ORIGINAL ARTICLE

The Clinical Characteristics and Outcomes of *JAK2/CALR/MPL* Mutation Related Myeloproliferative Neoplasms - A Single Centre Study from Malaysia

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ABSTRACT

INTRODUCTION: The pathogenesis of classical myeloproliferative neoplasm (MPN) driven by JAK2, CALR and MPL gene mutations affects the clinical course and survival. This study aimed to determine the prevalence of driver mutations in MPN patients and their association with clinical presentations and outcomes. **MATERIALS AND METHODS:** We conducted a retrospective study involving patients with classical MPN diagnosed from 2002 to 2019. The patient's clinical and laboratory information, as well as outcomes, were collected and reviewed. **RESULTS:** 267 patients with classical MPN were recruited into the study. Majority of these patients were Chinese (46.5%), followed by Malay (40.1%) and Indian (12.7%). Most of the patients had essential l thrombocythaemia (ET) (57.3%), followed by polycythaemia vera (PV) (30.0%) and primary myelofibrosis (PMF) (12.7%). JAK2V617F mutation was detected in PV (87.5%), ET (68.0%), and PMF (67.6%) patients whereas CALR mutation was present in 15.0% of ET and 8.8% of PMF patients and MPL mutation was present in 0.7% and 5.9% of ET and PMF patients respectively. CALR-mutated ET patients were less likely to develop vascular events compared to JAK2V617F mutated patients (Odds ratio 0.301, 95% confidence interval 0.097–0.939, p=0.039). As for clinical outcomes, triple negative PMF patients had shorter median overall survival than those with JAK2V617F mutation (24.0 months vs. 161.0 months, p=0.017). **CONCLUSION:** Majority of classical MPN patients were Chinese with ET being the most common MPN subtype. The mutation profiles, clinical features, and survival outcomes were comparable to previous reports. Mutation studies are therefore important for prognostication and should be performed routinely.

Keywords
myeloproliferative neoplasm, JAK2, MPL, CALR, clinical outcomes

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INTRODUCTION

The classical myeloproliferative neoplasm (MPN) which include polycythaemia vera (PV), essential thrombocythaemia (ET) and primary myelofibrosis (PMF) are clonal diseases of the hematopoietic stem cell, classified under *BCR::ABL1*-negative MPN subtypes according to World Health Organization (WHO) classification.[1] The incidence rates for ET, PV and PMF were 1.55, 1.57 and 0.44 per 100,000 person-years, respectively in the United States.[2] These are comparable to the ranges reported in other continents, including Europe, Australia and Asia.[3-5]

The key driver mutations involved in the pathogenesis for classical MPN include Janus kinase 2 *(JAK2)*, calreticulin *(CALR)* and myeloproliferative leukemia virus oncogene *(MPL)* mutation. Somatic mutations in *JAK2* gene, both in the form of *JAK2V617F* or *JAK2 exon 12* mutation, drive the pathogenesis of PV and are observed in more than 90% of PV patients. In contrary, *CALR* mutation is present mainly in ET and PMF, albeit at a lower frequency with prevalence rate of 15% to 24% and 25% to 35% respectively.[6] *MPL* mutation is present at an even lower rate at 4% of ET patients and 8% of PMF patients.[7]

Approximately 10% of *BCR::ABL1* negative MPN have none of these three mutation and is termed as triple negative MPN.[8]

Variation in mutation profiles has been shown to be associated with different clinical characteristics and clinical course. As an example, *CALR* mutated ET is associated with lower hemoglobin levels, higher platelet counts, lower leukocyte counts, and younger age compared to *JAK2V617F* mutated ET.[9] There are a few *CALR* mutation variants that have been identified and they are found to be associated with different phenotypes; Type 1 and type 1-like mutations variants have greater risk of MF transformation, while type 2 and type 2-like mutations variants are associated with more indolent clinical course.[10,11] On the other hand, *JAK2V617F* mutated ET was shown to be associated with increased risk of thrombosis.[12]

Among patients with PMF, those harboring *CALR* mutations are younger and has lower risk of cytopenias.[13] In contrast to *CALR* mutated PMF, those harboring *JAK2V617F* mutation are associated with increased risk of thrombosis.[14] Amid all genetic mutations, triple negative PMF has the worst prognosis where there is higher risk of leukemic transformation and poorer overall survival.[13]

Although the prevalence of these molecular mutations is widely reported in the developed nations especially in the Caucasian populations, there is paucity of epidemiological data in the Southeast Asia (SEA) regions and even scarcer is the clinical outcome of these patients. Even though there are few reports on epidemiological data of classical MPN from different parts of Malaysia, complete information on the prevalence of *CALR* and *MPL* mutation and patient survival are limited.[15-17]

In view of this, this study aims to determine the prevalence of *JAK2/CALR/MPL* mutations in MPN patients and to determine their association with clinical presentations and outcomes.

MATERIALS AND METHODS

This was a retrospective study where patients diagnosed with classical MPN (ET, PV and PMF) and treated at a tertiary academic centre in Kuala Lumpur, Malaysia were recruited from year 2002 to 2019. The study was approved by the local institution ethic committee with the registration number of MREC-202123-9792.

Patients were included if they were ≥18 years old, had available results of mutational studies (*JAK2V617F, MPL, CALR* and *JAK2 exon12*) at the time of diagnosis; and fulfilled the criteria of MPN according to 2022 WHO classification criteria.[18] Patients with incomplete clinical information were excluded.

The clinical information that was collected included patients' demographic data, clinical presentations, clinical outcome, and their survival status. Clinical outcome was defined as occurrence of hematological transformation to acute leukemia, and vascular related complications. Vascular related complications were defined as clinical and/or radiological confirmed thrombotic or bleeding event, occurred after the initial diagnosis of MPN. Time to progression was defined as the duration from the date of diagnosis to the date of disease progression. Overall survival (OS) was determined from the date of diagnosis to the date of death or date of last known follow up.

All statistical analysis was conducted using SPSS Statistics for Windows Version 26. Descriptive statistics were utilized for categorical variables. Results were presented as frequencies and percentage for categorical data. Numerical data which are normally distributed are presented as mean and standard deviation while median and interquartile ranges were presented for numerical data which are not normally distributed. Fisher's exact test and Chi Square test were used to compare categorical variables. Mann Whitney or Kruskal Wallis were used to test the non-parametric continuous variable and One Way Anova was used to test for parametric continuous variables. Nominal regression was used to estimate Odd's ratio for categorical

variables. Survival analysis was estimated using Kaplan Meier method and compared by the log rank test. P value of 0.05 was considered statistically significant.

RESULTS

Clinical characteristics of classical MPN patients

A total of 267 patients with a mean age of 59.5 years were included in this study. These patients were followed-up for a median duration of 40 months. Most patients were diagnosed with ET (57.3%), followed by PV (30.0%) and PMF (12.7%). The gender distribution for ET (54.2% females) differed from PV and PMF where the majority were males (67.5% and 58.8% males, respectively) (p=0.006). Majority of patients with classical MPNs were Chinese (46.5%), followed by Malay (40.1%) and Indian (12.7%). The mutation profile of the patients is shown in Table I.

Table I: Clinical characteristics of classical MPN patients

n (%)	Total	PV	ET	PMF	P value
Number of patients	267 (100.0)	80 (100.0)	153 (100.0)	34 (100.0)	
Age at diagnosis, mean (SD)	59.5 (15.5)	57.7 (13.8)	59.7 (16.6)	62.5 (14.1)	0.310
Gender					
Male	144 (53.9)	54 (67.5)	70 (45.8)	20 (58.8)	*0.006*
Female	123 (46.1)	26 (32.5)	83 (54.2)	14 (41.2)	
Ethnicity					
Malay	107 (40.1)	37 (46.3)	57 (37.3)	13 (38.2)	0.236
Chinese	124 (46.5)	29 (36.3)	76 (49.7)	19 (55.9)	
Indian	34 (12.7)	14 (17.5)	18 (11.8)	2 (5.9)	
Others	2 (0.7)	0 (0.0)	2 (1.3)	0 (0.0)	
Mutation profile					
JAK2V617F	197 (73.8)	70 (87.5)	104 (68.0)	23 (67.6)	*<0.001*
CALR	26 (9.7)	0 (0.0)	23 (15.0)	3 (8.8)	
MPL	3 (1.1)	0 (0.0)	1 (0.7)	2 (5.9)	
JAK2 Exon12	1 (0.4)	1 (1.3)	0 (0.0)	0 (0.0)	
No mutation	40 (15.0)	9 (11.3)	25 (16.3)	6 (17.6)	
Clinical presentation					
Asymptomatic	202 (75.7)	54 (67.5)	128 (83.7)	20 (58.8)	*0.001*
Thrombotic events	35 (13.1)	17 (21.3)	17 (11.1)	1 (2.9)	*0.017*
Bleeding events	12 (4.5)	3 (3.8)	5 (3.3)	4 (11.8)	0.109
Constitutional symptoms	18 (6.7)	6 (7.5)	3 (2.0)	9 (26.5)	*<0.001*
Complications					
Vascular events	11 (4.1)	4 (5.0)	5 (3.3)	2 (5.9)	0.618
Secondary myelofibrosis	19 (7.1)	6 (7.5)	13 (8.5)	-	0.233
Leukemic transformation	11 (4.2)	3 (3.8)	6 (3.9)	2 (5.9)	0.819

Abbreviations: N, numbers; SD, standard deviation; PV, Polycythaemia Vera; ET, Essential Thrombocythaemia; PMF, Primary Myelofibrosis
P<0.05 is statistically significant.

PMF patients who were included in the study were risk stratified using Dynamic International Prognostic Scoring System (DIPSS), where 17.6% and 50.0% were classified as low and intermediate-1 risk groups, while 29.4% and 2.9% were categorized as intermediate-2 and high-risk groups.

Table II: Blood parameters, clinical presentations and complications of classical MPNs

Characteristics	*JAK2V617F* mutation	*CALR* mutation	*MPL* mutation	*JAK2 Exon12* mutation	No mutation	p value
Polycythaemia Vera (n=80)						
Hemoglobin (g/L)*	183 ± 26	-	-	174	194 ± 19	0.434
WCC count (x10⁹/L)*	17.5 ± 9.8	-	-	10.9	10.0 ± 3.4	0.069
Platelet count (x10⁹/L)*	544 ± 222	-	-	117	259 ± 123	*<0.001*
Clinical presentation						
Asymptomatic	49 (70.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (55.6)	0.246
Thrombotic events	15 (21.4)	0 (0.0)	0 (0.0)	0 (0.0)	2 (22.2)	1.000
Bleeding events	1 (1.4)	0 (0.0)	0 (0.0)	1 (100.0)	1 (11.1)	*0.009*
Constitutional symptoms	5 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (11.1)	0.564
Complications						
No complication	60 (85.7)	0 (0.0)	0 (0.0)	1 (100.0)	6 (66.7)	0.298
Vascular events	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	3 (33.3)	*0.005*
Secondary myelofibrosis	6 (8.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
Leukemic transformation	3 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
Essential Thrombocythaemia(n=153)						
Hemoglobin (g/L)*	131 ± 25	120± 27	110	-	132 ± 16	0.176
WCC count (x10⁹/L)*	13.9 ± 8.0	13.8 ± 10.5	8.1	-	11.6± 5.5	0.555
Platelet count (x10⁹/L)*	882 ± 306	1128 ± 530	783	-	1050 ± 483	*0.022*
Clinical presentation						
Asymptomatic	88 (84.6)	17 (73.9)	1 (100.0)	0 (0.0)	22 (88.0)	0.478
Thrombotic events	9 (8.7)	5 (21.7)	0 (0.0)	0 (0.0)	3 (12.0)	0.284
Bleeding events	4 (3.8)	1 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	0.824
Constitutional symptoms	3 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
Complications						
No complication	89 (85.6)	20 (87.0)	1 (100.0)	0 (0.0)	19 (76.0)	0.547
Vascular events	2 (1.9)	1 (4.3)	0 (0.0)	0 (0.0)	2 (8.0)	0.208
Secondary myelofibrosis	9 (8.7)	2 (8.7)	0 (0.0)	0 (0.0)	2 (8.0)	1.000
Leukemic transformation	4 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)	2 (8.0)	0.468
Primary Myelofibrosis (n=34)						
Hemoglobin (g/L)*	104± 31	107 ± 44	109± 1	-	87± 22	0.636
WCC count (x10⁹/L)*	23.9± 21.2	15.5 ±5.0	15.5±9.0	-	16.2±14.0	0.733
Platelet count (x10⁹/L)*	466 ± 353	1090 ± 1394	830 + 684	-	304 + 360	0.142
Clinical presentation						
Asymptomatic	12 (52.2)	2 (66.7)	2 (100.0)	0 (0.0)	4 (66.7)	0.753
Thrombotic events	1 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
Bleeding events	3 (13.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	1.000
Constitutional symptoms	7 (30.4)	1 (33.3)	0 (0.0)	0 (0.0)	1 (16.7)	0.913
Complications						
No complication	21 (91.3)	3 (100.0)	2 (100.0)	0 (0.0)	4 (66.7)	0.367
Vascular events	1 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	0.549
Leukemic transformation	1 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	0.549

Abbreviations: WCC, white cell count
P<0.05 is statistically significant.
* Mean ± Standard Deviation

PV patients with *JAK2V617F* mutation presented with higher platelet count (544±222x10^9/L, p<0.001), whereas ET patients with CALR mutation was associated with significant thrombocytosis (1128 ±530 x10^9/L, p=0.022) compared to other mutations. (Table II).

There was no significant difference in clinical presentation among classical MPN patients with various mutations. Among patients with ET, *CALR* mutated patients were less likely to develop vascular events compared to *JAK2V617F* mutated patients (Odds ratio [OR] 0.301, 95% confidence interval [95% CI] 0.097–0.939, p=0.039). Disease progression to secondary myelofibrosis from PV occurred in 8.6% of patients with *JAK2V617F* mutation, at mean duration of 66.3 months from diagnosis. In comparison, mean time to progression to secondary myelofibrosis from ET occurred later at 72.7 months, 154.5 months and 114.5 months, for *JAK2V617F*, *CALR* and triple negative ET respectively (p=0.440).

Survival outcomes of classical MPNs

Among all patients with classical MPNs, PMF had the shortest median overall survival (66.0 months) compared to patients with ET and PV (median OS not reached, p<0.001) (Figure 1a). Further analyses among patients with ET and PV showed no difference in OS across various gene mutations. Among PMF patients, however, triple negative PMF patients had shorter median OS compared to those with *JAK2V617F* mutation (24.0 months vs. 161.0 months, p=0.017) (Figure 1b). There was no significant difference in OS among patients with various DIPSS risk scores.

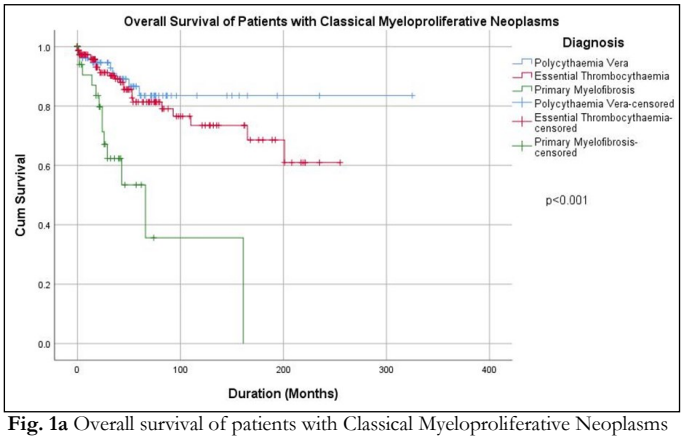

Fig. 1a Overall survival of patients with Classical Myeloproliferative Neoplasms

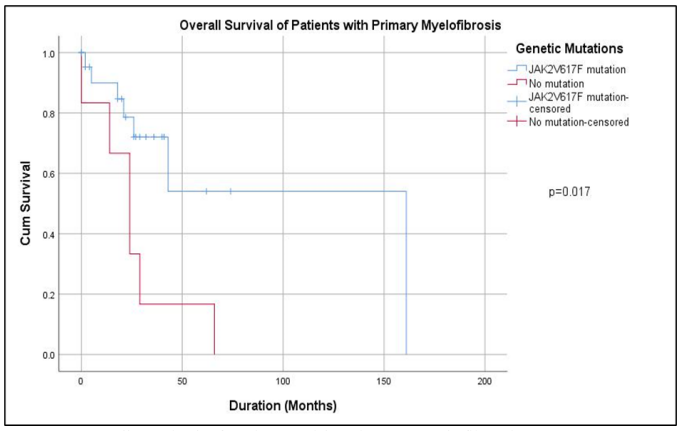

Fig 1b Overall survival of patients with Primary Myelofibrosis

DISCUSSION

Classical MPN is a group of diseases associated with several genetic alterations that activate the JAK/STAT pathway. It is caused by somatic mutations in the tyrosine kinase *JAK2*, the chaperone protein *CALR* or the thrombopoietin receptor *MPL* in majority of the patients.[19] Somatic mutations in myeloid cancer genes are often also mutated in MPN, such as *DNMT3A* and *TET2* mutations, among others. The occurrence of these concomitant mutations is found to affect the prognosis of patients with MPN.[20]

In our study, classical MPN is more prevalent in the Chinese ethnic group compared to Malay and Indian. The ethnic distribution is comparable with other studies from Malaysia as well as Singapore (between 43.2% and 65.8%), where the population comprise of multiple ethnicities.[15, 21] As pointed out by *Yap et al*, the inheritance of *JAK2* 46/1 haplotype that is linked to development of *JAK2V617F* mutation, has been found to be present among the populations in China and Japan. In fact, most Malaysian Chinese are originated from Southern China and its surrounding geographical regions, this may be the explanation for the higher prevalence of MPN compared to other ethnic groups. [15]

Among all classical MPNs, we found that ET was the most common classical MPN (57.3%) and majority of the patients were females. Although this is in contrast to reports from the USA where the most frequent MPN subtype was PV, our result is consistent with epidemiologic studies from this region, where ET

predominates (between 49.1 to 58.4%).[22-25] This observation can be explained by the distinct genetic makeup among Han Chinese, Japanese and Korean populations based on their genome wide data or ancestry informative markers.[26] The homogeneity in their ancestry genetic makeup could have translated into similarity in disease patterns in these nations and in our Chinese populations.

In contrast to Caucasian patients where most of the MPN patients were diagnosed in the elderly population (>70 years), the mean age at diagnosis in our cohort was 59.5 years, likely due to a generally younger population in our nation. Similarly, MPN patients from Japan and Korea also present at their late fifties,[24,27] and this observation again could be attributed to the analogous genetic makeup among the East Asian populations.[26]

Notably, 85% of our MPN population harboured at least one molecular mutation, with *JAK2V617F* mutation being the most common (73.8%). The finding is consistent with other studies where approximately 80% of their patients had at least one mutation identified.[16,28] However, this is contradictory to the study by Yap *et al* where only 63.7% of their MPN patients had detectable mutation.[15] The different findings could be due to the relatively high number of their patients did not have mutation study performed.

In another study with a smaller sample size conducted in Malaysia, 10.2% of their MPN cohorts harbored *CALR* mutation, similar to our findings.[17] Interestingly, the frequencies of *CALR* mutation (15.1% in ET and 8.8% in PMF) reported in our cohort were lower when compared to other Asian studies where the prevalence in their ET and PMF patients were 23.0% and 21.0% respectively. However, our finding is consistent with what was reported in the western countries where their prevalence was also at approximately 16.0%.[29] Similar to previous reports, our *CALR* mutated ET patients had significant higher platelet counts but lower thrombotic risk.[10,12] Although patients with *CALR* mutated PMF have been reported to have survival advantage, this was not seen in our study.[13] The relatively small sample size in this study may be the possible reason.

We reported a lower prevalence of *MPL* mutations among our ET patients (0.7%), compared to other countries where the prevalence was reported to be 2.7% to 4.3%.[30,31] In comparison, the prevalence of *MPL* mutated PMF in our cohort (5.9%) is similar to those in Argentina (6.1%), but lower compared to those reported in USA and Italy (8.1%).[32,33] *MPL* mutations have been shown to be associated with higher risk of transformation to secondary myelofibrosis among patients with ET, and those *MPL* mutated PMF were associated with low leukocytes counts, less cellular bone marrow (BM) and a higher number of BM megakaryocytes compared to those with *JAK2V617F* mutation.[34,35] We were not able to demonstrate these findings, again due to the relatively small sample size.

Previous literatures had reported lower vascular related event and lower risk of secondary myelofibrosis among triple negative ET patients.[36,37] In contrast, triple negative PMF patients have an aggressive clinical course and poor survival outcome. Our triple negative PMF cohort has significantly shorter median OS compared to those *JAK2*-mutated PMF. The median OS in triple negative PMF in this study was at a dismal 2 years compared to those with *JAK2*-mutated PMF, 13.4 years. This is consistent with the report by Rumi *et al.* where median OS was also at 3.2 years for triple negative PMF and 9.2 years for *JAK2*-mutated PMF.[13] Even with the availability of new therapeutic options such as *JAK2* inhibitors, it has thus far not shown to reduce the risk of mortality or disease transformation. At present, allogeneic haematopoietic stem cell transplant is the only curative treatment for PMF. In view of this, this group of patients may benefit from allogeneic stem cell transplant rather than *JAK2* inhibitor.

This study has several limitations. One of them was the relatively small sample size. In addition, this was a retrospective single centre study and hence may not truly be reflective of the Malaysian population. However, the strength of this study is the availability of the *CALR* and *MPL* mutations results where such information is scarce especially in this region, thus providing a more comprehensive overview of the epidemiological findings.

Secondly, the relatively long follow up allowed us to report on related complications and survival of the three MPNs.

CONCLUSION

In summary, our study showed that majority of classical MPN patients were Chinese, ET being the most common MPN subtype. The mutation profiles of these patients and their clinical features were comparable with previous reports. Among all mutations, triple negative PMF had the worst clinical outcome. Therefore, mutation studies should be incorporated as part of the routine diagnostic investigations for patients who are suspected to have MPNs even in a resource-limited nations such as Malaysia. This may allow one to have better understanding of the mutation landscape, aid in making the right diagnosis as well as to prognosticate patients.

CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interest to disclose.

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