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Clinical phenotype and prognosis of *JAK2* and *CALR* mutation in Asian patients with Essential Thrombocythemia

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Abstract

Objectives: Calreticulin mutated Essential Thrombocythemia (ET) has a distinct clinical phenotype when compared to *JAK2* V617F mutated ET. Here we determined the prevalence and compared the clinical phenotypes and outcomes of *JAK2* mutated, *CALR* mutated, and both *JAK2* and *CALR* unmutated (double-negative) genotypes in 331 Asian ET patients.

Methods: ET patients defined by BCSH 2010 criteria were selected from our institutional MPN database. Archived blood samples of wild type *JAK2* ET patients were screened for *CALR* mutation using Sanger sequencing. Clinical and laboratory data at diagnosis were collected and compared across the 3 different mutation groups. Outcomes including overall survival and cumulative thrombotic event at 4 years were compared between the three mutation groups and also between *JAK2* mutated versus *JAK2* unmutated patients. Competing risk analysis was used.

Results: *JAK2* V617F mutation was found in 61.9% and *CALR* mutation in 12.1% of our ET cohort. *CALR* mutated patients were more likely to be male, younger and had higher platelet count compared with patients with *JAK2* mutation, Additionally, *CALR* mutated patients had a lower cumulative incidence of thrombosis but similar overall survival compared with *JAK2* mutated patients. However, there was no difference in age, cumulative incidence of thrombosis or overall survival when *CALR* mutated ET patients to *CALR* unmutated patients.

CALR mutated ET patients were significantly more likely to be male with a higher platelet count, and higher LDH. Cumulative incidence of increased peripheral blasts of >5% (PB5) was also appeared to be higher in *CALR* mutated

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group (10 years cumulative incidence were 0%, 1% and 12% for *CALR* unmutated, Jak2 mutated, and CALR mutated, p=0.066)

Conclusion: The prevalence of *JAK2* V617F and *CALR* mutation in Asians is different from that reported in Caucasian patients with ET. The presence of *JAK2* mutation increases the risk of thrombosis, regardless of *CALR* mutation status. Although the presence of *CALR* mutation was associated with a higher platelet count, this did not appear to have any prognostic significance for cumulative incidence of thrombosis or overall survival when the *JAK2* status is taken into account.

CALR mutated ET maybe associated with an increased risk of myelobrotic or leukemic transformation given the increased incidence of rising peripheral blast. (descrever o significado da sigla ja no resumo, ja que não se trata de uma abreviatura já consagrada).

Background

Philadelphia negative Myeloproliferative Neoplasms (MPNs) that are associated with Janus kinase 2 (*JAK2*) mutation include Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF). The discovery of *JAK2* V617F mutation in MPNs has facilitated the diagnosis of MPNs and led to a better understanding of their pathophysiology [1-3]. The majority of PV patients are *JAK2* mutated, while 50-60% of ET and PMF are *JAK2* mutated [2,4-6]. Other driver mutations described in MPN include Myeloproliferative Leukemia (*MPL*) and calreticulin (*CALR*). MPL is mutated in 5-8% of *JAK2* wild type ET and PMF [7,8]. About 30-40% of ET and PMF have neither *JAK2* nor *MPL* mutation (double-negative). In December 2013, two groups simultaneously reported the presence of calreticulin mutation (*CALR*) in ET and PMF [9,10]. The incidence of *CALR* mutation is approximately 70% in *JAK2* wild type ET and PMF, but rare in PV. *CALR* mutation is almost mutually exclusive with *JAK2* mutation with rare concomitant mutations (<1%) reported in a number of studies [11-14]. More than 50 different *CALR* mutations are reported, but 80% of *CALR* mutated patients have one of two mutation variants: type 1 (52-bp deletion) or type 2 (5-bpinsertion) [15].

In Caucasians, *CALR* mutated ET is associated with a specific phenotype with younger age, male sex, higher platelet count (Plt), lower Hemoglobin (Hb) and a lower incidence of thrombotic events when compared to *JAK2* mutated ET [9,10,16]. Whether the lower thrombotic risk is conferred by the presence of the *CALR* mutation or due to the mere absence of the *JAK2* mutation is unclear and the prognostic effect of *CALR* on overall survival is conflicting [9,17]. In this retrospective study, we aim to describe the clinicopathologic features of ET in different genotypes in an Asian population and also determine the prognostic significance of *JAK2* and *CALR* mutation status on thrombotic events and overall survival.

Methods

Patients and samples

We retrospectively identified 331 consecutive patients with ET at National University Hospital of Singapore (NUH), Tan Tock Seng Hospital (TTSH), and Singapore General Hospital (SGH)between 1990-2015. Diagnosis of ET was in accordance with the British Committee of Society of Hematology (BCSH) guideline [18]. Test samples were archival genomic DNA samples in the Molecular Diagnostic Centre of NUH. Out of these 331 cases, 126 cases were *JAK2*-wild type and were subjected to *CALR* mutation testing. *CALR* mutation was detected by conventional Sanger Sequencing method on the *CALR* exon 9 gene by our Molecular Diagnostic Centre. This would result in three genotypes as follow: *Jak2*-mutated is *Jak2+/CALR-*, *CALR*-mutated is *Jak2-/CALR+*, and *CALR*-wild type is *Jak2-/CALR-*. Baseline characteristics and other clinical information were extracted from our Computerized Patient Support System. This study was approved by the respective Institutional Review Boards.

Statistical Analysis

Baseline demographic and clinical characteristics were summarized by mean and Standard Deviation (SD) for continuous variables with approximately normal distribution, and frequency and percentage for categorical variables. Features were compared across three genotypes (*JAK2* mutated, *CALR* mutated and both *JAK2* and *CALR* unmutated (double-negative) using one-way ANOVA for continuous variables and Fisher's exact test for categorical variables. Pair wise comparison (*JAK2* mutated versus *CALR* unmutated, *JAK2* mutated versus *CALR* mutated and *CALR* mutated versus *CALR* unmutated) was subsequently conducted using the independent two-sample *t*-test and the Fisher's exact test with Bonferroni correction. Baseline characteristics were also compared between *JAK2* mutated and *JAK2* wildtype (irrespective of *CALR* mutation status)by the independent two-sample *t* test and the Fisher's exact test for continuous and categorical variables respectively.

We adopted a competing risks approach where thrombotic event, peripheral blast and death were considered as competing events. The proportional sub-distribution hazards regression [19] was used to study the association between different genotypes and clinical outcomes of thrombotic event and peripheral blast. The corresponding cumulative incidence curves were plotted and compared using the Gray's test [20]. To look at the independent predictors of thrombotic event, we further performed a multivariable analysis by adjusting for age, White Blood Cell (WBC) and plts.

The Overall Survival (OS) across the three genotypes were plotted using the Kaplan-Meier survival curves and compared by the Log-rank test. The association between each genotype and overall survival was studied by the univariate Cox regression analysis.

All statistical analyses assumed a two-sided test with a significance level of 5%, and were performed using the statistical software Stata SE 14 (StataCorp LP, College Station, Texas, USA) and the statistical package *cmprsk* in R (www.r-project.org).

Results

Prevalence of *JAK2* and *CALR* mutation

JAK2 mutation was found in 61.9% of ET patients while *CALR* mutation was found in 12.1% (or 31.7% among *JAK2* wild type ET). The *MPL* mutation was only performed in 9 out of 331 cases, and no positive result was observed (qual resultado positive?). Type 1 and Type 2 *CALR* mutations constituted 83% of *CALR* mutations with each having a similar frequency of 41.5%. The remaining *CALR* mutations consisted of deletions and insertions of various lengths, or a combination of both (Table 1). There was no significant difference in gender, age, Hb, Plt, WBC, Lactate Dehydrogenase (LDH), thrombotic events, and in-

cidence of raised peripheral blast of >5% (PB5) between type 1 and type 2 mutations.

Demographic and clinical characteristics

Demographic and baseline clinical characteristics as well as the comparison between different genotypes are shown in Table 2. The mean age of the cohort was 61 years (SD=16) and 78.5% of all patients were Chinese ethnicity. Gender distribution was balanced in the overall group but male gender was more common in *CALR*-mutated ET (male: female ratio =1.5) while female sex was more common in *CALR* unmutated ET (male: female ratio =0.54). *CALR*-mutated group were significantly younger than *JAK2*-mutated group (mean±SD: 56±16 and 65±15 years respectively, p=0.005).

The mean presenting WBC count was 11.7 x 10^9/L (SD=5.9 x 10^9), Hb level was 13.3g/dL (SD=2.0), and Plt count was 804.7 x 10^9/L (SD=339.3x 10^9). 4 patients had low Hb which were unrelated to ET (one patient had severe B12 deficiency, one had severe iron deficiency and two patients had active gastrointestinal bleeding).

JAK2-mutated group had a significantly higher Hb than *JAK2*-wild type group (mean±SD: 13.5±2.0g/dL and 12.9±1.9g/dL respectively, p=0.002). There was no statistical difference in Hb levels between *CALR*-mutated and *CALR*-ET.

JAK2-mutated ET had a significantly higher presenting WBC count compared to *JAK2*-wild type ET (p=0.002) with no statistical difference between *CALR*-mutated and *CALR*- unmutated ET.

CALR-mutated group has a significantly higher platelet count at presentation compared to *Jak2*-mutated or *CALR*-groups (p<0.001). The mean Plt count for *JAK2*-mutated, *CALR*-mutated and *CALR*- unmutated ET were 784, 1066, and 734 x 10^9/L respectively. LDH level at diagnosis in double negative group was significantly lower when compared to *JAK2*-mutatedor *CALR*-mutated ET. Overall, 5.7% of ET patients had splenomegaly at diagnosis (8.2%, 2.8%, and 2.5% for *JAK2*-mutated, *CALR*-mutated, and *CALR*- unmutated ET respectively). Although *JAK2*-mutated ET appeared to have the higher rate of splenomegaly but this was not statistically significant.

Incidence of thrombotic events

A total of 78 thrombotic events were documented from the time of diagnosis until the last follow-up. 27 (8.2%) patients presented with a thrombotic event at diagnosis (24 *JAK2*-mutated, one *CALR*-mutated and two *CALR*-wild type). *JAK2*-mutated ET had a significantly higher incidence of thrombotic event sat presentation when compared to *Jak2*-wild type ET, p=0.002 (Table 2). Similarly, *JAK2*-mutated ET had a higher cumulative incidence of thrombosis at 10 years compared with *CALR*-mutated (crude sub-distribution hazard ratio (SHR) 5.59, p=0.011) or *CALR*-wild type ET (SHR 6.55, p=0.002) (Table 3). There was no difference in thrombotic event at diagnosis (p=1.0) or cumulative incidence of thrombosis at 10 years (SHS 1.17, p=0.856) between *CALR* mutated and *CALR*-wild type ET patients (Table 2&3). The 10-year estimated cumulative incidence of thrombotic events for *JAK2*-mutated, *CALR*-mutated and *CALR*-wild type ET were 34%, 3% and 6% respectively (Figure 1).

In multivariate analysis, after adjusting for age, WBC and platelet count, *JAK2* mutation status remained an independent predictor of subsequent thrombotic events (adjusted SHR = 5.98, 95% CI: 1.84-19.44, p=0.003) (Table 4).

Increase in peripheral blast

The overall incidence of PB5% (from the time of diagnosis until the last follow-up) was 1.5% (5patients). The PB5% incidence was 7.3% (n=3), 1.0% (n=2) and 0% for *CALR*-mutated, *JAK2*-mutated and *CALR*-unmutated groups respectively (p=0.020, Fisher's exact test). This incidence was significantly higher in *CALR*-mutated ET. With competing risk analysis, the crude SHR for *CALR*-mutated group compared to *JAK2*-mutated group was 10.2 (95%CI: 0.86-121.11, p=0.066). The estimated 10 years cumulative incidence of PB5% were 12%, 1%, and 0% for *CALR*-mutated, *Jak2*-mutated and *CALR*-wild type groups respectively (p=0.013) (Figure 2).

Total weekly dose of hydroxyurea

The total weekly dose of hydroxyurea needed to control the platelet count below 600 x 10^9/L was calculated. The mean±SD (g) for *CALR*-mutated, *JAK2*-mutated, and *CALR*-wild type ET were 6.02 ± 2.95, 4.05 ± 2.14, and 4.37 ± 1.82 respectively, p<0.001 (Table 2). *CALR*-mutated ET patients required a significantly higher total weekly dose of hydroxyurea. Pair wise comparison (with Bonferroni correction) confirmed that *CALR* mutated ET patients required a significantly higher dose of hydroxurea when compared to *JAK2* mutated ET(p<0.001) or *CALR* unmutated ET patients (p=0.019) (Table 1).

Overall survival for ET

Median follow-up for the overall cohort was 4.3 years (*JAK2*-mutated 4.3 years, *CALR*-mutated 5.1 years, and *CALR*-wild type 3.4 years). The estimated 10-year OS for *CALR*-mutated, *JAK2*-mutated and *CALR*-wild type ET were 89%, 76%, and 84% respectively (p=0.217) (Figure 3).

Discussion

The main purpose of this study was to clarify the thrombotic risk of *CALR* mutated ET as compared to *CALR* wild type ET. It was often reported in the initial studies that *CALR* mutated ET has lower thrombotic risk compared to *Jak2* mutated ET, however the comparison with *CALR* wild type ET was mostly not reported [9,10,14]. We also seek to ascertain the prevalence of difference genotypes of ET in the Asian population and their clinical phenotypes.

The prevalence of *JAK2* mutation on a large cohort of ET patients was first reported in year 2005 [21]. The prevalence was reported to be around 53% in this large European series, while a North America group reported a prevalence of 50% (n=605) [22]. In the Asian context, Lin and co-workers reported a prevalence of 58.4% in cohort of 428 Chinese patients [23]. In a separate group, Taiwanese investigators reported a prevalence of 63.9% [16]. We have also reported a higher prevalence (61.9%) in this predominantly Chinese population. These could suggest that Chinese population has a relatively higher prevalence of *JAK2*-mutated ET compared to European or North American populations. On the other hand, the Japanese group reported the incidence that was quite similar to Western report [13].

CALR mutation was first discovered almost concurrently by two groups of investigators. Nangalia et al. reported an incidence of 82% in the *JAK2*-wild type ET [10], and Klampfl et al. reported a rate of 67% with the latter having a larger cohort [9]. A North America group has also reported an incidence close to 67% in *JAK2*- wild type ET [17]. On the other hand, the Chinese group reported an incidence of 54.5% within *JAK2*-wt ET [23]. However, the Taiwanese group reported a slightly higher

incidence of 62.3% in their 147 case of *JAK2* non-mutated ET. The Japanese group reported a much lower incidence (39.6%) [13] while our cohort showed the lowest incidence (31.7%). It appeared that the prevalence of Jak2 mutation and CALR mutation among ET patients in the Far East Asia, in particular Chinese population are quite different from the Western population. The differences in prevalence of *JAK2* and *CALR* mutations in our study may reflect our use of BCSH diagnostic criteria while the other studies used WHO 2008 to diagnose ET. Differences in *CALR* assay sensitivity may also play a role.

Even though the gender distribution in our cohort was quite equal (male to female ratio of 1.0), a striking gender discrepancy between different mutation groups were observed. Males appeared to be more prevalent in the *CALR*-mutated group (male 60% and female 40%), while females appeared to be more prevalent in the *CALR*-wild type group (male 34.9% and female 65.1%), p=0.009. This finding concurred with a meta-analysis that looked into gender distribution between *CALR*-mutated and *JAK2*-mutated group [24]. *CALR*-mutated MPN are often younger compared to other genotypes. Rumi and co-workers from Italy reported a median age of 45 and 50 years for *CALR*-mutated and *JAK2*-mutated ET respectively (p=0.001) [25]. We found a similar pattern with our cohort. The mean age for *JAK2*-mutated, *CALR*-mutated and *CALR*- unmutated ET were 65, 56 and 53 years respectively (Table 2). It is important to note that there was no significant difference between *CALR*-mutated and *CALR*-wild type groups. This would suggest that *JAK2*-mutated ET was associated with older age compared to *Jak2*-wild type group.

CALR-mutated ET has been shown repeatedly to have lower Hb compared to *JAK2*-mutated ET in a number of studies [16,25-29]. However in our cohort, we could only demonstrate that *JAK2*-mutated ET had a significantly higher Hb at diagnosis when compared to *Jak2*- wt ET (p=0.002) or *CALR*- wild type ET (p=0.01), but not when compared to *CALR*-mutated ET. The difference between *CALR*-mutated and *CALR*- wild type ET was also not significant. *Jak2*- mutated ET was almost always associated with elevated WBC count, while *CALR*- mutated ET was often associated with high platelet count [23,25-29]. We found the same association, but in addition, we have also showed that *CALR*-mutated ET required higher dose of Hydroxyurea (Hydroxycarbamide) to control the platelet count below 600.

JAK2-mutated myeloproliferative neoplasm is often associated with an increased thrombotic risk [30-33]. The overall incidence of thrombosis in *JAK2*-mutated ET was reported to be around 21%-41% compared to *CALR*-mutated ET (10-31%). [9,17,28]. The prevalence of *JAK2* mutation was found to be as high as 40% among patients with splanchnic vein thrombosis without clinical manifestation of MPN [34]. The prevalence of *CALR* mutation was reported to be 0.7% to 2.4% in patients with splanchnic vein thrombosis [35-39]. Since the discovery of *CALR* mutation in MPNs, it has been widely reported that the incidence of thrombotic in this group was significantly lower compared to *JAK2*-mutated group, and many have concluded that *CALR* mutation is associated with lower risk of thrombotic event [17,25,29]. However, none of these previous studies compared the incidence of thrombosis between *CALR*-mutated and *CALR*-wild type ET. We have demonstrated that the incidence of thrombotic event at diagnosis or the cumulative incidence of subsequent thrombotic events was clearly highest among *JAK2*-mutated ET compared to *CALR*-mutated or *CALR*-wild type ET. However, the incidence was almost identical between *CALR*-mutated and *CALR*-wild type ET. It was mere presence of Jak2 mutation that conferred an increased risk of thrombosis irrespective CALR mutation status. (see Figure 1, Table 2 & 3).

In the multivariable analysis that included, age, *JAK2* mutation status, WBC, and platelet count as covariates, only *JAK2* mutation status stood out as an independent predictor for subsequent thrombotic event. Though there were study groups reported an association of leukocytesis with increased risk of thrombosis [30,40,41], we were not able to demonstrate that in our cohort. Palandri and co-workers further demonstrated that WBC of more than 11.0 x 10^9/L was significantly associated with thrombotic events, which we have failed to demonstrate. One of the reasons for this difference may be that we included *JAK2* mutation status in the multivariable model, and it is well recognized that *JAK2* mutation is associated with higher WBC count [42,43].

It is well reported that platelet count in ET does not correlate well with thrombotic events [44-46]. In fact, extreme thrombocytosis is a risk factor for bleeding due to acquired von Willebrand disorder. Similarly, one group reported that a platelet count >1000 x 10^6/micro L was associated with a significantly decreased risk for arterial thrombosis (HR 0.42; 95% CI 0.22-0.78) [30]. With the discovery of *CALR* mutation in myeloproliferative neoplasm, which is often associated with high platelet count but with lower risk of thrombosis could explain this paradox [17,25,29]. This raises the question of the role cytoreduction therapy in preventing thrombotic events. An anti-platelet therapy may be adequate as long as the platelet count is not in a range of increased risk of bleeding, i.e more than 1000 x 10^9/L.

The association of *CALR* mutated ET with higher risk of myelofibrotic progression and leukemic transformation is inconclusive. The Italian group reported no significant increase in leukemic or myelofibrotic transformation in *CALR* mutated ET [25], so did Tefferi and co-workers [17]. The latter reported an incidence of leukemic transformation of 5% and 8.4% for *JAK2* mutated and *CALR* mutated ET respectively. A Belgian cohort reported an increase risk of progression to myelofibrosis in *CALR* mutated compared to *JAK2* mutated ET [47]. In another separate report, the investigators found the type 1 *CALR* mutation was associated with myelofibrotic progression in ET [48]. We were unable to fully determine the incidence of myelofibrotic or leukemic transformation accurately as most patients declined a repeat bone marrow examination at the point of suspected disease progression and were on palliative management. However, we did observe that *CALR* mutated ET had a higher incidence of increased peripheral blasts on long-term follow-up. Estimated 10 years cumulative incidence of PB5% were 12%, 1% and 0% for *CALR*-mutated, *JAK2* mutated and *CALR*-wild type ET respectively, p=0.013) suggesting an increased risk of myelofibrotic or leukemic transformation in *CALR* mutated ET. Further confirmation is required from a larger cohort with well-documented blast or myelofibrotic transformation.

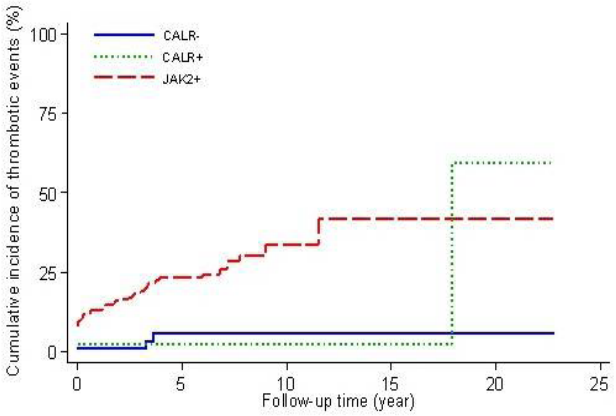
After two initial reports showing superior survival in *CALR*-mutated ET [9,47], most groups could not demonstrate the superior survival of CALR mutated ET [16,17,25-27,29]. In our cohort the estimated 10-year OS for *CALR* mutated, *JAK2* mutated and double-negative groups were 89%, 76%, and 84% respectively (p=0.217).

More than 80% of the mutations were reported to be due to the two commonest mutations, type 1 and 2 [9,49,50] with

type 1 being the most common (45-63.3%). We reported a similar incidence for both the type 1 and 2 – 41.5%. In 114 cases of *CALR* mutated ET, Tefferi and co-workers demonstrated that type 2 mutation had a significantly higher platelet count compared to type 1 [49]. Riera's group with a smaller cohort (n=60) demonstrated that type 1 mutation was associated with male gender while type 2 mutation was associated with younger age [50]. In our cohort, we were unable to demonstrate any of these differences between type 1 and type 2. There was also no difference in term of other clinical parameters or thrombotic event. We have detected several complex mutations of various lengths (deletions and/or insertions). In almost all mutants, CALR proteins possess an altered C-terminus with a longer peptide stretch caused by a disrupted reading frame due to these frame-shift mutations.

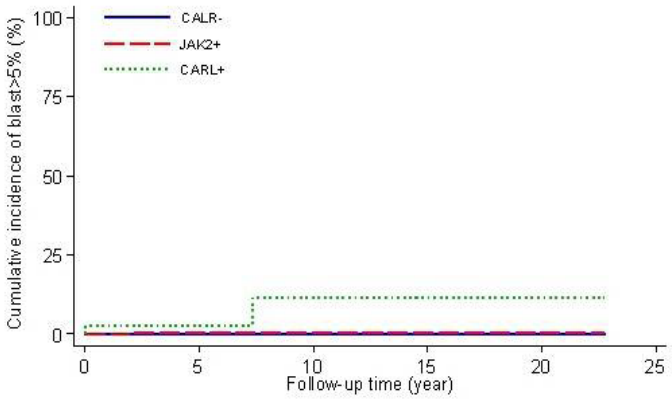
The main limitation of this study was the missing MPL mutation status. It was only performed in 9 out of 331 cases. The possible reasons being the test was introduced not long before the CALR mutation testing and not many physician were aware of it or feel the need to perform it. Nevertheless, the reported incidence of MPL mutated ET was less than 5% [51,52], and that would not have significant impact on the analysis.

Figures



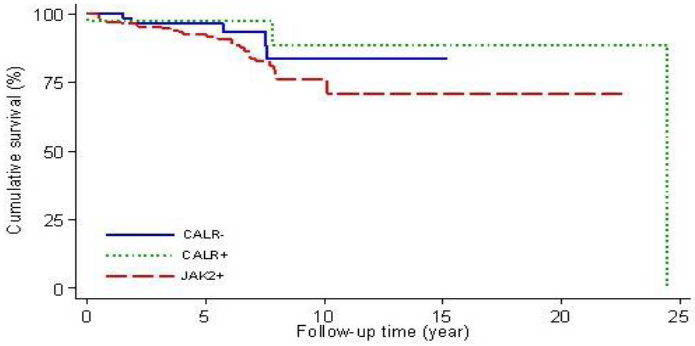
Mutation	10-year Cumulative Incidence	Crude SHR	95% CI	*p*-value
CALR-	6%	1.00	-	-
CALR+	3%	1.17	0.21-6.48	0.856
JAK2+	34%	6.55	2.05-20.97	0.002

Figure 1: Cumulative incidence of thrombotic event in different genotypes



Mutation	10-year Cumulative Incidence	Crude SHR	95% CI	*p*-value
CALR-	0%	-	-	-
JAK2+	1%	1.00	-	-
CALR+	12%	10.20	0.86-121.11	0.066

Figure 2: Cumulative incidence of Blast >5%



Mutation	10-year Overall Survival	Crude HR	95% CI	*p*-value
CALR-	84%	1.00	-	-
CALR+	89%	0.67	0.13-3.45	0.631
JAK2+	76%	1.78	0.69-4.63	0.236

Mutation	Median follow-up time (year)
CALR-	3.4
CALR+	5.1
Jak2+	4.3
Overall	4.3

Figure 3: Cumulative survival

Tables

Table 1: Type of CALR Mutations Identified in JAK2- ET Patients

Nucleotide change	Amino acid change	Amino acid sequence	Frequency	
			n	%
Wild-type CALR	ns	AAEKQMKDKQDEEQRLKEEEEDKKRKEEEEAEDKEDDEDKDEDEEDEEDKEEDEEEDVPGQAKDEL-	ns	ns
c.1099_1150del	p.Leu367fs*46	AAEKQMKDKQDEEQR**TRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWILD-TYPEEA-**	17	41.5
c.1154_1155 insTTGTC	p.Lys385fs*47	AAEKQMKDKQDEEQRLKEEEEDKKRKEEEEAED**NCRRMMRTKMRMRRMRRTRRKMRRKMSPAR-PRTSCREACLQGWILDTYPEEA-**	17	41.5
c.1093_1126del	p.Gln365fs*54	AAEKQMKDKQDEE**AKRRRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQG-WILDTYPEEA-**	1	2.4
c.1102_1104delAAG	p.Lys368fs*	AAEKQMKDKQDEEQRL_EEEEDKKRKEEEEAEDKEDDEDKDEDEEDEEDKEEDEEEDVPGQAKDEL-	1	2.4
c.1103_1136del	p.Lys368fs*51	AAEKQMKDKQDEEQRL**RRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQG-WILDTYPEEA-**	1	2.4
c.1106_1139del	p.Glu369fs*50	AAEKQMKDKQDEEQRLK**GRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQG-WILDTYPEEA-**	1	2.4
c.1120A>G	p.Lys374Arg	Genetic variant of uncertain significance	1	2.4
c.1122_1125delGAAA	p.Lys374fs*55	AAEKQMKDKQDEEQRLKEEEED**NAKRRRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPAR-PRTSCREACLQGWILDTYPEEA-**	1	2.4
c.1129_1153delins	p.Lys377fs*	AAEKQMKDKQDEEQRLKEEEEDKKR**LCVSF**EDDEDKDEDEEDEEDKEEDEEEDVPGQAKDEL-	1	2.4

Bold and underline indicate altered amino acid

Table 2: Baseline dermographic and clinical features, and comparison between different genotypes

	Overall	*JAK2*+	*CALR*+	*CALR*-	*p*-value	p value			
	(n = 331)	(n = 205)	(n = 40)	(n = 86)		*Jak2*+ vs *CALR*-	*CALR*+ vs *CALR*-	*Jak2*+ vs *CALR*+	*Jak2*+ vs *Jak2*-
Age, mean (SD) (year)	61 ± 16	65 ± 15	56 ± 16	53 ± 14	< 0.001	< 0.001	0.752	0.005	< 0.001
Gender (%)					0.009	0.03	0.036	1	0.141
Male	160 (48.3)	106 (51.7)	24 (60.0)	30 (34.9)					
Female	171 (51.7)	99 (48.3)	16 (40.0)	56 (65.1)					
Ethnicity (%)					0.659	1.000	1.000	1.000	0.529
Chinese	260 (78.5)	160 (78.1)	35 (87.5)	65 (75.6)					
Malay	44 (13.3)	26 (12.7)	4 (10.0)	14 (16.3)					
Indian	12 (3.7)	7 (3.3)	1 (2.5)	4 (4.7)					
Others	15 (4.5)	12 (5.9)	0 (0.0)	3 (3.4)					
Haemoglobin (g/dL) (SD)	13.3 ± 2.0	13.5 ± 2.0	13.0 ± 1.9	12.8 ± 1.9	0.008	0.01	1	0.294	0.002
WBC ($\times 10^9$/L) (SD)	11.7 ± 5.9	12.4 ± 6.6	10.2 ± 4.0	10.6 ± 4.6	0.014	0.049	1	0.093	0.002
Platelet ($\times 10^9$/L) (SD)	804.7 ± 339.3	783.6 ± 343.9	1065.9 ± 319.9	733.5 ± 279.2	< 0.001	0.695	< 0.001	< 0.001	0.149
LDH (U/L) (SD)	622 ± 267	649 ± 291	685 ± 283	526 ± 167	0.018	0.038	0.044	1	0.139
Splenomegaly (%)	15 (5.7)	12 (8.2)	1 (2.8)	2 (2.5)	0.195	0.438	1	1	0.064
Baseline thrombosis (%)	27 (8.2)	24 (11.7)	1 (2.5)	2 (2.3)	0.009	0.036	1	0.27	0.002
Dose of hydroxyurea (g) (SD)	4.37 ± 2.32	4.05 ± 2.14	6.02 ± 2.95	4.37 ± 1.82	< 0.001	1	0.019	< 0.001	0.001

Jak2+: *Jak2*-mutated and *CALR*-wildtype; *Jak2*-: *Jak2*-wildtype irrespective of *CALR* status; *CALR*+: *CALR*-mutated and *Jak2*-wildtype; *CALR*-: *Jak2*-wildtype and *CALR*-wildtype

Table 3: Comparison of thrombotic risk between different genotypes

Genotypes	Crude SHR	95%CI	*p*-value
Jak2+ vs Jak2-wildtype	6.16	2.49-15.24	<0.001
Jak2+ vs CALR+	5.59	1.48-21.08	0.011
Jak2+ vs CALR-	6.55	2.05-20.97	0.002
CALR+ vs CALR-	1.17	0.21-6.48	0.856

* Comparison of thrombotic risk was done between *Jak2* mutated (also *CALR* wild type) and *Jak2* wild type (include both *CALR* mutated and wild type); *Jak2* mutated and *CALR* mutated (also *Jak2* wild type); *Jak2* mutated and *CALR* wild type (also *Jak2* wild type); and *CALR* mutated and *CALR* wild type.

Table 4: Variables Associated with Subsequent Thrombotic Events by Multivariable Proportional Subdistribution Hazards Regression, n = 304

Variable	Adjusted SHR*	95% CI#	*p*-value
JAK2/CALR mutation			0.002
CALR-	1.00	-	-
CALR+	1.32	0.23-7.57	0.753
JAK2+	5.98	1.84-19.44	0.003
Age	1.01	0.99-1.03	0.593
WBC	1.03	0.98-1.09	0.280
Platelet	1.00	0.99-1.01	0.450

* SHR: subdistribution hazard ratio. # CI: confidence interval.

Conclusion

We reconfirmed some of the unique clinical features of CALR mutated ET reported in the literature. In addition, we have also demonstrated that the prevalence of *JAK2* mutation and *CALR* mutation in Asians with ET differs from studies related to Western population. The presence of *JAK2* mutation increases the risk of thrombosis, regardless of *CALR* mutation status. Although the presence of *CALR* mutation was associated with a higher platelet count, this did not appear to have any prognostic significance for cumulative incidence of thrombosis or overall survival when the JAK2 status is taken into account. Moving forward, we hope to better characterize the incidence of MPL in relation to Jak2 and CALR mutation status. The test has been incorporated as a triple test for MPN cases in particular ET and MF cases since year 2015.

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