplasms

	PMF	ET	PV
Number of patients	28	14	12
Sex (male/female)	18/10	8/6	7/5
Age (year), mean±SD	53±14.7	46±13.2	52±14.7
Splenomegaly (%)	82	71	72
Thrombosis/hemorrhage	0/0	4/1	5/2
WBC ($\times 10^9/l$), median	11.7 (1100-64,000)	15.2 (7.5-40.0)	22.8 (8.6-33.5)
Hb (g/dl), mean±SD	8.7±2.9	14.7±2.8	16.7±2.42
Platelet ($\times 10^9/l$), median	150.5 (10-1310)	970.5 (650-1610)	370.5 (180-980)
JAK2 V617F (+), n/N (%)	5/7 (71)	2/4 (50)	3/3 (100)

SD – Standard deviation; PMF – Primary myelofibrosis; ET – Essential thrombocythemia; PV – Polycythemia vera; WBC – White blood cell; Hb – Hemoglobin; JAK2 – Janus-associated kinase 2

Essential thrombocytenia

Fourteen patients were diagnosed as ET as per the revised WHO criteria 2008, of which 8 (57.14%) were males and 6 (42.8%) were females. Their ages ranged from 26 to 70 years, with a mean age of 46 years. Most of the cases 9 (64.28%) had thrombocytosis with thrombotic event seen in 4 (28.57%) patients, which included digital infarction in 2 (14.28%), portal vein thrombosis in 1 (7.14%), and cerebrovascular accidents in 1 (7.14%) case. Bleeding (epistaxis) was noted in 1 (7.14%) case. The mean Hb was 14.7 ± 2.8 g/dl, median WBC count was $15.2 \times 10^9/l$, and median platelets was 9.7 lakh/mm.^[3] JAK2 mutation analysis was available in four cases, out of which 2 (50%) were positive.

Polycythemia vera

Twelve patients were diagnosed as PV, their ages ranged from 28 to 78 years with a mean age of 52 years, of which, 7 (58%) were males and 5 (41%) were females. Of these, 86% of the patients had conjunctival plethora and ruddy cyanosis. Thrombotic events were seen in 5 (41%) patients including digital infarction in 2 (16%), cortical sinus thrombosis in 1 (8%), portal vein thrombosis in 1 (8%), and cerebrovascular accidents in 1 (8%) patient. Two cases (16%) had bleeding episodes (epistaxis and hematemesis, respectively). The mean Hb was 16.7 ± 2.42 g/dl, median WBC count was $22.8 \times 10^9/l$, and median platelets was 3.7 lakh/mm.^[3] JAK2 mutation analysis was available in 3 cases, and all of them (100%) were positive.

Bone marrow histology analysis

All patients of PMF, PV, and ET had increased cellularity for their age; however, absolute cellularity was found to

be higher in polycythemic patients (mean: 95%) than in ET (mean: 76%) and PMF patients (mean: 66%). Trilineage hyperplasia was seen in PV, whereas PMF showed myeloid hyperplasia and in ET, E/M ratio was normal [Table 4].

Megakaryocytes were increased in all the patients of PMF, PV, and ET. In PMF cases, the majority of megakaryocytes (100%) showed "PMF-like" features, the most striking being atypia with increased nuclear:cytoplasmic ratio and abnormal patterns of chromatin clumping, with bulbous, "cloud-like" or "balloon-shaped" nuclei [Figure 1]. In ET, majority of megakaryocytes showed "ET-like" features displaying large hypermature megakaryocytes with abundant mature cytoplasm deeply hyperlobated nuclei. Some of them showed emperipolesis [Figure 2]. In PV, 92% of the patients showed "PV-like" megakaryocytes with

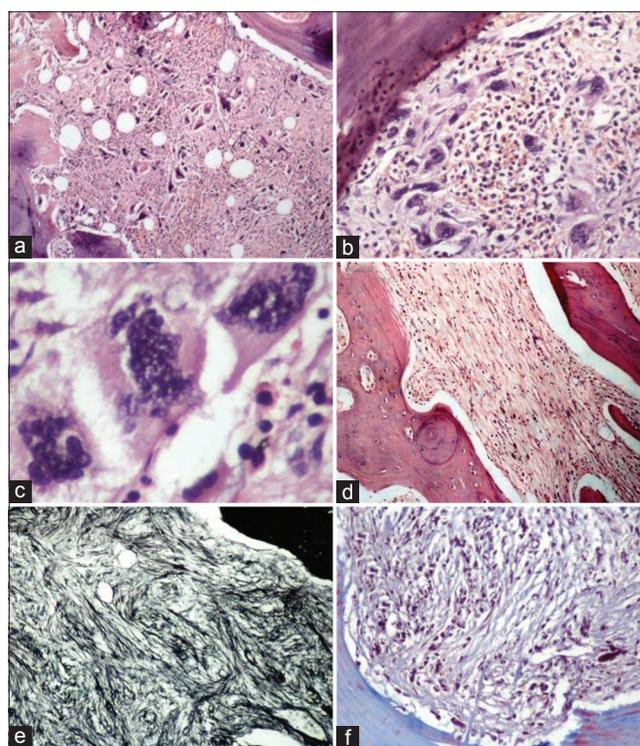


Figure 1: Primary myelofibrosis trephine histology. (a) Early stage (H and E, ×40). (b) Paratrabeular megakaryocytes (H and E, ×400). (c) Megakaryocytes with "cloud-like" nuclei (H and E, ×1000). (d) Fibrotic stage (H and E, ×400). (e) Reticulin Grade 3-4 (Reticulin, ×400). (f) Coarse collagen fibers (Masson Trichrome, ×400)

Table 2: Clinicohematological parameters of Janus-associated kinase 2 positive and negative cases (n=14)

	JAK2 positive (n=10)	JAK2 negative (n=4)	P
Age (year), mean±SD	52±10.2	46±15.1	<0.05
Hb (g/dl), mean±SD	13.8±3.59	10.2±2.33	<0.05
WBC ($\times 10^9/l$), median	16.7	10.1	<0.05
Platelets ($\times 10^9/l$), median	5.6	5.4	0.348

SD – Standard deviation; WBC – White blood cell; Hb – Hemoglobin; JAK2 – Janus-associated kinase 2

Table 3: Clinicohematological parameters of patients (n=28) with primary myelofibrosis

	Prefibrotic/early	Fibrotic	Blast transformation
Number of patients	3	21	4
Age (year), mean±SD	59±24.4	52±14.2	56±11.1
Sex (male/female)	2/1	12/9	4/0
Hb (g/dl), mean±SD	11.4±2.37	8.7±2.70	6.4±3.37
WBC ($\times 10^9/l$), median	40,200 (38,900-48,100)	9400 (1100-64,000)	17,600 (1600-40,600)
Platelet ($\times 10^9/l$), median	500.1 (150-550)	150.0 (10-1310)	150.1 (22-420)
Leukoerythroblastic picture, (%)	0	100	50
JAK2 V617F (+)	2/2	2/3	1/2

SD – Standard deviation; WBC – White blood cell; Hb – Hemoglobin; JAK2 – Janus-associated kinase 2

Table 4: Marrow histopathological features of patients (n=54) with non chronic myeloid leukemia myeloproliferative neoplasms

	PMF (n=28)	ET (n=14)	PV (n=12)
Cellularity, % (mean)	64% (15%-75%)	76% (40%-95%)	92% (70%-95%)
M:E ratio (median)	4.1:1	2.5:1	2.0:1
Megakaryocytes (%)			
<i>n</i>	8-10/hpf	13-15/hpf	5-6/hpf
IMF-like megakaryocytes	100	0	8
PV-like megakaryocytes	0%	0	92
ET-like megakaryocytes	0	100	0
Marrow fibrosis (%)			
Grade 1	7	95	92
Grade 2	3	5	8
Grade 3	42		0
Grade 4	46		0
Blasts (%)	14	0	0

PMF – Primary myelofibrosis; ET – Essential thrombocythemia; PV – Polycythemia vera; M:E ratio – Myeloid/erythroid ratio; IMF – Idiopathic myelofibrosis

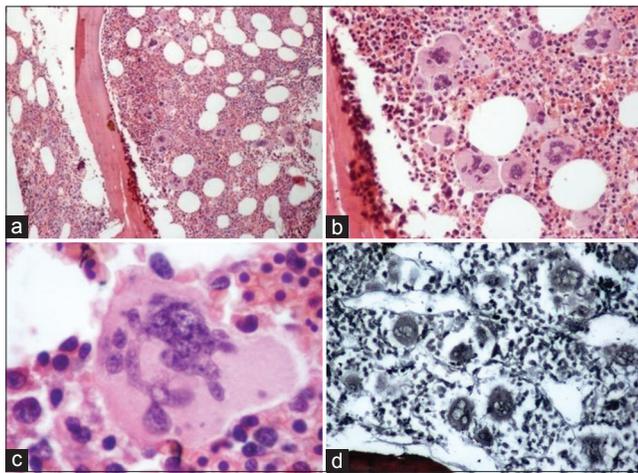


Figure 2: Essential thrombocytopenia trephine histology (a) Normocellular marrow with increased giant megakaryocytes (H and E, ×40). (b) Hyperlobated megakaryocytes (H and E, ×400). (c) A large, staghorn megakaryocyte (H and E, ×1000). (d) Reticulin fibers, Grade I condensation (Reticulin stain, ×400)

variable degree of pleomorphism, with normally folded or deeply lobulated nuclei, lacking significant cytologic abnormality [Figure 3]. However, 1 case (8%) showed atypical megakaryocytes with Grade 2 fibrosis. Tight clustering and paratrabeular localization were the constant features noted in PMF.

Discussion

In the present study, we analyzed the clinicopathological spectrum of the most common BCR-ABL-negative MPNs-PMF, essential thrombocythemia, and PV, along with JAK2 mutation correlation.

In the study, the incidence of PMF was higher than ET and PV, and there were more males than females. An Indian study also showed male preponderance.^[9] The mean age of presentation was slightly higher in PMF patients,

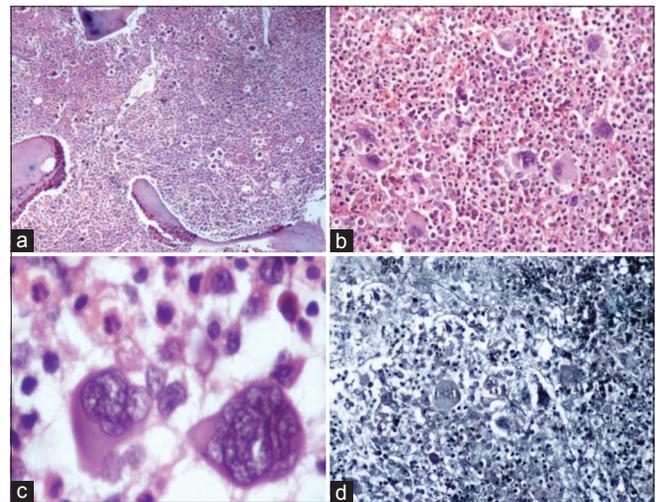


Figure 3: Polycythemia vera trephine histology. (a) Hypercellular marrow with large megakaryocytes (H and E, ×40). (b) Panmyelosis (H and E, ×400). (c) Megakaryocytes with deeply lobulated and bulbous nuclei (H and E, ×1000). (d) Reticulin fibers, Grade 1–2 (Reticulin stain, ×400)

followed by PV and ET. Splenomegaly was found in 82% of PMF patients, whereas ET and PV had 71% and 72%, respectively, comparable to other studies.^[9,10,11]

Thrombotic events were seen in patients of PV (41%) and ET (28%), in concordance with other studies.^[12-14] None of the idiopathic myelofibrosis (IMF) patient showed thrombosis/hemorrhage. Hence, basic investigations should be done for the consideration of MPNs in all patients presenting with thrombotic events. The median leukocyte count was high in all the three MPNs, marked in PV, and mild in PMF, whereas median platelet count was much higher in ET, followed by PV and PMF. These findings are in line with other studies.^[11,15]

In this study, the overall JAK2 V617F mutation was detected in 71% of BCR-ABL-negative MPNs. This is comparable to studies done by Sazawal *et al.* in the Indian

Table 5: Comparison with the literature

Parameters	Present study		Muhammad Arif <i>et al.</i> ^[9]		Sazawal <i>et al.</i> ^[11]	
	JAK2 positive	JAK2 negative	JAK2 positive	JAK2 negative	JAK2 positive	JAK2 negative
n (%)	10 (71)	4 (29)	64 (69)	29 (31)	51 (68)	24 (32)
Age (mean±SD)	52±10.2	46±15.1	54.2±15.5	41.2±13.7	53±11.4	44±17.3
Hb (g/dl), mean±SD	13.8±3.59	10.2±2.33	14.8±3.9	11.2±2.6	13.9±4.6	11.4±5.2
WBC (×10 ⁹ /l), median	16.7	10.1	18.7	14.5	24.8	12.5
Platelet (×10 ⁹ /l), median	5.6	5.4	8.2	7.9	3.6	2.5

SD – Standard deviation; WBC – White blood cell; Hb – Hemoglobin; JAK2 – Janus-associated kinase 2

population and by Suksomyos *et al.* in Thai patients. They reported 68% and 68.8% prevalence of JAK2V617F mutation in BCR-ABL-negative MPNs, respectively.^[11,16] In this study, JAKV617F mutations in PV, ET, and PMF was 100%, 50%, and 71%, respectively. It was interesting to note that in our study the frequency of JAK2 mutation in IMF was higher, which is different from that reported in Western literature; however, the frequency of JAK2 in patients with PV and ET was comparable with other studies.^[5,6] In this study, JAK2-positive group was associated with higher age, higher hemoglobin, and higher white blood cell count than JAK2-negative group, similar to other studies.^[9,11,15] [Table 5].

Of the PMF patients, three cases were in prefibrotic/early stage, and they were associated with higher mean Hb, WBC count, and platelets compared to 21 cases of fibrotic stage. All (100%) patients in fibrotic phase displayed leukoerythroblastic picture comprising of immature granulocytes, nucleated RBCs, polychromatophils, and tear drop cells. In this study, 14% of IMF cases transformed into AML. However, none of the patients in PV and ET showed blastic transformation. In a study conducted by Cervantes *et al.* showed 11% blast transformation in IMF, 4% in PV, and 1% in ET.^[17] Mesa *et al.* reported that all leukemic transformations were myeloid.^[18]

Morphological differences among the BCR/ABL-negative MPNs have not been extensively studied in the Indian subcontinent. These results show that there are morphologic differences and megakaryocytic morphology represent useful clue for differential diagnosis. In particular, the majority of megakaryocytes of PV and ET retained the typical morphology, that is, “PV-like” and “ET-like” megakaryocytes with no, or only occasional nuclear maturational defects without significant megakaryocytic atypia. On the contrary, prominent atypical changes in megakaryocytes, such as the presence of abnormal N/C ratio together with the occurrence of tight clusters and bizarre nuclei, were the hallmarks of PMF. A study conducted by Boiocchi *et al.* also showed similar findings and showed megakaryocyte morphology is a reliable histological feature.^[10,19,20]

Reticulin grade was 3–4 in IMF, whereas in ET and PV, it was 1–2, similar to other studies.^[10,20,21] A study conducted by Bridget *et al.* showed that marrow topography,

cellularity, and degree of fibrosis are more reliable histological features of marrow.

Conclusion

JAK2 gene mutation is seen in a significant proportion in BCR-ABL-negative MPNs (71%); thus, our findings support the recommendation that peripheral blood mutation screening for JAK2V617F be incorporated into the initial evaluation of patients with suspected MPNs. It also helps in excluding a large number of reactive causes. However, since the mutation may be absent in a few cases of PV, ET, and IMF, it cannot be used as a single test for making the diagnosis. This study also showed that megakaryocyte morphology and marrow fibrosis are reliable histological features and represent a useful clue for differential diagnosis of these BCR-ABL-negative MPN subtypes.

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Conflicts of interest

There are no conflicts of interest.

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