# Mutational subtypes of *JAK2* and *CALR* correlate with different clinical features in Japanese patients with myeloproliferative neoplasms

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Running title: *JAK2* and *CALR* mutation subtypes in MPNs

Original article

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**Abstract**

The majority of patients with Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs) harbor *JAK2*, *CALR*, or *MPL* mutations. We compared clinical manifestations of different subtypes of *JAK2* and *CALR* mutations in Japanese patients with MPNs. Within our cohort, we diagnosed 166 patients as polycythemia vera (PV), 212 patients as essential thrombocythemia (ET), 23 patients as pre-primary myelofibrosis (PMF), 65 patients as overt PMF, and 27 patients as secondary myelofibrosis following the 2016 WHO criteria. Compared to patients with *JAK2*V617F-mutated PV, *JAK2* exon 12-mutated PV patients were younger, showed lower white blood cell (WBC) counts, lower platelet counts, higher red blood cell counts, and higher frequency of thrombotic events. Compared to *JAK2*-mutated ET patients, *CALR*-mutated ET patients were younger, showed lower WBC counts, lower hemoglobin levels, higher platelet counts, and fewer thrombotic events. *CALR* type 1-like mutation was the dominant subtype in *CALR*-mutated overt PMF patients. Compared with *JAK2*V617F-mutated ET patients, *JAK2*V617F-mutated pre-PMF patients showed higher LDH levels, lower hemoglobin levels, higher *JAK2*V617F allele burden, and higher frequency of splenomegaly. In conclusion, Japanese patients with MPNs grouped by different mutation subtypes exhibit characteristics similar to those of their Western counterparts. In addition, ET and pre-PMF patients show different characteristics, even when restricted to *JAK2*V617F-mutated patients.

Keywords: *CALR*, driver mutations, *JAK2* exon 12, *JAK2*V617F, *MPL*

## 1. Introduction

Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs) are clonal hematopoietic stem cell disorders presenting with proliferation of myeloid lineage cells. Major entities of MPNs include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). Recurrent somatic mutations in *JAK2*, *CALR* and *MPL* genes have been identified in patients diagnosed with MPNs.(1) *JAK2* mutations can be subclassified into the *JAK2*V617F mutation and *JAK2* exon 12 mutations. *CALR* mutations can be subclassified into two major subgroups according to their predicted effect on the calreticulin C-terminal, *CALR* type 1-like mutations and type 2-like mutations. PV patients are known to potentially harbor *JAK2*V617F or *JAK2* exon 12 mutations. In contrast, patients with ET and PMF can present with *JAK2*V617F, *CALR* type 1-like, *CALR* type 2-like, or *MPL* mutations. Mutation subtypes in specific entities of MPNs are known to be associated with certain clinical features in Caucasian cohorts.(2–4) We applied the 2016 WHO diagnostic criteria to a large cohort of Japanese patients with suspected MPNs and verified whether previous findings are the same in Japan.

## 2. Material and Methods

### 2.1 Study population

This study involved 2219 individuals with suspected MPNs from the Department of Hematology at Juntendo University School of Medicine and other participating institutions in Japan between April 2010 and December 2016. This study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of Juntendo University School of Medicine (IRB#2013020). Written informed consent was obtained prior to the use of samples and collection of clinical records.

### *2.2* Mutation analysis

Genomic DNA was isolated from peripheral blood using a QIAamp DNA Mini Kit (QIAGEN) or Gentra Puregene Blood Kit (QIAGEN). DNA concentration was determined by using a NanoDrop LITE spectrophotometer (Thermo Scientific), and the

samples were stored at -80°C until use. *JAK2*V617F allele burdens were first determined via ABC-PCR.(5) If *JAK2*V617F allele burdens were below 10%, we added allele-specific PCR (AS-PCR), which is more accurate for measuring low allele burdens below 10%.(6) If *JAK2*V617F was negative in a patient clinically suspected of PV, *JAK2* exon 12 mutations were additionally analyzed by direct sequencing method or by Miniseq Sequencing System (Illumina) using primers forward; 5'-TGGAGCAATTCATACTTTCAGTG-3', reverse; 5'-AACACAAGGTTGGCATATTTTTC-3'. *MPL*W515K/L mutations were assessed using an allele-specific PCR technique called dual amplification refractory mutation system PCR (DARMS-PCR) and subsequent capillary electrophoresis.(7) The *CALR* mutation on exon 9 was examined using our in-house fragment analysis method.(8) If the detected *CALR* mutation was other than *CALR*del52 and *CALR*ins5 mutations, we additionally carried out direct sequencing method to classify the *CALR* mutation subtype based on the definition by Pietra et al (9) by using primers forward; 5'-CTGGTCCTGGTCCTGATGTC-3', reverse; 5'-CAGTCCAGCCCTGGAGGCAG-3'. Although *JAK2, MPL, CALR* mutations have been proposed to be mutually exclusive, we identified two ET patients with *JAK2*V617F coinciding with either *MPL*W515L or *MPL*W515K mutations, and these patients were excluded from analysis.

2.3 Diagnosis

Patients were diagnosed as PV, ET, prefibrotic PMF (pre-PMF), overt PMF, or secondary myelofibrosis (sMF) according to the 2016 WHO diagnostic criteria with available indices.(10) Clinical and laboratory parameters were obtained from the time of diagnosis. We set an erythropoietin (EPO) concentration of less than 12.5 mU/mL as subnormal serum EPO level.(11)

2.4 Statistics

For comparing different patient groups, we applied the Fisher exact test for categorical variables and the Mann-Whitney *U* test for continuous variables. All statistical

analysis were carried out with EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan) , which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).(12) P values less than 0.05 were considered significant.

## 3. Results

### 3.1 Diagnosis of MPNs and driver mutation frequencies

In a cohort of patients with suspected MPNs, we diagnosed 166 patients with PV, 212 patients with ET, 23 patients with pre-PMF, 65 patients with overt PMF, and 27 patients with sMF consisting of nine post-polycythemia vera myelofibrosis (post-PV MF) patients and 18 post-essential thrombocythemia myelofibrosis (post-ET MF) patients. Clinical characteristics of PV, ET, pre-PMF, overt PMF and sMF were similar compared to previous reports (Table 1).(4,13,14) Of 166 PV patients, 161 (97.0%) harbored *JAK2* mutations, and five (3.0%) patients were negative for *JAK2*, *CALR* and *MPL* mutations (triple-negative). Of 212 ET patients, 127 (59.9%) harbored *JAK2* mutations, 57 (26.9%) harbored *CALR* mutations, 10 (4.7%) harbored *MPL* mutations and 18 (8.5%) patients were triple-negative. Of 23 pre-PMF patients, 18 patients (78.3%) harbored *JAK2* mutations, one patient (4.3%) harbored a *CALR* mutation, and four patients (17.4%) were triple-negative. Of 65 overt PMF patients, 35 patients (53.8%) harbored *JAK2* mutations, 18 patients (27.7%) harbored *CALR* mutations, one patient (1.5%) harbored an *MPL* mutation and 11 patients (16.9%) were triple-negative. Of 27 sMF patients, 16 patients (59.3%) harbored *JAK2*V617F, nine patients (33.3%) harbored *CALR* mutations and one patient (3.7%) harbored an *MPL* mutation and one patient (3.7%) was triple-negative.

### 3.2 *JAK2*V617F allele burdens in PV, ET, pre-PMF, overt PMF and sMF

Median *JAK2*V617F allele burdens calculated within *JAK2*V617F mutation-positive patients were 77.6% in PV, 30.7% in ET, 38.0% in pre-PMF, 48.2% in overt PMF, 94.5% in post-PV MF, 72.7% in post-ET MF. *JAK2*V617F allele burdens were significantly higher in PV compared to ET, pre-PMF, and overt PMF ($p<0.001$). Pre-PMF

patients showed significantly higher *JAK2*V617F allele burdens compared to ET patients (p=0.036). Post-PV MF patients showed significantly higher *JAK2*V617F allele burdens compared to PV patients (p=0.022). Post-ET MF patients tended to show higher allele burdens compared to ET patients (p=0.095). Overt PMF patients tended to show higher *JAK2*V617F allele burdens compared to pre-PMF patients (p=0.372) (Figure 1).

3.3 Clinical characteristics of PV patients with different *JAK2* mutation subtypes.

Of 161 *JAK2*-mutated PV patients, we identified 152 patients with *JAK2*V617F mutations and nine patients with *JAK2* exon 12 mutations. *JAK2* exon 12 mutation variations of the nine patients were present in the following distributions: two c.1615_16 AA>TT, two c.1611_16 delTCACAA, one c.1615_16 AA>CT, one c.1614_16 CAA>ATT, one c.1613_16 ACAA>T, one c.1623_28 delAAATGA and one c.1627_32 delGAAGAT. Compared with *JAK2*V617F patients, *JAK2* exon 12-mutated patients were significantly younger (exon 12: 53years vs V617F: 64years, p=0.024), showed lower levels of white blood cell (WBC) counts (exon 12: $8.4\times10^9$/L vs V617F: $14.2\times10^9$/L, p=0.028), higher red blood cell (RBC) counts (exon 12: $7.6\times10^{12}$/L vs V617F: $7.1\times10^{12}$/L, p=0.019), lower mean corpuscular volume (MCV) (exon 12: 75.3fl vs V617F: 82.6fl, p=0.012), lower platelet counts (exon 12: $357\times10^9$/L vs V617F: $565\times10^9$/L, p=0.016), and higher frequency of thrombotic events (exon 12: 55.6% vs V617F: 23.4%, p=0.046). There were no differences between the two groups concerning EPO levels, presence of splenomegaly and bone marrow fibrosis (Table 2).

3.4 Clinical characteristics of ET and PMF patients with different mutational status

Compared to *JAK2*-mutated ET patients (*JAK2*-ET), *CALR*-mutated ET patients (*CALR*-ET) were significantly younger (*CALR*-ET: 60years vs *JAK2*-ET: 67years, p=0.036), showed lower WBC counts (*CALR*-ET: $8.0\times10^9$/L vs *JAK2*-ET: $9.8\times10^9$/L, p<0.001), lower hemoglobin (Hb) levels (*CALR*-ET: 13.4g/dL vs *JAK2*-ET: 14.3g/dL, p<0.001), lower hematocrit (Hct) levels (*CALR*-ET: 40.0% vs *JAK2*-ET: 43.3%, p<0.001), higher platelet counts (*CALR*-ET: $1040\times10^9$/L vs *JAK2*-ET: $847\times10^9$/L, p<0.001), and lower frequency of

thrombotic events (*CALR*-ET: 5.3% vs *JAK2*-ET; 20.5%, p=0.008). Compared to *JAK2*-ET, triple-negative ET patients also showed significantly lower WBC counts (triple-negative ET: $7.8\times10^9$/L vs *JAK2*-ET: $9.8\times10^9$/L, p=0.009), lower Hb levels (triple-negative ET: 13.2g/dL vs *JAK2*-ET: 14.3g/dL, p=0.003) and lower Hct levels (triple-negative ET: 40.1% vs *JAK2*-ET: 43.3%, p=0.003), but no difference was observed concerning age, platelet counts and thrombotic events. Triple-negative ET patients showed significantly lower rate of bone marrow fibrosis (MF-1) compared to *JAK2*-ET and *CALR*-ET (triple-negative ET: 0% vs *JAK2*-ET: 20.5% p=0.044, triple-negative ET: 0% vs *CALR*-ET: 22.8% p=0.030, respectively). Compared to *JAK2*-ET, *MPL*-mutated ET patients (*MPL*-ET) showed significantly lower Hb levels (*MPL*-ET: 12.9g/dL vs *JAK2*-ET: 14.3g/dL, p=0.016), lower Hct levels (*MPL*-ET: 40.0% vs *JAK2*-ET: 43.3%, p=0.023) and higher platelet counts (*MPL*-ET: $1296\times10^9$/L vs *JAK2*-ET: $847\times10^9$/L, p=0.002). No significant differences in gender ratio, lactate dehydrogenase (LDH) levels and frequency of splenomegaly were seen between different mutation groups of ET (Table 3a).

No significant differences were seen in clinical characteristics between different mutation groups within pre-PMF patients (Table S1).

Concerning overt PMF, triple-negative overt PMF patients were younger than *JAK2*-mutated patients (*JAK2*-overt PMF) (triple-negative overt PMF; 51years vs *JAK2* overt PMF; 71years, p=0.017). Also, LDH levels differed significantly between *JAK2*V617F-mutated, *CALR*-mutated, and triple-negative overt PMF patients, with *CALR*-mutated patients (*CALR*-overt PMF) showing highest LDH levels (median LDH: 610IU/L), *JAK2*-overt PMF showing intermediate LDH levels (median LDH: 465IU/L), and triple-negative overt PMF showing lowest LDH levels (median LDH: 276IU/L) (*JAK2*-overt PMF vs *CALR*-overt PMF, p=0.036; *JAK2*-overt PMF vs triple-negative overt PMF, p=0.006; *CALR*-overt PMF vs triple-negative overt PMF, p=0.007) (Table 3b).

3.5 Clinical characteristics of ET and MF (PMF+sMF) patients with different *CALR* mutation subtypes.

Of 57 *CALR*-ET, 35 patients harbored *CALR*del52 (type 1 mutation), 15 patients

harbored *CALR*ins5 (type 2 mutation) and seven patients harbored other *CALR* exon 9 mutations. Based on the definition by Pietra et al,(9) we recategorized all patients into 38 type 1-like mutations, 16 type 2-like mutations, and three other type mutations. As previously reported,(8) patients with *CALR* type 2-like mutations tended to show higher platelet counts compared to patients with type 1-like mutations. LDH levels were significantly higher in patients with *CALR* type 1-like mutations (315IU/L vs 244IU/L, p=0.041) (Table 4a).

In myelofibrosis (MF) patients, which included overt PMF and sMF patients, 27 *CALR*-mutated patients were identified and categorized into 22 (81.5%) type 1-like and five (18.5%) type 2-like mutations. In accordance with previous reports, *CALR* type 1-like mutations were more common in MF patients compared to ET patients (MF: 81.5% vs ET: 70.4%).(9,15) In particular, *CALR* type 1-like mutated patients constituted the vast majority of *CALR*-overt PMF (15/17: 88.2%) (data not shown). As previously reported, there were no significant differences in patient characteristics between *CALR* type 1-like and *CALR* type 2-like mutated MF patients (Table 4b).(9)

## 4. Discussion

We compared clinical characteristics between different driver mutation groups and different mutation subtype groups in Japanese patients with PV, ET, pre-PMF, overt PMF and sMF diagnosed by the 2016 WHO criteria. The results were for the most part in line with previous reports from Western countries,(4,9,13,14) but some differences were found in our cohort. Concerning PV patients, we found that *JAK2* exon 12 mutated patients had higher frequency of thrombotic events compared to *JAK2*V617F mutated patients. Previous reports of Caucasian cohorts showed that frequency of thrombotic events were equal between the two groups.(16,17) This is the first study comparing clinical characteristics of Japanese PV patients with *JAK2*V617F and *JAK2* exon 12 mutations, and these conflicting results may be due to differing ethnicity. Larger studies in Asian patients are needed to verify these results. Analysis of ET patients showed that frequency of bone marrow fibrosis (MF-1) was significantly lower in triple-negative ET compared to

*JAK2*-ET and *CALR*-ET. This was rather surprising, because triple-negative ET patients are reported to have shorter time to myelofibrosis when compared to *CALR*-ET.(18) Within *CALR*-mutated ET, myelofibrotic transformation is known to be higher in *CALR* type 1-like mutations.(19,20) The current study found no difference between *CALR* type 1-like and type 2-like patients concerning presence of bone marrow fibrosis, but this may be due to the small number of patients studied. However, patients with *CALR* type 1-like mutation showed significantly higher LDH levels in the current study which may reflect a higher myelofibrotic transformation potential. Also concerning ET, we found no gender difference between *CALR* type 1-like and type 2-like mutation groups, but male gender was reported to be associated with *CALR* type 1-mutation in a previous study.(21)

Compared to other mutation groups, *CALR*-overt PMF are reported to present with younger age, lower WBC counts and higher platelet counts. We found conflicting results, with triple-negative PMF patients presenting with youngest age. When compared to *JAK2*-overt PMF, *CALR*-overt PMF are reported to have lower risk of thrombotic events, but we found no difference between the two groups.(3) Also, we found that LDH levels significantly differed between *JAK2*V617F-mutated, *CALR*-mutated, and triple-negative PMF patients, which has not been previously reported.

*JAK2*V617F allele burdens are reported to differ between different MPNs, and our study verifies these findings.(4,22,23) *JAK2*V617F allele burdens cannot be directly compared between studies when methods of analysis differ, and thus analysis of a broad range of MPNs within the same study are valuable. ET and pre-PMF showed lowest *JAK2*V617F allele burdens whereas PV and sMF showed highest *JAK2*V617F allele burdens. *JAK2*V617F allele burdens are low in ET but are approximately twice as high in post-ET MF, and these findings further support a previous report by Shirane et al showing that an increase of *JAK2*V617F allele burdens during the disease course of ET might correlate with myelofibrotic transformation.(24)

The 2016 WHO diagnostic criteria newly recognized pre-PMF as a distinct entity of the MPNs. It is important to distinguish pre-PMF from ET because the two entities present with different clinical characteristics and pre-PMF carries a worse prognosis,(25)

but cautious discrimination is necessary because both entities can show thrombocytosis. Compared to ET patients, pre-PMF patients are known to show higher WBC counts, lower Hb levels, higher platelet counts, higher LDH levels, higher *JAK2*V617F allele burdens and higher frequency of splenomegaly.(14) However, comparison of the two entities based on driver mutations is limited. We compared *JAK2*V617F-mutated ET patients and *JAK2*V617F-mutated pre-PMF patients and found that the latter showed significantly lower Hb levels (p=0.006), lower platelet counts (p=0.004), higher LDH levels (p=0.043) and higher *JAK2*V617F allele burdens (p=0.036). *JAK2*V617F-mutated pre-PMF patients also showed a tendency of higher WBC counts (p=0.200) and higher frequency of splenomegaly (p=0.079) (Table S2). All of these findings with the exception of platelet counts are in line with previous reports comparing entire ET and pre-PMF groups,(14) and suggest that even when the comparison is done restricted to *JAK2*V617F-mutated patients, the result is similar, and further supports that ET and pre-PMF are separate entities.

Pre-PMF and overt PMF are basically considered to be a single entity only looked upon at different time points of the disease. Half of surviving pre-PMF patients are reported to proceed to overt PMF over a time of approximately twenty years.(26) Within *JAK2*V617F-mutated pre-PMF, *JAK2*V617F allele burdens did not differ significantly between pre-PMF and overt PMF patients. However, to our surprise, *JAK2*V617F-mutation frequencies of pre-PMF and overt PMF differed significantly, with pre-PMF patients showing higher mutations rates of *JAK2*V617F (pre-PMF: 78.3% vs overt PMF: 53.8%, p=0.049) (data not shown). A smaller percentage of *JAK2*V617F mutation in overt PMF may suggest that pre-PMF patients with non-mutated *JAK2*V617F have higher myelofibrotic potential.

We demonstrate that mutational status has a large effect on clinical characteristics of MPN patients. The findings were basically in line with previous reports, and we confirm that Japanese MPN patients grouped by different mutation subtypes show similar characteristics as that of their Western counterparts. Also, we show that patient characteristics differ between ET and pre-PMF patients even when restricted to *JAK2*V617F-mutated patients, which further supports that ET and pre-PMF are two distinct

entities.

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**Disclosure statement**

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Figure Legend

Figure 1. Diversity of *JAK2*V617F allele burdens among MPNs.

PV: polycythemia vera; ET: essential thrombocythemia; pre-PMF: prefibrotic primary myelofibrosis, overt PMF: overt fibrotic stage primary myelofibrosis; MF: myelofibrosis. Median *JAK2*V617F allele burdens of each disease were as follows, PV 77.6% (range; 8.5%-100%), post-PV MF 94.5% (range; 48.8%-100%), ET 30.7% (range; 5.2%-100%), post-ET MF 72.7% (range; 3.9%-99.8%), pre-PMF 38.0% (range: 14.9%-100%), overt PMF 48.2% (range; 11.2%-99.3%).

Table Legends

Table 1. Clinical characteristics of patients with MPNs diagnosed by the 2016 WHO criteria.

PV: polycythemia vera; ET: essential thrombocythemia; pre-PMF: prefibrotic primary myelofibrosis, overt PMF: overt primary myelofibrosis. WBC: white blood cells; RBC: red blood cells; Hb: hemoglobin; Hct: hematocrit; Epo: erythropoietin levels; LDH: lactate dehydrogenase.

For continuous variables, data are shown as median values and brackets represent range. For categorical variables, right side of / represents the total number of patients studied and left side represents the number of positive patients, and brackets represent positive percentages.

Table 2. Clinical characteristics of polycythemia vera patients with *JAK2*V617F or *JAK2* exon 12 mutations.

PV: polycythemia vera; WBC: white blood cell; RBC: red blood cell; Hb: hemoglobin; Hct: hematocrit; MCV: mean corpuscular volume; Epo: erythropoietin.

For continuous variables, data are shown as median values and brackets represent range. For categorical variables, right side of / represents the total number of patients studied and left side represents the number of positive patients, and brackets represent positive percentages.

Table 3. Clinical characteristics of patients with different mutational status in essential thrombocythemia (3a) and overt primary myelofibrosis (3b).

ET: essential thrombocythemia; pre-PMF: prefibrotic primary myelofibrosis, overt PMF: overt fibrotic stage primary myelofibrosis. WBC: white blood cell; Hb: hemoglobin Hct: hematocrit; LDH: lactate dehydrogenase. For continuous variables, data are shown as median values and brackets represent range. For categorical variables, right side of / represents the total number of patients studied and left side represents the number of positive patients, and brackets represent positive percentages.

*There was only one *MPL*-mutated overt PMF patient, and thus was removed from analysis here.

Table 4. Clinical characteristics depending on *CALR* type 1-like or type 2-like mutations in patients with essential thrombocythemia (4a) and myelofibrosis (4b).
ET: essential thrombocythemia; PMF: primary myelofibrosis.
WBC: white blood cell; Hb: hemoglobin: Hct: hematocrit; LDH: lactate dehydrogenase.
For continuous variables, data are shown as median values and brackets represent range. For categorical variables, right side of / represents the total number of patients studied and left side represents the number of positive patients, and brackets represent positive percentages.

Supporting Information Legends
Table S1. Clinical characteristics of *JAK2*V617F-mutated and triple-negative pre-PMF patients.
ET: essential thrombocythemia; pre-PMF: prefibrotic primary myelofibrosis. WBC: white blood cell; Hb: hemoglobin Hct: hematocrit; LDH: lactate dehydrogenase.
For continuous variables, data are shown as median values and brackets represent range. For categorical variables, right side of / represents the total number of patients studied and left side represents the number of positive patients, and brackets represent positive percentages.
*There was only one *CALR*-mutated pre-PMF patient, and thus was removed from analysis here.

Table S2. Clinical characteristics of *JAK2*V617F-mutated ET and *JAK2*V617F-mutated pre-PMF patients.
*JAK2*-ET: *JAK2*V617F-mutated essential thrombocythemia; *JAK2*-pre-PMF: *JAK2*V617F-mutated prefibrotic primary myelofibrosis. WBC: white blood cell; Hb: hemoglobin; Hct: hematocrit; LDH: lactate dehydrogenase.

For continuous variables, data are shown as median values and brackets represent range. For categorical variables, right side of / represents the total number of patients studied and left side represents the number of positive patients, and brackets represent positive percentage.

Table S1. Clinical characteristics of *JAK2*V617F-mutated and triple-negative pre-PMF patients.

| | pre-PMF (n=22)* | | p value |
|---|---|---|---|
| | | | *JAK2*V617F vs |
| mutation profiles | *JAK2*V617F | triple negative | triple negative |
| number of patients, n= | 18 (78.3%) | 4 (17.4%) | |
| male, n= | 9 (50.0%) | 2 (50.0%) | p=1 |
| age, years | 73 (31-94) | 71 (35-76) | p=0.639 |
| WBC, $\times 10^9$/L | 12.9 (3.5-27.7) | 14.4 (6.7-68.7) | p=0.774 |
| Hb, g/dL | 13.1 (7.5-15.8) | 11.3 (8.7-13.3) | p=0.195 |
| Hct, % | 42.2 (24.1-47.1) | 35.6 (26.9-40.2) | p=0.173 |
| Platelets, $\times 10^9$/L | 656 (102-1974) | 387 (135-1162) | p=0.443 |
| LDH, IU/L | 323 (176-997) | 258 (161-411) | p=0.275 |
| thrombotic events, n= | 5/16 (31.3%) | 1/4 (25.0%) | p=1 |
| splenomegaly, n= | 6/14 (42.9%) | 1/4 (25.0%) | p=1 |

Table S2. Clinical characteristics of *JAK2*V617F-mutated ET and *JAK2*V617F-mutated pre-PMF patients.

| Disease | *JAK2*-ET | *JAK2*-pre-PMF | p value |
|---|---|---|---|
| number of patients, n= | 127 | 18 | |
| male, n= | 58 (45.7%) | 9 (50.0%) | p=0.803 |
| age, years | 67 (19-87) | 73 (31-94) | p=0.109 |
| WBC, $\times 10^9$/L | 9.8 (1.4-30.9) | 12.9 (3.5-27.7) | p=0.200 |
| Hb, g/dL | 14.3 (9.0-18.4) | 13.1 (7.5-15.8) | **p=0.006** |
| Hct, % | 43.3 (26.7-54.8) | 42.2 (24.1-47.1) | **p=0.028** |
| Platelets, $\times 10^9$/L | 847 (458-2470) | 656 (102-1974) | **p=0.004** |
| LDH, IU/L | 253 (170-635) | 323 (176-997) | **p=0.043** |
| *JAK2*V617F allele burdens, % | 30.7 (5.2-100) | 38.0 (14.9-100) | **p=0.036** |
| thrombotic events, n= | 26/127 (20.5%) | 5/16 (31.3%) | p=0.340 |
| splenomegaly, n= | 23/119 (19.3%) | 6/14 (42.9%) | p=0.079 |

Table 1. Clinical characteristics of patients with MPNs diagnosed by the 2016 WHO criteria.

| | PV | ET | pre-PMF | overt PMF | sMF |
|---|---|---|---|---|---|
| number of patients, n= | 166 | 212 | 23 | 65 | 27 |
| male, n= | 82 (49.4%) | 96 (45.3%) | 11 (47.8%) | 38 (58.5%) | 15 (55.6%) |
| age, years | 63 (20-90) | 64 (15-87) | 72 (31-94) | 66 (21-88) | 67 (31-91) |
| laboratory data | | | | | |
| WBC, $\times 10^9$/L | 14.0 (4.5-45.2) | 9.3 (1.4-30.9) | 12.3 (3.5-68.7) | 7.3 (0.6-52.8) | 10.3 (1.7-61.8) |
| Hb, g/dL | 18.8 (15.8-24.3) | 13.9 (8.5-18.4) | 12.7 (7.5-15.8) | 9.9 (4.5-17.5) | 11.8 (5.0-20.5) |
| Hct, % | 57.6 (49.2-72.7) | 42.3 (26.7-54.8) | 40.2 (24.1-47.1) | 31.0 (14.1-58.4) | 35.7 (14.8-61.2) |
| Platelets, $\times 10^9$/L | 538 (126-1815) | 917 (458-3817) | 634 (102-1974) | 285 (6-2690) | 364 (51-1117) |
| LDH, IU/L | 303 (176-847) | 262 (162-686) | 310 (161-997) | 465 (220-3585) | 688 (222-1085) |
| mutation profiles | | | | | |
| *JAK2*, n= | 161 (97.0%) | 127 (59.9%) | 18 (78.3%) | 35 (53.8%) | 16 (59.3%) |
| *CALR*, n= | 0 (0%) | 57 (26.9%) | 1 (4.3%) | 18 (27.7%) | 9 (33.3%) |
| *MPL*, n= | 0 (0%) | 10 (4.7%) | 0 (0%) | 1 (1.5%) | 1 (3.7%) |
| triple negative, n= | 5 (3.0%) | 18 (8.5%) | 4 (17.4%) | 11 (16.9%) | 1 (3.7%) |

Table 2. Clinical characteristics of polycythemia vera patients with *JAK2*V617F or *JAK2* exon 12 mutations.

| | PV (n=166) | | | p value |
|---|---|---|---|---|
| | *JAK2*V617F | *JAK2* exon12 mutation | triple negative | V617F vs exon12 |
| number of patients, n= | 152 (91.6%) | 9 (5.4%) | 5 (3.0%) | |
| male, n= | 74 (48.7%) | 5 (55.6%) | 3 (60.0%) | p=0.743 |
| age, years | 64 (22-90) | 53 (20-74) | 47 (44-80) | **p=0.024** |
| WBC, $\times 10^9$/L | 14.2 (6.3-45.2) | 8.4 (4.5-25.1) | 10.4 (8.7-13.4) | **p=0.028** |
| RBC, $\times 10^{12}$/μL | 7.1 (5.2-9.3) | 7.6 (6.6-9.7) | 6.3 (5.7-6.6) | **p=0.019** |
| Hb, g/dL | 18.8 (15.8-24.3) | 18.7 (17.3-20.6) | 20.0 (18.2-20.6) | p=0.988 |
| Hct, % | 57.6 (49.2-72.7) | 58.2 (55.8-67.2) | 56.6 (53.0-61.3) | p=0.231 |
| MCV, fl | 82.6 (63.2-107.6) | 75.3 (69.3-85.0) | 92.0 (88.5-93.2) | **p=0.012** |
| Platelets, $\times 10^9$/L | 565 (126-1815) | 357 (259-703) | 212 (180-251) | **p=0.016** |
| Epo, mIU/mL | 7.2 (0.6-23.1) | 7.2 (1.4-10.2) | 8.2 (1.9-8.6) | p=0.832 |
| thrombotic events, n= | 34/145 (23.4%) | 5/9 (55.6%) | 1/5 (20.0%) | **p=0.046** |
| splenomegaly, n= | 65/146 (44.5%) | 2/6 (33.3%) | 0/5 (0%) | p=0.695 |
| bone marrow fibrosis, n= | 15/64 (23.4%) | 1/5 (20.0%) | 0/5 (0%) | p=1 |

Table 3. Clinical characteristics of patients with different mutational status in essential thrombocythemia (3a) and overt primary myelofibrosis (3b).

3a

| | ET（n=212） | | | | p value | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *JAK2*V617F vs | | | *CALR* vs | | *MPL* vs |
| mutation profiles | *JAK2*V617F | *CALR* | *MPL* | triple negative | *CALR* | *MPL* | triple negative | *MPL* | triple negative | triple negative |
| number of patients, n= | 127 (59.9%) | 57 (26.9%) | 10 (4.7%) | 18 (8.5%) | | | | | | |
| male, n= | 58 (45.7%) | 23 (40.4%) | 4 (40.0%) | 11 (61.1%) | p=0.525 | p=1 | p=0.313 | p=1 | p=0.175 | p=0.433 |
| age, years | 67 (19-87) | 60 (24-86) | 65 (45-81) | 64 (15-86) | **p=0.036** | p=0.966 | p=0.566 | p=0.351 | p=0.931 | p=0.737 |
| WBC, $\times 10^9$/L | 9.8 (1.4-30.9) | 8.0 (4.1-15.1) | 8.7 (4.9-17.1) | 7.8 (4.5-13.5) | **p<0.001** | p=0.059 | **p=0.009** | p=0.692 | p=0.637 | p=1 |
| Hb, g/dL | 14.3 (9.0-18.4) | 13.4 (9.7-16.8) | 12.9 (10.9-15.3) | 13.2 (8.5-16.1) | **p<0.001** | **p=0.016** | **p=0.003** | p=0.532 | p=0.399 | p=1 |
| Hct, % | 43.3 (26.7-54.8) | 40.0 (30.6-48.8) | 40.0 (36.4-45.0) | 40.1 (26.9-47.0) | **p<0.001** | **p=0.023** | **p=0.003** | p=0.860 | p=0.775 | p=0.905 |
| Platelets, $\times 10^9$/L | 847 (458-2470) | 1040 (464-3817) | 1296 (825-2053) | 851 (474-2244) | **p<0.001** | **p=0.002** | p=0.670 | p=0.256 | p=0.094 | p=0.051 |
| LDH, IU/L | 253 (170-635) | 298 (170-686) | 285 (214-398) | 253 (162-386) | p=0.401 | p=0.916 | p=0.290 | p=1 | p=0.148 | p=0.573 |
| thrombotic events, n= | 26/127 (20.5%) | 3/57 (5.3%) | 1/10 (10.0%) | 1/18 (5.6%) | **p=0.008** | p=0.686 | p=0.196 | p=0.485 | p=1 | p=1 |
| splenomegaly, n= | 23/119 (19.3%) | 5/50 (10.0%) | 2/10 (20.0%) | 2/15 (13.3%) | p=0.176 | p=1 | p=0.736 | p=0.330 | p=0.658 | p=1 |
| bone marrow fibrosis (MF-1), n= | 26/127 (20.5%) | 13/57 (22.8%) | 1/10 (10.0%) | 0/18 (0%) | p=0.702 | p=0.686 | **p=0.044** | p=0.675 | **p=0.030** | p=0.357 |

3b

| | overt PMF (n=64)* | | | p value | | |
|---|---|---|---|---|---|---|
| | | | | *JAK2*V617F vs | | *CALR* vs |
| mutation profiles | *JAK2*V617F | *CALR* | triple negative | *CALR* | triple negative | triple negative |
| number of patients, n= | 35 (54.7%) | 18 (28.1%) | 11 (17.2%) | | | |
| male, n= | 23 (65.7%) | 9 (50.0%) | 5 (45.5%) | p=0.375 | p=0.296 | p=1 |
| age, years | 71 (38-88) | 63 (45-81) | 51 (21-76) | p=0.263 | **p=0.017** | p=0.074 |
| WBC, $\times 10^9$/L | 9.7 (0.6-52.8) | 5.3 (2.0-36.1) | 5.8 (1.8-44.1) | p=0.097 | p=0.257 | p=0.906 |
| Hb, g/dL | 10.4 (5.6-17.5) | 9.5 (5.7-14.7) | 10.8 (4.5-15.7) | p=0.110 | p=0.615 | p=0.424 |
| Hct, % | 31.9 (16.9-58.4) | 30.3 (16.9-43.5) | 33.5 (14.1-46.3) | p=0.175 | p=0.487 | p=0.611 |
| Platelets, $\times 10^9$/L | 293 (6-2690) | 252 (34-1491) | 158 (29-855) | p=0.960 | p=0.150 | p=0.208 |
| LDH, IU/L | 465 (230-1789) | 610 (244-2741) | 276 (220-3585) | **p=0.036** | **p=0.006** | **p=0.007** |
| thrombotic events, n= | 2/34 (5.9%) | 1/17 (5.9%) | 0/11 (0%) | p=1 | p=1 | p=1 |
| splenomegaly, n= | 23/31 (74.2%) | 12/17 (70.6%) | 7/11 (63.6%) | p=1 | p=0.699 | p=1 |

Table 4. Clinical characteristics depending on *CALR* type 1-like or type 2-like mutations in patients with essential thrombocythemia (4a) and myelofibrosis (4b).

4a

| | ET (n=54) | | p value |
|---|---|---|---|
| mutation profiles | *CALR* type 1-like | *CALR* type 2-like | |
| number of patients, n= | 38 (70.4%) | 16 (29.6%) | |
| male, n= | 15 (39.5%) | 7 (43.8%) | p=0.772 |
| age, years | 59 (24-83) | 61 (34-86) | p=0.719 |
| WBC, $\times 10^9$/L | 8.0 (5.3-15.1) | 7.8 (4.1-12.7) | p=0.629 |
| Hb, g/dL | 13.4 (9.7-16.8) | 13.5 (11.0-15.8) | p=0.879 |
| Hct, % | 40.0 (30.6-48.8) | 40.0 (34.4-46.2) | p=0.977 |
| Platelets, $\times 10^9$/L | 1027 (464-3817) | 1191 (744-2832) | p=0.137 |
| LDH, IU/L | 315 (199-686) | 244 (170-442) | **p=0.041** |
| thrombotic events, n= | 1/38 (2.6%) | 2/16 (12.5%) | p=0.206 |
| splenomegaly, n= | 4/35 (11.4%) | 1/13 (7.7%) | p=1 |
| myelofibrosis, n= | 10/38 (26.3%) | 3/16 (18.8%) | p=0.732 |

4b

| | overt PMF+post ET myelofibrosis (n=27) | | p value |
|---|---|---|---|
| mutation profiles | *CALR* type 1-like | *CALR* type 2-like | |
| number of patients, n= | 22 (81.5%) | 5 (18.5%) | |
| male, n= | 10 (45.5%) | 3 (60.0%) | p=0.648 |
| age, years | 62 (31-81) | 67 (53-68) | p=0.948 |
| WBC, $\times 10^9$/L | 6.3 (2.0-36.1) | 4.7 (1.7-15.1) | p=0.345 |
| Hb, g/dL | 9.9 (5.7-14.7) | 9.8 (6.9-13.5) | p=1 |
| Hct, % | 31.5 (16.9-43.5) | 30.4 (21.0-39.7) | p=1 |
| Platelets, $\times 10^9$/L | 325 (34-1491) | 410 (51-789) | p=0.950 |
| LDH, IU/L | 668 (244-2741) | 527 (346-685) | p=0.241 |
| thrombotic events (%) | 2/21 (9.5%) | 0/5 (0%) | p=1 |
| splenomegaly (%) | 16/21 (76.2%) | 3/5 (60.0%) | p=0.588 |