JAK2 p.V617F detection and allele burden measurement in peripheral blood and bone marrow aspirates in patients with myeloproliferative neoplasms

Short Title: JAK2 p.V617F in peripheral blood and bone marrow

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Key Points

1. The sensitivity and specificity of detecting the JAK2 p.V617F mutation in peripheral blood are both 100% compared to bone marrow.

2. The JAK2 p.V617F allele burden measured in peripheral blood is equivalent to that in bone marrow aspirate (R²=0.991; P<0.0001).

Abstract

Detection of the JAK2 p.V617F mutation and measurement of its allele burden can be performed using both peripheral blood (PB) and bone marrow (BM) samples from patients with myeloproliferative neoplasms (MPNs). However, the diagnostic accuracy of detecting the JAK2 p.V617F mutation and quantifying its allele burden in PB and BM samples have not been systematically compared. We retrospectively analyzed 388 patients with MPN who had been tested for JAK2 p.V617F allele burden using both PB and BM samples within 3 months of each other. The sensitivity and specificity of detecting JAK2 p.V617F in PB when compared with BM were both 100%. Furthermore, the JAK2 p.V617F allele burden measured in PB and BM were equivalent by linear regression analysis (R²=0.991; P<0.0001). We therefore conclude that PB is a reliable source for testing for the JAK2 p.V617F mutation and quantifying its allele burden in patients with MPN.
Introduction

Somatic mutation of the JAK2 gene (JAK2 p.V617F) can be detected in a variable proportion of patients with myeloproliferative neoplasms (MPNs).1 The JAK2 p.V617F mutation is observed in up to 96% of patients with polycythemia vera (PV) and approximately 55-65% of those with essential thrombocythemia (ET) and primary myelofibrosis (PMF), and testing for its presence is an integral part of the diagnostic workup.2 In addition, quantitative measurement of the JAK2 p.V617F allele burden (ratio of mutant allele to total allele) has been associated with certain clinical phenotypes, such as higher incidence of pruritus, splenomegaly, and increased risk of thrombosis in patients with PV and ET, although the exact association between allele burden and long-term outcome remains controversial, particularly in patients with MF.3-6 DNA extracted from peripheral blood (PB) granulocytes or bone marrow (BM) aspirates is most commonly used for testing for JAK2 p.V617F. In this study, we sought to answer two questions: 1. whether the sensitivity and specificity of detecting JAK2 p.V617F in PB and BM are equivalent, and 2. whether the JAK2 p.V617F allele burden measured in PB and BM are equivalent. Previous studies suggested the feasibility of detecting JAK2 p.V617F in both PB and BM samples.4,7-9 Furthermore, quantification of JAK2 p.V617F allele burden in PB and BM samples appeared to be equivalent in a small number of MPN cases.8,10 However, to date no systematic large-scale analysis comparing qualitative (sensitivity and specificity) and quantitative (allele burden) JAK2 p.V617F testing in PB and BM samples has been conducted for patients with MPN.
Patients and Methods

By retrospective chart review, we identified 388 patients with a diagnosis of MPN (primary and secondary MF [N=329] as well as ET and PV [N=59]) who were referred to The University of Texas MD Anderson Cancer Center between January 2004 and July 2012 and were tested for the JAK2 p.V617F mutation using samples obtained from PB and BM during the same time period. For the purpose of this study, the sample analysis was considered to be conducted in the same time period if the analyses of PB and BM samples were conducted within 3 months of each other. Diagnosis of MF, ET and PV strictly followed the criteria established by the World Health Organization (WHO) in 2008. The study was based on a chart review protocol approved by the Institutional Review Board at MD Anderson Cancer Center and was conducted in accordance with the Declaration of Helsinki.

Genomic DNA was extracted from freshly obtained PB or BM aspirate samples using a semi-automated DNA-extraction method following the manufacturer’s instructions (Gentra Autopure; Qiagen, Valencia, CA). The JAK2 p.V617F mutation was detected using PCR-based pyrosequencing, as previously described. The sensitivity of detecting the JAK2 p.V617F mutation by this assay was 5%. Correlation between the JAK2 p.V617F allele burden measured in PB and BM was evaluated by scatter plot. Linear regression was conducted to calculate coefficient of determination ($R^2$) as well as the statistical significance of the correlation. SPSS Statistics, version 21 (IBM Corp, Armonk, NY) was used for all statistical analyses.
Results and Discussion

Among the 388 patients included in our analysis, 243 (63%) had a diagnosis of PMF, 48 (12%) had MF secondary to PV (post-PV MF), 38 (10%) had MF secondary to ET (post-ET MF), 32 (8%) had ET, and 27 (7%) had PV. Median time from diagnosis of MPN to JAK2 p.V617F testing was 5.9 months (range: 0-382 months) (Table 1).

All patients who were negative for JAK2 p.V617F in PB samples were also negative in BM samples and vice versa, making the sensitivity and specificity of JAK2 p.V617F mutation detection in PB samples both 100% in the current MPN cohort (using BM testing as a reference).

Among patients who tested positive for JAK2 p.V617F, the median JAK2 p.V617F allele burden in PB and BM were 52.7% (range; 3.3-100) and 51.4% (range; 3.1-98.7), respectively, in all MPN patients (Table 1). Patients with post-PV MF had the highest JAK2 p.V617F allele burden (median 92.6% in PB and 91.5% in BM), while patients with primary ET had the lowest (median 18.3% in PB and 18.9% in BM). The correlation between the JAK2 p.V617F allele burden measured in PB and BM samples in all MPN patients was very strong (R²=0.991; P<0.0001, Figure 1A). The correlation remained strong for each MPN subtype (Figures 1B-D).

In summary, our data from a large cohort of patients with MPN (MF, ET and PV) confirmed the consistency of detecting JAK2 p.V617F between PB and BM samples by the method used in our study. Moreover, our data confirmed that the JAK2 p.V617F allele burden measured in PB is equivalent to that measured in BM, which is in agreement with results from earlier published studies.8,10 These findings are valuable
from a clinical perspective. Our results show that BM testing is not required to detect 
*JAK2* p.V617F mutation or quantify *JAK2* p.V617F allele burden because PB testing is 
highly reliable. Hence, a negative result of the *JAK2* p.V617F mutation in a PB sample 
can be considered to be reliable, and obtaining a BM sample for further testing does not 
seem to be necessary. In the other words, our results dispel a notion that one needs to 
obtain a bone marrow aspirate and biopsy to be able to verify the presence or absence 
of the *JAK2* p.V617F mutation. Further, our results strongly suggest that heterogeneity 
of sampling (PB vs. BM) can be essentially ignored when interpreting clinical correlation 
studies of *JAK2* p.V617F allele burden. One could comparably compare levels done 
from the BM with the PB and be able to use the values interchangeably for those in 
which initial values were obtained from the BM. This makes it much more feasible for 
the long-term monitoring of *JAK2* p.V617F allele burden in clinical trials with new agents, 
as well as in a setting of post hematopoietic stem cell transplant, as *JAK2* p.V617F 
allele burden seems to function as minimal residual disease marker. It should be 
noted, however, that the sensitivity of the assay used in the current study is 5%. 
Because the presence of a very low level of residual *JAK2* p.V617F allele burden can 
be clinically significant in a post-transplant setting, a future study to confirm the 
qualitative and quantitative accuracy between PB and BM samples using more sensitive 
assays is needed.
Acknowledgements

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Authorship Contributions

K.T. designed the study, collected and analyzed the data and wrote the manuscript. H.K. and J.C. treated the patients and reviewed the manuscript. K.P.P. and R.L. conducted molecular analysis and reviewed the manuscript. S.P. collected the data and reviewed the manuscript. S.V. designed the study concept, guided the project, and wrote the manuscript.

Conflict of Interest Disclosure

The authors declare no conflict of interest.
References


Table 1. Summary of JAK2 p.V617F testing in patients with MPN

<table>
<thead>
<tr>
<th></th>
<th>N (% total)</th>
<th>JAK2 p.V617F - positive N (% each diagnosis)</th>
<th>JAK2 p.V617F allele burden*</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PB (N)</td>
<td>BM (N)</td>
</tr>
<tr>
<td>All patients</td>
<td>388</td>
<td>264 (68)</td>
<td>264 (68)</td>
</tr>
<tr>
<td>PMF</td>
<td>243 (63)</td>
<td>156 (64)</td>
<td>156 (64)</td>
</tr>
<tr>
<td>Post-ET MF</td>
<td>38 (10)</td>
<td>13 (34)</td>
<td>13 (34)</td>
</tr>
<tr>
<td>Post-PV MF</td>
<td>48 (12)</td>
<td>48 (100)</td>
<td>48 (100)</td>
</tr>
<tr>
<td>Primary ET</td>
<td>32 (8)</td>
<td>22 (69)</td>
<td>22 (69)</td>
</tr>
<tr>
<td>Primary PV</td>
<td>27 (7)</td>
<td>25 (93)</td>
<td>25 (93)</td>
</tr>
</tbody>
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Abbreviations: PMF, primary myelofibrosis; PV, polycythemia vera; MF, myelofibrosis; ET, essential thrombocythemia; PB, peripheral blood; BM, bone marrow.

*Median and range are shown.
**Figure Legend:**

**Figure 1.** Correlation between JAK2 p.V617F allele burden measured in peripheral blood granulocytes and bone marrow aspirates from (A) all MPN patients (N = 388), (B) MF patients (N = 329), (C) ET patients (N = 32), and (D) PV patients (N = 27).

Coefficient of determination ($R^2$) was calculated by linear regression.
Figure 1.

A.
D.

![Graph showing the relationship between JAK2 p. V617F allele burden in bone marrow and peripheral blood. The graph includes a linear regression line with an $R^2$ value of 0.984.](image-url)
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