Original Research Article

The clinicopathological spectrum and driver mutation profile in classic BCR-ABL1 negative myeloproliferative neoplasms: a three-year study from a tertiary care center in Kerala, South India

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ABSTRACT

Background: Myeloproliferative neoplasms (MPNs) are clonal hematopoietic stem cell disorders primarily of the adults. The 2016 World Health Organization (WHO) classification of MPNs include the molecular landscape as one of the diagnostic criteria. JAK2 exon14 (JAK2 V617F), JAK2 exon12, Myeloproliferative leukemia virus oncogene exon 10 (MPL 515), and calreticulin exon 9 (CALR) mutations are the main somatic driver mutations detected in classic BCR-ABL1 negative MPNs.
Methods: A retrospective, cross-sectional study was conducted including 99 patients diagnosed with classic BCR-ABL1 negative MPNs during a 3-year time period, from March 2018 to February 2021 in the departments of pathology and clinical haematology- haemato oncology of a tertiary care teaching hospital. Clinical, haematological and morphological features were analysed and correlated with MPN associated mutation studies done in blood/bone marrow samples.
Results: The prevalence of polycythaemia vera (PV) was found to be higher than other MPN, two third of which were JAK2 positive. More than half of the cases of primary myelofibrosis (PMF) and essential thrombocythemia (ET) also showed JAK2 mutation. CALR was positive in 17.4% of ET and 31.3% of PMF; MPL in 4.4% of ET and 3.1% of PMF.
Conclusions: The prevalence of triple-negative MPN point towards the need for whole-exome sequencing of triple-negative MPN.

Keywords: Myeloproliferative neoplasms, BCR-ABL1 negative, Polycythaemia vera, Primary myelofibrosis, Essential thrombocythemia, Driver mutations

INTRODUCTION

Myeloproliferative neoplasms (MPNs) are clonal hematopoietic stem cell disorders characterized by the proliferation of cells of one or more myeloid lineages. They primarily occur in adults, with incidence peaking in the fifth to seventh decades of life and the annual incidence is 6 cases per 1,00,000 population.[1]

The World Health Organization (WHO) postulate of an integrated approach that includes haematological, morphological, and molecular genetic findings influenced the revision of the 2008 criteria for the classification of MPNs. The 2016 WHO diagnostic criteria for MPN subtypes include the molecular landscape as one of the diagnostic criteria. 2016 WHO classification of MPNs includes BCR-ABL1 positive chronic myeloid leukemia (CML), polycythaemia vera (PV), chronic neutrophilic leukemia (CNL), primary myelofibrosis (PMF), essential thrombocythemia (ET), chronic eosinophilic leukemia not otherwise specified (CEL NOS), and myeloproliferative

neoplasm, unclassifiable (MPN-U). The classic BCR-ABL1 negative MPNs includes PV, characterized by excessive erythroid series production, ET in which an abnormal increase of platelet count is observed, and PMF, which is characterized by leuco-erythroblastic anemia and marrow fibrosis.

The main somatic driver mutations detected in classic BCR-ABL1 negative MPNs are JAK2 exon14 (JAK2 V617F), JAK2 exon12, Myeloproliferative leukemia virus oncogene exon 10 (MPL 515), and calreticulin exon 9 (CALR) mutations.[2] The discovery of driver mutations has led to a more specific approach to diagnosis and treatment. These mutations are mutually exclusive. The prevalence of JAK2 V617F mutations is higher than 95% in PV, 50-75% in ET, and 40-75% in PMF. JAK2 exon12 mutations are specific for PV. CALR mutations are seen in 20-30% of patients with ET and PMF. MPL mutations are detected in less than 10% of patients with ET or PMF.

The clinicopathological and the molecular profile data are limited in India, where challenges remain in diagnosing these conditions. In this study, we describe the clinicopathological profile and driver mutational analysis in classic BCR-ABL1 negative MPNs presenting in a tertiary care center in Kerala state of Indian subcontinent.

Aim and objective

Aim and objective of the research was to study the clinicopathological and molecular profile of classic BCR-ABL1 negative MPNs.

METHODS

The study was a retrospective and cross-sectional study conducted in the departments of clinical hematology and haemato-oncology and pathology of Rajagiri hospital in Kerala, South India including 99 patients who were diagnosed with classic BCR-ABL1 negative MPNs from the time period March 2018 to February 2021. Cases of CML, MPN unclassifiable, MPN/MDS (myelodysplastic syndrome) and reactive causes of polycythaemia, thrombocytosis and myelofibrosis were excluded from the study.

After obtaining consent from the institutional ethics committee, each patient's clinical and laboratory data were collected from electronic medical records. Clinical details that were collected include age, gender, presenting symptoms and duration and assessment of organomegaly. The complete blood counts, serum (S.) erythropoietin, S. LDH, and peripheral smear findings at the time of diagnosis were evaluated. In addition, bone marrow aspiration and bone marrow biopsy were examined after staining with routine stains and special stains for iron, reticulin and collagen. MPN associated mutation studies done in blood/bone marrow samples were analysed. Samples were outsourced and tested by SYBR green real time ARMS PCR with melt curve analysis with positive

and negative controls for the V617F mutations (G1849T) in exon 14 of the JAK2 gene. In cases where JAK2 V617F variant was not detected by ARMS PCR, custom targeted paired-end NGS testing of JAK2 exons 12 and 14, CALR exon 9, and MPL exon 10 by accredited laboratory-developed protocol was performed.

Data were entered in Microsoft excel and analysed using statistical package for the social sciences (SPSS) version 26. Quantitative data was expressed as mean and standard deviation, and qualitative data as proportion or percentage. Association between qualitative variables was assessed using Chi-square and quantitative variables by t-test. A p value <0.05 was considered significant.

RESULTS

A total of 199 patients were referred for evaluation of suspected myeloproliferative neoplasms from March 2018 to February 2021. After initial screening, 65 patients who were not completely evaluated were excluded. 33 patients diagnosed as CML and two cases as MPN- U were also excluded. After strict compliance with WHO 2016 diagnostic criteria, 99 patients were included in the final analysis of classic BCR-ABL1 negative MPNs.

Baseline characteristics and different disease subtypes are summarized in Table 1 and Figure 1.

Table 1: Baseline characteristics of patients.

Characteristics	N (%)
Age, y (range)	61.8 (26-88)
Gender	
Male	62 (63.4)
Female	37 (36.6)
Initial symptoms	
Constitutional symptoms	31 (31.3)
Incidentally diagnosed	22 (22.2)
Neurological symptoms	5 (5.1)
Vascular symptoms	12 (12.1)
Respiratory symptoms	10 (10.1)
Gastrointestinal symptoms	7 (7.1)
Others	12 (12.1)
Final diagnosis	
PV	44 (43.5)
ET	23 (22.8)
PMF	32 (31.7)

A total of 44 patients (43.5%) were diagnosed with PV. The mean age of the patient was 58.6 years (range, 36-88 years), of which 31 were males, and 13 were females. The most common presentation was incidental in 12 (28.5%) followed by vascular symptoms in 9 (20.4 %), respiratory symptoms in 7 (15.9%), constitutional symptoms in 5 (11.3%), pain in lower limb in 4 (9%), pruritis in 1 (2.3%), headache in 1 (2.3%), epistaxis in 1 (2.3%) and other symptoms in 4 (9%). Splenomegaly was noted in 9 patients

(20.4%), and thrombotic history was noted in 7 patients (15.9%). Two patients had bleeding history. The study revealed that the mean value of Hb was 18.1±2 g/dl, the mean total count was 11.3±6.1×10⁹/l, and the mean platelet count was 3.9 lakh/mm3. The mean value of serum erythropoietin (EPO) was 3.8±2.7 U/l, and S. LDH was 327.8 U/l.

The baseline bone marrow examination was available for 34 patients. All patients had increased cellularity for their age. Hypercellular marrow with panmyelosis was noted in 32 patients (94.1%). Megakaryocytes were increased in all patients and arranged in loose clusters in 19 patients (55.9%), scattered in 14 patients (41.1%), and tight clusters in 1 (3%). The majority of the megakaryocytes showed pleomorphic morphology (82.3%), followed by mature lobated (11.7%) and atypical (6%). More than half of the cases, bone marrow showed grade 1 marrow fibrosis (47%) and grade 0 marrow fibrosis (32.3%). JAK2 mutational analysis was available in all cases, 29 of them (65.9%) were positive. Sixteen patients were negative for all driver mutations but had characteristics morphological features of PV and met the WHO 2016 diagnostic criteria. Two cases (4.5%) transformed to post polycythaemia myelofibrosis, and one case (2.3%) showed blast transformation to acute erythroid leukemia. All three patients were JAK2 positive.

A total of 23 patients (17.2%) were diagnosed with ET. The mean age of the patient was 58.8 years (range, 26-86 years), of which 11 were males, and 12 were females. The most common presentation was incidental in 9 patients (39.1%), followed by vascular symptoms in 7 (30.4%) and fatigue in 3 (13%). Splenomegaly was noted in 3 patients (13%), and thrombotic history was noted in 8 patients (34.8%). Mutational analysis was available in all cases. 12 patients (52.2%) were positive for JAK2 V617F, CALR was positive in 4 patients (17.4%), MPL in 1 patient (4.4%). Six patients (26%) were negative for all driver mutation tested (Figure 3). One patient (4.3%) transformed to myelofibrosis.

Primary myelofibrosis was diagnosed in 32 patients, of which 11 cases were in prefibrotic stage and 20 cases were in the overt fibrotic stage. The mean age of 68.5 years (range, 31-86 years), of which 20 were males and 12 were females. More than half of the cases presented with constitutional symptoms. Splenomegaly was noted in 14 patients and hepatomegaly in 9 patients. Thrombotic history was noted in 4 patients. The bone marrow examination was available for 31 patients. Most of the patients had increased cellularity for their age. Megakaryocytes were increased in all patients and arranged in loose clusters in 19 patients (55.9%), scattered in 14 patients (41.1%), and tight clusters in 1 (3%). The majority of the megakaryocytes showed pleomorphic morphology (82.3%), followed by mature lobated (11.7%) and atypical (6%). More than half of the cases, bone marrow showed grade 1 marrow fibrosis (47%) and grade 0 marrow fibrosis (32.3%).
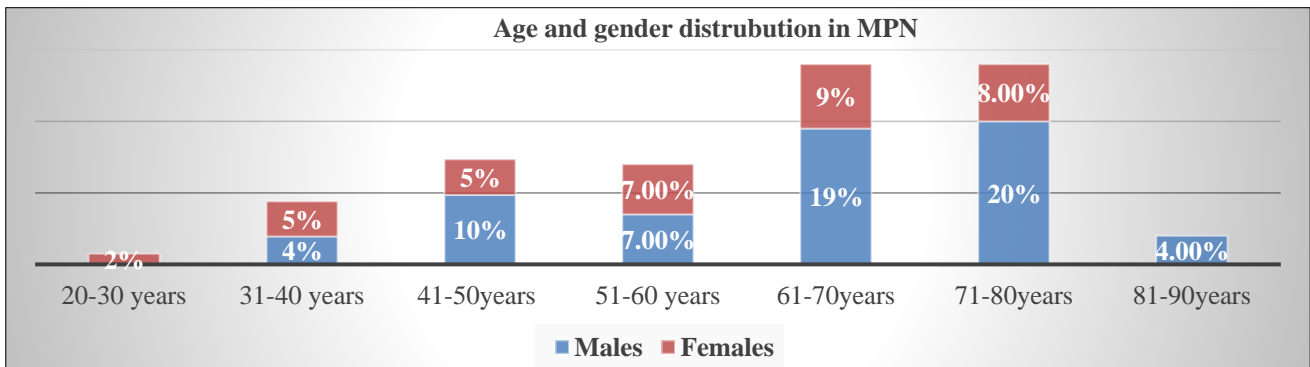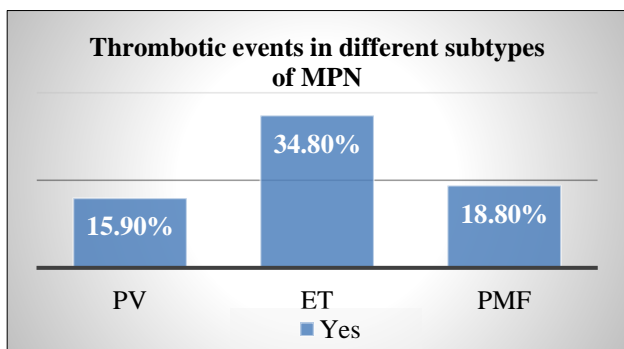

Figure 1: Age and gender distribution in MPN.

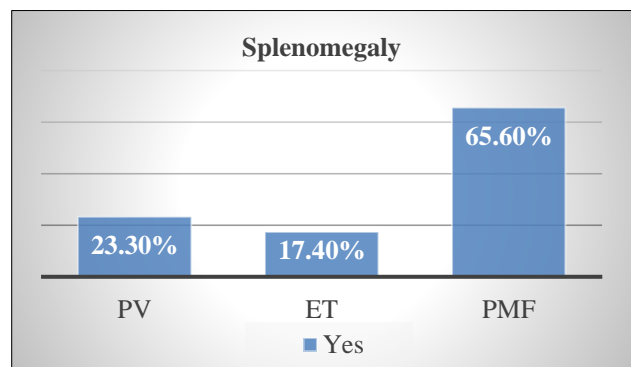**Figure 2: Thrombotic events in different subtypes of MPN.**

Figure 3: Splenomegaly.

Mutational analysis was available in all cases; 17 patients (53.1%) were positive for JAK2 V617F, CALR was positive in 10 patients (31.3%), MPL in 1 patient (3.1%). Among 10 CALR positive patients, 6 were type 1 CALR, and 4 were type 2 CALR. 4 patients (12.5%) were negative for all driver mutation tested (Figure 3). One case (3.2%) showed blast transformation to acute myeloid leukemia.
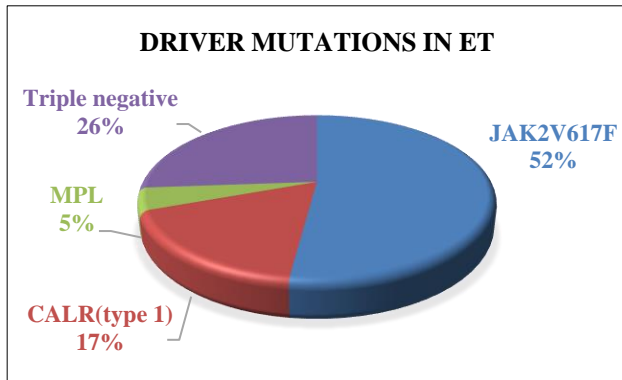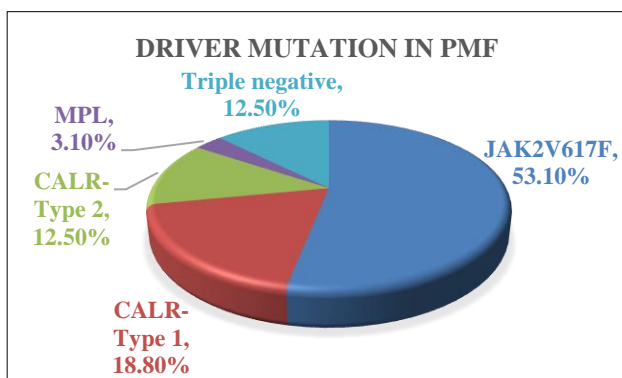

Figure 4: Driver mutations in ET.

Figure 5: Driver mutations in PMF.

DISCUSSION

In this study, we observed that the prevalence of PV was higher than other MPN, which is different from the data in a multinational, multicenter observational registry for myeloproliferative neoplasms in Asia by Yassin et al.[3] There is male preponderance in all subtypes of MPN except in ET. The mean age of presentation of MPN was 60.5 years, which agrees with the published data with an older age presentation noted in PMF. We observed that our cohort demonstrates a younger age at diagnosis for PV, i.e., 58.6 years compared to 60 years in a study conducted by Yassin et al.[3] We observed that splenomegaly was noted in 65.6% patients with PMF, whereas PV and ET had 23.3% and 17.4% respectively.

This finding is comparable to other studies.[4] According to Kaifie et al and Alvarezlarren et al, thrombotic events were seen more in patients with PV and ET.[5,6] In our study, thrombotic events were seen in 34.8% of patients with ET, 15.9% with PV, 18.8% with PMF, and 3% with CML. The difference in the percentage of thrombotic events in different subtypes of MPN and higher frequency in ET was statistically significant with a p value less than 0.05. Hence, the patients presenting with thrombotic events should be considered for basic investigations of MPN. In our study, the JAK2 V617F mutation was detected in 58.6% of patients diagnosed with BCR-ABL1 negative MPNs. This frequency of JAK2 V617F was lower than 71% in a survey conducted by Dixith et al and 68% in a study conducted by Sazawal et al.[7,8] The frequency of CALR and MPL was 13.9% and 2%, which correlates with the published data. In this study, JAK2 V617F mutation was noted in 29 patients (65.9%) with PV, which is lower than 90% in a study conducted by Maddali et al 74% in a study conducted by Singh et al and 85% in a study conducted by Yassin et al.[3,9,10] A possible reason may be due to the overdiagnosis of JAK2 negative PV. 2016 WHO diagnostic PV criteria were less precise and lacked a separate criterion for labeling JAK2 negative PV. Although 15 cases were categorized as JAK2 negative PV based on the 2016 WHO diagnostic criteria for PV, only two of these cases met the BSH 2019 criteria, which include other additional criteria like splenomegaly and WBC counts.

Furthermore, research focusing on understanding the possible genetic alterations in JAK2 negative PV is required. However, few studies from India noted a lower incidence of JAK2 mutated PV, which emphasizes need for further large-scale evaluation to confirm whether there is a true disparity between the Asian and western data.[3,11-13] Our study also compared the clinical phenotype of JAK2 V617F positive and negative PV, ET, and PMF cases. The JAK2 V617F positive PV patients have older age and higher leucocyte count. These findings are in correlation with other published data.[11] But in our study, it was noted that JAK2 mutated PV has a higher platelet count and is different from the published data.[11] In ET and PMF, patients with JAK2 V617F mutation have older age, increased risk of thrombotic events, and higher leukocyte count, which was statistically significant with a p value less than 0.05.[12,13] They were also noted to have higher hemoglobin and lower platelet count which were not statistically significant. This finding was in concordance with studies conducted by Tefferi et al.[11] In our study, the frequency of CALR mutation in ET and PMF was 17.4% and 31.3%. In ET, all CALR mutation was type 1. In PMF, six patients (18.8%) were type 1 CALR, and four patients (12.5%) were type 2 CALR. The frequency of CALR in ET was lower than the findings of Singh et al, Maddali et al and Tefferi et al.[9,10,12,13] Our study also compared the clinical phenotype of CALR positive and negative ET and PMF cases. We found that CALR positive cases present at a younger age with lower hemoglobin and higher platelet count in ET and PMF. These findings are in concordance with studies conducted by Maddali et al.[9] In our study, MPL was found in 3.1% of patients with PMF and 4.4% patients with ET, similar to published data.[14] A comparison of driver mutation analysis with other studies is given in Table 2.

Table 2: Comparison of frequencies of driver mutations in MPN.

MPN subtypes and studies	Median age, years	JAK2V617F (%)	JAK2 Exon12	CALR (%)	MPL (%)	Triple negative (%)
PV						
Tefferi et al[11]	61	77	3	-	-	-
Singh et al[10]	57	74	-	-	-	-
Maddali et al[9]	51	90.3	5.7	-	-	-
Rabade et al[15]	50	100	-	-	-	-
Dixith et al[7]	52	100	-	Not studied	Not studied	Not studied
Sazawal et al[8]	52	82	-	Not studied	Not studied	Not studied
Present study	58.6	64.4	-	-	-	-
ET						
Tefferi et al[12]	56	53	-	32	3	12
Singh et al[10]	50	33	-	33	4.7	28.5
Maddali et al[9]	36	37.8	-	33.3	2.9	26
MPN subtypes						
Rabade et al[15]	46	61.7	-	15.1	9.1	15.2
Dixith et al[7]	46	50	-	Not studied	Not studied	Not studied
Sazawal et al[8]	49	70	-	Not studied	Not studied	Not studied
Present study	58.8	52.2	-	17.4	4.4	26
PMF						
Tefferi et al[13]	64	58	-	25	8.3	8.7
Singh et al[10]	65	16	-	16	33	33
Maddali et al[9]	52	48	1.4	32.9	2.7	15
Rabade et al	53	57.6	-	23.7	3.4	15.3
Dixith et al[7]	53	71	-	Not studied	Not studied	Not studied
Sazawal et al[8]	47	52	-	Not studied	Not studied	Not studied
Present study	68.5	53.1	-	31.3	3.1	12.5
PV						
Tefferi et al[13]	61	77	3	-	-	-
Singh et al[10]	57	74	-	-	-	-
Maddali et al[9]	51	90.3	5.7	-	-	-
Rabade et al[15]	50	100	-	-	-	-
Dixith et al[7]	52	100	-	Not studied	Not studied	Not studied
Sazawal et al[8]	52	82	-	Not studied	Not studied	Not studied
Present study	58.6	64.4	-	-	-	-
ET						
Tefferi et al[11]	56	53	-	32	3	12
Singh et al[10]	50	33	-	33	4.7	28.5
Maddali et al[9]	36	37.8	-	33.3	2.9	26
MPN subtypes						
Rabade et al[15]	46	61.7	-	15.1	9.1	15.2
Dixith et al[7]	46	50	-	Not studied	Not studied	Not studied
Sazawal et al[8]	49	70	-	Not studied	Not studied	Not studied
Present study	58.8	52.2	-	17.4	4.4	26
PMF						
Tefferi et al[11]	64	58	-	25	8.3	8.7
Singh et al[10]	65	16	-	16	33	33
Maddali et al[9]	52	48	1.4	32.9	2.7	15
Rabade et al[15]	53	57.6	-	23.7	3.4	15.3
Dixith et al[7]	53	71	-	Not studied	Not studied	Not studied
Sazawal et al[8]	47	52	-	Not studied	Not studied	Not studied
Present study	68.5	53.1	-	31.3	3.1	12.5

Limitations

The limitations of this study include small sample size and being a single-center study and the findings must be seen in the light of these limitations.

CONCLUSION

The confirmation of JAK2 negative PV has been a challenge; compiling 2016 diagnostic criteria for PV with BSH 2019 criteria will be more reliable. The frequency of triple-negative MPN in our study was comparable with Asian data but was a little higher compared to the western data. These findings point towards the whole-exome sequencing of triple-negative MPN. In addition, it needs validation on larger datasets.

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