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Original Article

Comparability of JAK2 p.V617F allele burden in peripheral blood and that in bone marrow in patients with suspected myeloproliferative neoplasms: a single-center prospective study

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Abstract: Purpose: To detect JAK2 p.V617F and measure allele burden in peripheral blood (PB) and bone marrow (BM) aspirates in patients with suspected myeloproliferative neoplasms (MPNs). Methods: Patients with suspected MPNs were prospectively enrolled between August 2017 and May 2019, and their PB and BM were collected during the same period. Quantitative fluorescence polymerase chain reaction (PCR) was used to detect the copy number of JAK2 wild type and the V617F mutant; the JAK2 V617F proportion was also calculated. The JAK2 p.V617F proportion in PB was compared to that in BM by Chi-square test. Results: Among 54 patients with suspected MPNs, 43 of them were eligible for analysis. The JAK2 p.V617F in PB had the same sensitivity and specificity as BM (all P>0.05). The Chi-square test suggested that the JAK2 p.V617F allele burden of PB was comparable to that of BM (Spearman Correlation =0.986; P=0.000). Conclusion: PB could be used as an alternative to BM for JAK2 p.V617F measurement in patients with suspected MPNs.

Keywords: Myeloproliferative neoplasms, JAK2 p.V617F allele, peripheral blood, bone marrow

Introduction

Myeloproliferative neoplasms (MPNs) are a group of malignant clonal diseases characterized by the proliferation of one or more lineages of myeloid cells in the bone marrow. The classic BCR/ABL-negative MPNs include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). Several studies indicated that JAK2V617F mutation has an important role in the development of MPNs in 2005 [1-3]. Also, subsequent studies have confirmed that the JAK2 V617F mutation is one of the most common mutation genes in MPNs. JAK2V617F mutation was detected in about 95% of PV patients, 50% of ET patients, and 50% of PMF patients [4-8], while it was not detected in the normal population and patients with secondary erythrocytosis. Therefore, JAK2 V617F mutation is one of the main diagnostic indicators for MPNs.

Bone marrow sampling is commonly used to detect gene mutation. Yet, it remains unclear whether peripheral blood (PB) could be used as an alternative sample to detect the mutation. Previously, two studies reported that the JAK2 V617F allele burden was equivalent in PB and BM [9, 10]. However, none of these studies were prospective. Here we evaluated the comparability of the JAK2 p.V617F allele burden in PB and BM in patients with suspected MPNs.

Materials and methods

Patients

Fifty-four patients suspected of MPNs according to clinical manifestations and blood routines in Taizhou Central Hospital were enrolled. All were of Han ethnicity and living in Taizhou city. All patients signed the informed consent. The present study was approved by the Ethics

Table 1. The sequence of JAK2 primers

Sequence of JAK2 primers
Forward primer 1 (F1, wild-type): 5'-ATCTATAGTCATGCTGAAAGTAGGAGAAAG-3'
Forward primer 2 (F2, mutated): 5'-AGCATTTGGTTTTAAATTATGGAGTATGTT-3'
Reverse primer (R): 5'-CTGAATAGTCCTACAGTGTTTTCAGTTTCA-3'

Committee of Taizhou Central Hospital (Taizhou University Hospital) and was conducted in accordance with the Declaration of Helsinki.

Sample collection

A total of 4 ml of BM and 4 ml of PB were collected from each patient with suspected MPNs, after which an ethylenediamine tetraacetic acid (EDTA) anticoagulation was performed.

Key reagents and instruments

The QIAamp DNA Blood Mini Kits were purchased from Guangzhou Jianlun Biological Technology Co., Ltd. The SYBR Green PCR Kits were acquired from Beijing Bulader Technology Development Co., Ltd. The fusion gene kits were obtained from Shanghai Yuanqi Bio-Pharmaceutical Co., Ltd. The primers and probes were bought from Shanghai Generay Biotech Co., Ltd., and the Platinum SuperMix-UDG was purchased from Invitrogen, USA.

The spectrophotometer was purchased from Beijing Cossim Scientific Instrument Co., Ltd. The gene sequencer was obtained from Guangzhou KingCreate Biotechnology Co., Ltd. The PCR machine and the ABI 7500 real-time fluorescence-based quantitative PCR machine were purchased from Applied Biosystems, USA.

Genomic DNA extraction

A total of 4 ml of BM and 4 ml of PB were collected from each patient and placed into two EDTA anticoagulant tubes. Red blood cells were lysed with erythrocyte lysate in order to collect white blood cells in BM or PB. The QIAamp DNA Blood Mini Kit was used to extract genomic DNA, and the spectrophotometer was used to measure the concentration and purity of the DNA. After that, the DNA concentration was adjusted to 50 ng/μl and kept in a -80°C refrigerator for later use.

Screening of JAK2V617F mutation

The PCR machine was used to detect JAK2V617F mutation with two forward primers and one backward primer (**Table 1**). Briefly, 1 μL of the sample DNA was added to a 25 μo reaction mixture containing 1.2 μL of F1, 1.3 μL of F2, 2.5 μL of IR, 12.5 μL of SYBR PCR Master Mix, and water. Also, the mixture reacted at 94°C for 11 minutes for pre-degeneration. After that, the mixture was degenerated at 94°C for 30 seconds, annealed at 55°C for 30 seconds, and maintained at 72°C for 30 seconds. After 36 cycles, the mixture reacted at 72°C for 6 minutes and maintained at 10°C. The amplification products were separated by electrophoresis with 2% agarose gel, sequenced by the BigDye Terminator, and subsequently analyzed in the gene sequencer.

Determination of JAK2 V617F allele burden

The ABI 7500 real-time fluorescence-based quantitative PCR machine was used to detect the positive samples. The amplification primers and probes are shown in **Table 2**. Amplification conditions were the following: 12.5 μL of Platinum SuperMix-UDG, 0.1 μL of ROX, 0.5 μL of the 10 μM forward primer, 0.5 μL of the 10 μM reverse primer, 0.3 μL of the 10 μM probe T, 0.3 μL of the 10 μM probe G and about 100 ng of the DNA template, deionized water that was added until the final volume of 25 μL. The amplification was performed 40 times according to the standard procedure recommended by the manufacturer (at 50°C for 2 minutes, at 95°C for 10 minutes, at 95°C for 15 seconds, at 55°C for 20 seconds, and at 72°C for 1 minute). Each sample was treated 3 times. The cycle threshold (CT) was used to calculate the JAK2 V617F proportion. The ΔCT value of each sample was defined as the difference in CT between the two probes. The ratio of mutated JAK2 V617F and wild-type JAK2 V617F was $2^{-\Delta CT}$. The proportion of mutated JAK2V617F was $2^{-\Delta CT}/(1+2^{-\Delta CT})$.

Statistical analyses

The SPSS 25.0 software was used for all statistical analyses. Independent-samples t-test was used to analyze the JAK2 p.V617F allele bur-

Table 2. Amplification primers and probes of JAK2

Amplification primers and probes of JAK2
Forward primer: 5'-AGCTTTCTCACAAGCATTTGGTT-3'
Reverse primer: 5'-CAAAAACAGATGCTCTGAGAAAGG-3'
TaqMan MGB probe T (for the mutated): 5'-VIC-AATTATGGAGTATGTTTCTGTGGA-3'MGBNFQ
TaqMan MGB probe G (for the wild-type): 5'-FAM-TAAATTATGGAGTATGTGTCTGT-3'MGBNFQ

Table 3. Clinical characteristics of the 27 patients with JAK2 p.V617F mutation in BM

	number of cases	mean JAK2 p.V617F allele burden (%)	*p*
Age (years)			
≤60	n=10	32.56±28.62	0.124
>60	n=17	48.63±23.26	
Gender			
Male	n=15	52.36±28.98	0.021*
Female	n=12	30.58±15.78	
White Blood Cell (×10^9/L)			
>10	n=16	49.23±25.14	0.117
≤10	n=11	33.16±25.49	
Hemoglobin (g/L)			
>150	n=18	49.91±26.79	0.039*
≤150	n=9	28.23±18.16	
Platelet (×10^9/L)			
>300	n=24	40.64±26.06	0.258
≤300	n=3	58.99±23.84	

Note: *denotes $P<0.05$. Bone Marrow (BM).

den. The Chi-square test was used to analyze the correlation between JAK2 p.V617F in PB and that in BM. A two-tailed P<0.05 was considered statistically significant.

Results

Patients

Forty-three patients with suspected MPNs were eligible for analysis. Twenty-eight acquired JAK2 p.V617F mutation but did not have JAK2 exon 12 mutation. There were 25 males with a median age of 57.0 years (range: 16-82) and 18 females with a median age of 60.1 years (range: 29-86).

Clinical characteristics of MPN patients with JAK2 p.V617F mutation

Of the 28 patients with positive JAK2 p.V617F mutation, 1 was diagnosed with MPN/MDS syndrome; the other 27 were MPNs, including 15 males with a median age of 63.33 years (range: 37-82) and 12 females with a median age of 62.58 years (range: 43-85). Male patients had a higher allele burden of JAK2 p.V617F (P=0.021), as well as patients with high hemoglobin (HGB >150 g/L) (P=0.039). There was no significant difference in JAK2 p.V617F allele burden between patients of different ages, white blood cell counts, and platelet counts (**Table 3**).

Correlation between JAK2 p.V617F in PB and JAK2 p.V617F in BM in patients with MPNs

JAK2 p.V617F mutation was confirmed in all 28 patients after analyzing both PB and BM samples. In all MPNs patients with the mutation, the median allele burden in PB and BM was 42.24% (range: 4.95-92.50) and 43.01% (range: 7.72-91.74), respectively. The Chi-square test suggested a strong correlation between the allele burden of JAK2 p.V617F detected in PB and BM (Spearman Correlation =0.986; P=0.000).

Discussion

With the wide application of next-generation sequencing over recent years, many MPNs-related genes have been identified, thus furthering the understanding of the pathogenesis of the disease. Previous studies have confirmed that PB could be used as an alternative to BM for purposes of diagnosis if the blast cell percentage in PB exceeds 30% [11]. Due to the high sensitivity and specificity of quantitative fluorescence, PCR has been widely used for diagnosing, managing, and minimal residual disease monitoring in patients with hematological malignancies [12]. Studies on the consistency of gene mutation in PB and BM are main-

ly reported in the field of de novo acute myeloid leukemia. Also, several studies have shown that the allele burden in PB is strongly correlated with that in BM in patients with acute myeloid leukemia [13, 14]. Lee *et al.* [15] quantitatively detected Ph-positive cells in PB and BM in 75 patients with chronic myelogenous leukemia who underwent stem cell transplantation and found a strong correlation between the two samples. A recent study on MPNs patients also suggested a high consistency of JAK2 p.V617F in PB and BM. In the retrospective analysis carried out by Takahashi *et al.* [10], 388 MPNs patients were detected with JAK2 p.V617F allele burden in BM and PB. Also, the median allele burden of JAK2 p.V617F in PB and BM was 52.7% (range: 3.3-100) and 51.4% (range: 3.1-98.7), respectively, which suggested that the JAK2 p.V617F allele burden measured in PB and BM was equivalent ($R^2=0.991$, $P<0.0001$). Our prospective study confirmed that in MPNs patients, the allele burden of JAK2 V617F in PB was highly correlated with that in BM, irrespective of ethnicity.

We have shown that male patients had a higher allele burden of JAK2 V617F ($P=0.021$), which was consistent with the previous reports [16-19]. Karantanos *et al.* [16] found that the male gender was an independent predictor for adverse outcomes in MPNs. Their study showed that male patients had a higher allele burden of JAK2 V617F in CD34+ cells ($P=0.001$) and were more likely to acquire other gene mutations of higher allele burden, such as additional sex combs-like 1 (ASXL1), Enhancer of zeste homolog 2 (EZH2), Serine/arginine-rich splicing factor 2(SRSF2), nuclear RNA auxiliary factor 1, and Isocitrate dehydrogenase 1 and 2(IDH1/2) mutations. Stein *et al.* [18] found that female MPNs patients, particularly female patients with PV, had a lower allele burden than male patients. Also, male MPNs patients had a higher incidence of homozygous JAK2 V617F mutation than female patients. Consequently, it was hypothesized that the low allele burden of JAK2 V617F might contribute to the low frequency of mitotic recombination events in female patients.

Our data suggest that gender may affect the outcome of MPNs. However, the mechanism through which gender affects the phenotype of MPNs remains unclear, as there are few studies on the correlation between gender and the allele burden of JAK2 V617F. Whether gender should be adapted in risk stratification remains to be determined.

Anemia is one of the frequent treatment-related AEs of ruxolitinib. Shi *et al.* [20] found that JAK2 V617F stimulates the proliferation of erythroid progenitors and blocks their differentiation by activating Stat1 and other non-erythroid signals. The RESPONSE study indicated that ruxolitinib, a JAK2 inhibitor, reduces the JAK2 p.V617F allele burden in patients with polycythemia vera [21]. A study of ruxolitinib in the treatment of PMF showed that mean hemoglobin levels in the ruxolitinib group declined from the baseline level of 109.3 g per liter to a nadir of 94.1 g per liter. Also, the percentage of patients who required transfusions of packed red cells was higher among those who started ruxolitinib at a dose of 20 mg twice daily compared to those who started at 15 mg twice daily (58% vs. 41%) [22]. This might explain why the high hemoglobin group had a higher JAK2 V617F allele burden in the present study.

In summary, our findings revealed that the JAK2 V617F mutation in PB had comparable sensitivity and specificity to that in BM in patients with newly diagnosed MPNs. Furthermore, the allele burden of JAK2 p.V617F in PB and BM was equivalent. Hence, PB could be a rational alternative to BM regarding JAK2 V617F measurement in MPNs. Particularly, it is much more feasible to follow up patients with MPNs. It should be noted that this is a small-scale study, and 59.6% (16/27) of the patients in the study had a white blood cell count over 10×10^9/L. Future prospective and large-scale studies are needed.

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Disclosure of conflict of interest

None.

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