Iron deficiency in JAK2 exon12 and JAK2-V617F mutated polycythemia vera

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Dear Editor,

Somatic driver mutations in JAK2 (JAK2V617F and exon 12 mutations) are detected >95% of persons with polycythemia vera (PV) [1–4]. Iron deficiency is universal in persons with PV at diagnosis and can be worsened by phlebotomy [5]. Precise mechanisms of iron deficiency in persons with PV at diagnosis are unknown. A previous study reported heterogeneous bone marrow expression of erythroferrone (ERFE) and hepcidin, important regulators of iron metabolism, in mice with JAK2E617F or JAK2exon12 mutation [6].

The relationship between iron deficiency and the type of JAK2 mutations in persons with PV is unknown. We studied this issue in 305 subjects who were >18 years old with newly diagnosed PV seen at Blood Diseases Hospital, Chinese Academy of Medical Sciences from June 1, 2007 to February 28, 2020. Diagnosis of PV was based on the 2016 World Health Organization (WHO) criteria [7]. All subjects provided informed consent in compliance with the Declaration of Helsinki.

The median age was 59 years (IQR, 49–66 years), 158 (52%) were men. 11 (5%) of 228 subjects had abnormal diagnosis cytogenetics. 293 (96%) had JAK2V617F and 12 (4%), a JAK2exon12 mutation. The median JAK2V617F variant allele frequency (VAF) was 54% (IQR, 33–73%). Subjects with a JAK2exon12 mutation had higher RBC concentrations (medians, 8.60 versus 7.11 × 1012/L; p < 0.001) and hematocrits (medians, 64.7% versus 59.7%; p = 0.002) compared with subjects with JAK2V617F but lower concentrations of WBC (medians, 8.75 versus 12.95 × 109/L; p = 0.005), platelet (medians, 273 versus 474 × 109/L; p = 0.011) and serum erythropoietin (EPO) (medians, 0.68 versus 1.16 mIU/L; p = 0.005), which were consistent with previous studies [8–10]. There was no significant difference in hemoglobin concentration (medians, 194 g/L versus 194 g/L; p = 0.616).

Subjects with transferrin saturation (TSAT) <20% or ≥20% were defined as iron-deficient and iron-sufficient, respectively [11]. 159 (52%) were iron deficient at diagnosis. Detail clinical and laboratory co-variables of subjects with iron deficiency are displayed in Table 1. Subjects with iron deficiency had higher concentrations of RBCs (medians, 7.51 versus 6.67 × 1012/L; p < 0.001) and hematocrits (medians, 60.9% versus 58.9%; p = 0.003) and lower serum EPO concentrations (medians, 1.07 versus 1.35 mIU/mL; p = 0.021) compared with subjects, not iron deficient. There were no significant differences in diagnosis hemoglobin, WBC or platelet concentrations (p > 0.05; Table 1).

The severity of iron deficiency differed based on JAK2 mutation types. Subjects with a JAK2exon12 mutation were more likely to be iron deficient (92% versus 51%; p = 0.006) and had lower serum iron (medians, 5.1 versus 11.9 μmol/L; p = 0.002) and ferritin concentrations (medians, 13.9 versus 32.2 ng/mL; p = 0.004) compared with subjects with JAK2V617F (Fig. 1A). Declined mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were significantly more frequent in subjects with JAK2exon12 mutation (p < 0.05), consistent with their more severe iron deficiency (Fig. 1B). JAK2exon12 mutation was an independent factor associated with iron deficiency by multivariable analysis that adjusted by age and sex (HR = 11.185, 95% confidence interval [CI] 1.404–89.089, p = 0.023; Supplementary Table 1).

The severity of iron deficiency is also correlated with the JAK2V617F allele burden. Subjects with JAK2V617F VAFs ≥50% were more likely to be iron deficient (61% versus 44%, p = 0.013) and had lower serum iron (medians, 10.4 versus 13.7 μmol/L; p = 0.002) compared with subjects with JAK2V617F VAFs <50% (Fig. 1C). JAK2V617F VAF was weakly negatively correlated with iron deficiency (Fig. 1E, F). JAK2V617F VAF was significantly higher in iron-deficient subjects (medians, 61% versus 47%; p = 0.003; Table 1). Consistently, declined MCVs, MCHs, and MCHCs were more frequent in subjects with JAK2V617F VAFs ≥50% (Fig. 1D). JAK2V617F VAF >50% was an independent factor associated with iron deficiency by multivariable analysis that adjusted by age and sex (HR = 2.022, 95% CI 1.186–3.447, p = 0.010; Supplementary Table 2).

Before the publication of the 2016 WHO diagnostic criteria of PV, there were people defined as masked PV with JAK2V617F or JAK2exon12 mutations and with bone marrow histological features of PV, but not meeting hemoglobin concentration or hematocrit threshold defined in the World Health Organization (WHO) or British Criteria for Standards in Hematology (BCSH) PV diagnostic criteria [12, 13]. These low values were likely the result of iron deficiency [12, 13]. As discussed above, hematocrits were higher in subjects with iron deficiency compared with those without iron deficiency inconsistent with their comparable hemoglobin concentrations. Consequently, we compared the diagnostic accuracy of these co-variates according to 2016 WHO diagnostic criteria in subjects with and without iron deficiency stratified for sex [7]. We stratified subjects by sex because females are more often iron deficient because of menstruation. In the iron-deficient cohort, all subjects met the hematocrit thresholds for PV as defined in the 2016 WHO diagnostic criteria [7], but there were 7% of subjects not meeting the threshold of hemoglobin concentration for both sexes (Supplementary Fig. 1A, D). These data indicate hematocrit is more sensitive than hemoglobin concentration as an indicator of PV in persons who are iron deficient (sensitivities, 100% [86/86] versus 93% [80/86], p = 0.013 for females; 100% [73/73] versus 93% [68/73], p = 0.023 for males; Supplementary Fig. 1C, F). But in subjects without iron deficiency, there were comparable percentages of subjects not meeting the diagnostic threshold of HCT or HB (Supplementary Fig. 1B, E), and the diagnostic sensitivities were not.

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significantly different between HCT and HB (97% [59/61] versus 98% [60/61], p = 0.559 for females; 94% [80/85] versus 97% [82/85], p = 0.469 for males; Supplementary Fig. 1C, D).

We also found the diagnostic sensitivity of hematocrit was superior to hemoglobin concentration in males with a JAK2 V617F VAF ≥ 50% (98% [66/67] versus 91% [61/67], p = 0.052; Supplement Fig. 2F).

44 (14%) subjects lost follow-up. Among the remaining patients, the median follow-up for subjects with or without iron deficiency was 35 months (IQR, 15–68 months) and 36 months (IQR, 20–69), respectively. The 5-year accumulative incidence of death and thrombotic events were not significantly different between subjects with or without iron deficiency (4% versus 11%, p = 0.233; 3% versus 6%, p = 0.389; Supplementary Fig. 3).

Our study has limitations. For example, this is a retrospective study from our single-center, and data of iron metabolism were available in part of newly diagnosed subjects in our center.

In summary, as far as we know, this is the first report of the relationship between iron deficiency and type of JAK2 mutations in patients with PV in the English literature until now, our data showed that iron deficiency is more common in subjects with PV and JAK2 exon12 mutation compared with those with JAK2 V617F, and JAK2 V617F allele burden correlates with the probability of iron deficiency. Also, hematocrit was more sensitive than hemoglobin concentration as a basis to diagnosis PV in persons with iron deficiency. Regardless of these data, the mechanism(s) by which JAK2 mutations affect iron metabolism needs further study.

### Table 1. Co-variates of PV patients with and without TSAT < 20% at diagnosis.

| Variables                  | TSAT < 20% (n = 159) | TSAT ≥ 20% (n = 146) | p    |
|----------------------------|----------------------|----------------------|------|
| Female, n (%)              | 86 (54%)             | 61 (42%)             | 0.032|
| Age, n (%)                 | 60 (24–86)           | 58 (27–84)           | 0.217|
| RBC, ×10¹²/L; median (range)| 7.51 (5.48–10.94)    | 6.67 (4.65–9.39)     | <0.001|
| Hemoglobin, g/L; median (range) | 193 (150–241)       | 195 (157–245)       | 0.855|
| Hematocrit, %; median (range) | 60.9 (49.3–79.0)    | 58.9 (47.2–74.3)    | 0.003|
| WBC, ×10⁹/L; median (range) | 13.95 (3.97–45.59)   | 12.49 (3.75–34.28)  | 0.350|
| Platelets, ×10⁹/L; median (range) | 458 (127–1866)      | 506 (65–1609)       | 0.134|
| MCV, fL; median (range)    | 80.4 (61.4–100.4)    | 87.5 (75.7–106.2)   | <0.001|
| MCH, pg; median (range)    | 25.7 (16.0–34.0)     | 29.0 (23.3–36.8)    | <0.001|
| MCHC, g/L; median (range)  | 318 (248–354)        | 331 (299–365)       | <0.001|
| JAK2 V617F VAF, %; median (range) (N = 229)* | 61 (6–92)          | 47 (5–91)           | 0.003|
| JAK2 exon12 mutation, n (%) | 11 (7%)              | 1 (1%)              | 0.006|
| EPO, mIU/mL; median (range) (N = 194) | 1.07 (0.08–5.02)    | 1.35 (0.40–7.21)    | 0.021|
| Abnormal cytogenetics, n (%) (N = 228) | 5/122 (4%)          | 6/106 (6%)          | 0.759|

PV polycythemia vera, TSAT transferrin saturation, RBC red blood cell, MCV mean corpuscular volume, MCH mean corpuscular hemoglobin, MCHC mean corpuscular hemoglobin concentration, VAF variant allele frequency, EPO erythropoietin, UIBC unsaturated iron-binding capacity, TIBC total iron-binding capacity.

*In JAK2 V617F mutated patients.
The relationship between iron deficiency and JAK2 mutations in PV patients. A Serum iron, TSAT, and ferritin were significantly lower in JAK2exon12 mutated patients than in JAK2V617F mutated patients. B Declined MCV, MCH, MCHC was more frequent in JAK2exon12 mutated patients compared with JAK2V617F mutated patients. C Serum iron, TSAT were significantly lower in patients with low JAK2V617F VAF (<50%) than in patients with high JAK2V617F VAF (≥50%) among JAK2V617F mutated patients, but the ferritins were comparable between two these two cohorts. D Declined MCV, MCH, MCHC was more frequent in subjects with high JAK2V617F VAFs compared with low JAK2V617F VAFs. E and F JAK2V617F VAF negatively correlated with serum iron and TAST in JAK2V617F mutated patients. PV polycythemia vera, TSAT transferrin saturation, MCV mean corpuscular volume, MCH mean corpuscular hemoglobin, MCHC mean corpuscular hemoglobin concentration, VAF variant allele frequency. *p < 0.05; **p < 0.01; ***p < 0.001.
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AUTHOR CONTRIBUTIONS

ZJX designed the study. DL, ZFX collected and interpreted the data and performed the statistical analysis. PHZ and Q.S analyzed the bone marrow histology. TJQ, SQQ, LJP, WYC, JQL, HJW, XJS, MJ, QYG recruited subjects and collected the data. DL prepared the typescript with contributions from ZJX, ZFX, BL, GH, RPG, ZXS, and HJH. All authors reviewed the typescript, approved this version, and agreed to submit it for publication.

COMPETING INTERESTS

RPG is a consultant to BeGene Ltd., Fusion Pharma LLC, LaJolla NanoMedical Inc., Mingsight Pharmaceuticals Inc., and CStone Pharmaceuticals; advisor to Antegene Biotech LLC, Medical Director, FFF Enterprises Inc.; partner, AZAC Inc.; Board of Directors, Russian Foundation for Cancer Research Support; and Scientific Advisory Board: StemRad Ltd. There was no competing interest of other authors.

ADDITIONAL INFORMATION

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Correspondence and requests for materials should be addressed to Zhijian Xiao.

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