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Janus Kinase 2 V617F Allele Burden and its Correlation with Thrombotic Risk in Myeloproliferative Neoplasms: A Prospective Observational Study

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Abstract

Background: Janus Kinase 2 (JAK2) V617F mutation is a key molecular marker in Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs), but the clinical relevance of allele burden in thrombotic risk stratification remains under evaluation. **Methods:** This prospective observational study included 120 patients with MPNs at a tertiary hospital from June 2025 to May 2026. JAK2 V617F allele burden was assessed and categorized as negative, low (<25%), intermediate (25–50%), and high (>50%). Associations with clinical, hematological, and thrombotic outcomes were analyzed. **Results:** JAK2 V617F mutation was detected in 94 patients (78.3%). Median allele burden was highest in polycythemia vera (59.0%), followed by primary myelofibrosis (40.1%) and essential thrombocythemia (18.1%) ($P < 0.001$). High allele burden was present in 42 patients (35.0%) and was associated with leukocytosis, splenomegaly, high thrombotic risk category, prior thrombosis, venous thrombosis, and overall thrombotic events. Overall thrombotic events increased across allele burden categories and were highest in the high allele burden group (59.5%). Allele burden correlated positively with hemoglobin, hematocrit, leukocyte count, lactate dehydrogenase, and thrombotic risk score and inversely with platelet count. On multivariable analysis, allele burden remained independently associated with high thrombotic risk category (adjusted odds ratio [OR] 1.38/10% increase) and overall thrombotic events (adjusted OR 1.34/10% increase). **Conclusion:** Higher JAK2 V617F allele burden was associated with proliferative disease features and increased thrombotic risk, particularly venous and overall thrombotic events, supporting its role as an adjunctive biomarker in MPN risk stratification.

Keywords: Essential thrombocythemia, Janus Kinase 22 V617F allele burden, Myeloproliferative neoplasms, Polycythemia vera, Thrombosis

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Introduction

Myeloproliferative neoplasms (MPNs) are a group of clonal hematopoietic stem cell disorders that are associated with increased production of one or more myeloid cell lineages and have variable risks of thrombosis, hemorrhage, myelofibrotic progression, and leukemic transformation. The classical Philadelphia chromosome-negative MPNs include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). Current classification schemes focus on integrated diagnosis based on clinical characteristics, blood counts, bone marrow morphology, and molecular markers, including driver mutations such as Janus Kinase 2 (JAK2), Calreticulin (CALR) and Myeloproliferative leukemia oncogene (MPL).[1]

The identification of the JAK2 V617F mutation revolutionized the understanding of the biology and diagnosis of MPNs. This acquired gain-of-function mutation causes constitutive activation of JAK-STAT signalling, which causes cytokine-independent proliferation and survival advantage of myeloid progenitor cells.[2] JAK2 V617F is present in the vast majority of patients with PV and in a significant proportion of patients with ET and PMF and is the most common driver mutation in classical MPNs.[3]

Thrombosis is a clinically important complication of MPNs and a major cause of morbidity and mortality. During the course of the disease, arterial events such as stroke, transient ischemic attack, myocardial infarction, and peripheral arterial thrombosis can happen, as well as venous events such as deep vein thrombosis, pulmonary embolism, and splanchnic vein thrombosis. The conventional thrombotic risk assessment has been based primarily on age, previous thrombosis, and cardiovascular risk factors, and more recent models have included JAK2 V617F mutation status as an additional prothrombotic variable.[4]

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In addition to the presence or absence of the mutation, the JAK2 V617F allele burden, typically reported as variant allele frequency (VAF), can offer quantitative data on clonal disease burden. In various MPN types, higher allele burden has been correlated with increased myeloproliferation, leukocytosis, splenomegaly, microvascular symptoms, and vascular complications.[5,6] In ET, Antonioli *et al*. found that the higher the JAK2 V617F allele burden, the higher the risk of arterial thrombosis at diagnosis, with a relative risk of 3.0 in patients with an allele burden >25%.[5]

The association of allele burden with thrombosis has also been studied in the different MPN subtypes. Carobbio *et al*. directly compared ET and PV and found that the incidence of thrombosis increased from JAK2 wild-type ET to JAK2-mutated ET to PV, suggesting a biological relationship between the presence of a JAK2-mutated clone and vascular risk.[6] In PV, the presence of

the JAK2 V617F variant allele has been linked to the risk of venous thrombosis, with higher VAF associated with an increased risk of venous thrombosis, as demonstrated by Guglielmelli *et al*., who found that VAF >50% was an independent risk factor for venous thrombosis, whereas conventional cardiovascular risk factors were more important for arterial thrombosis.[7]

The prothrombotic effect of JAK2 V617F-positive disease can be explained by several mechanisms. These include leukocytosis, platelet activation, hyperviscosity due to erythrocytosis, endothelial interaction, activation of inflammatory cytokines, and abnormal formation of neutrophil extracellular traps. Coucelo *et al*. showed that JAK2 V617F allele burden correlated with activation of thrombotic mechanisms in PV and ET, suggesting that quantitative mutation burden may not only reflect clonal size but also biological thrombogenicity.[8]

The clinical value of JAK2 V617F allele burden in the routine assessment of thrombotic risk, however, is not fully established. Recent systematic review evidence indicates that increased JAK2 V617F allele burden is linked to thrombosis, splenomegaly, pruritus, leukocytosis, myelofibrotic transformation, and leukemic transformation in PV, but there is heterogeneity in methods and cutoffs that preclude uniform interpretation.[9] Thus, additional prospective data, particularly in real-world tertiary care settings, are required to determine if allele burden can be used to better predict thrombotic risk than traditional clinical and hematological factors.

The present study aimed to assess the JAK2 V617F allele burden and its association with thrombotic risk in patients with MPNs. This study may help to clarify whether allele burden can be a useful biomarker for thrombotic risk stratification in clinical practice in addition to MPN subtype, blood counts, cardiovascular risk factors, prior thrombosis, and thrombotic events.

Objectives

1. To estimate JAK2 V617F allele burden in patients diagnosed with MPNs
2. To assess the association between JAK2 V617F allele burden and thrombotic risk, including arterial and venous thrombotic events
3. To correlate JAK2 V617F allele burden with clinical and hematological parameters, including age, MPN subtype, hemoglobin, leukocyte count, platelet count, and splenomegaly
4. To identify whether higher JAK2 V617F allele burden predicts increased thrombotic risk after considering conventional risk factors such as age, prior thrombosis, leukocytosis, and cardiovascular risk factors.

Methods

Study Design and Setting

This prospective observational study was conducted at a tertiary hospital over 1 year, from June 2025 to May 2026. Patients with Philadelphia chromosome-negative MPNs were evaluated for JAK2 V617F mutation status, allele burden, clinical phenotype, and thrombotic risk.

Study Population

A total of 120 patients diagnosed with MPNs were included. Patients with PV, ET, or PMF were enrolled. Diagnosis was made according to the 2022 World Health Organization classification criteria, using integrated clinical, hematological, bone marrow morphological, and molecular findings.

Patients with BCR-ABL1-positive chronic myeloid leukemia, secondary erythrocytosis, reactive thrombocytosis, reactive leukocytosis, incomplete molecular data, or inadequate clinical information regarding thrombotic history were excluded.[12]

Data Collection

Baseline demographic, clinical, and hematological data were recorded, including age, sex, body mass index, disease duration, MPN subtype, hemoglobin, hematocrit, total leukocyte count, platelet count, lactate dehydrogenase (LDH), splenomegaly, cardiovascular risk factors, prior thrombosis, and cytoreductive treatment status. Leukocytosis was defined as total leukocyte count $>11 \times 10^9$/L.

Thrombotic events were classified as arterial or venous. Arterial events included ischemic stroke, transient ischemic attack, myocardial infarction, and peripheral arterial thrombosis. Venous events included deep vein thrombosis, pulmonary embolism, and splanchnic or other objectively documented venous thrombosis. Overall thrombotic event was defined as any documented arterial or venous thrombotic event before enrolment or during follow-up.

JAK2 V617F Allele Burden Assessment

Peripheral blood samples were collected in ethylenediaminetetraacetic acid vacutainers for molecular analysis. Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp DNA Blood Mini Kit (QIAGEN, Germany), according to the manufacturer's instructions. DNA quantity and purity were assessed before amplification.

JAK2 V617F mutation and allele burden were assessed using the ipsogen JAK2 MutaQuant Kit (QIAGEN, Germany), a quantitative real-time polymerase chain reaction (PCR)-based assay for detection and quantification of the JAK2 V617F/G1849T mutation in genomic DNA. The assay was performed on an Applied Biosystems 7500 real-time PCR system according to the manufacturer's protocol. The assay provides quantitative estimation of mutant and wild-type JAK2 copy numbers using standard-curve-based real-time PCR analysis.

JAK2 V617F allele burden was expressed as percentage VAF using the formula:

$$\text{JAK2 V617F allele burden (\%)} = (\text{JAK2 V617F mutant copy number}/[\text{JAK2 V617F mutant copy number} + \text{wild-type JAK2 copy number}]) \times 100$$

The manufacturer-reported limit of detection for the assay is approximately 0.061%, with optimal sensitivity achieved when adequate total JAK2 copy number is present. Results below the detection threshold were considered JAK2 V617F-negative.

For comparative analysis, patients were categorized as JAK2-negative, low allele burden (<25%), intermediate allele burden (25–50%), and high allele burden (>50%). These cutoffs were selected based on previous studies demonstrating clinically

relevant phenotypic and thrombotic differences across increasing JAK2 V617F allele-burden strata, with >50% generally representing high clonal burden.

Thrombotic Risk Assessment

Thrombotic risk was assessed using conventional clinical and hematological factors, including age ≥60 years, prior thrombosis, leukocytosis, and cardiovascular risk factor burden. Patients were classified into thrombotic risk categories based on these variables. Cardiovascular risk factor burden was calculated from the presence of established risk factors such as hypertension, diabetes mellitus, smoking, dyslipidemia, and obesity.

Outcome Measures

The primary outcome was the association between JAK2 V617F allele burden and thrombotic risk or thrombotic events. Secondary outcomes included the association of allele burden with MPN subtype, hemoglobin, hematocrit, total leukocyte count, platelet count, LDH, leukocytosis, splenomegaly, prior thrombosis, arterial thrombosis, venous thrombosis, and overall thrombotic events.

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation or median with interquartile range and categorical variables as frequency and percentage. Comparisons across MPN subtypes or JAK2 allele-burden categories were performed using one-way analysis of variance, Kruskal–Wallis test, or Chi-square test, as appropriate. Spearman correlation was used to assess associations between JAK2 V617F allele burden and clinical or hematological variables. Mann–Whitney U test was used for two-group comparisons of allele burden. Multivariable logistic regression was performed to assess independent associations of JAK2 V617F allele burden with high thrombotic risk category and overall thrombotic events, adjusting for age, leukocytosis, and cardiovascular risk factor burden. Adjusted odds ratios (OR) with 95% confidence intervals (CI) were calculated. A $P < 0.05$ was considered statistically significant.

Ethical Considerations

The study was conducted after approval from the Institutional Ethics Committee of the tertiary hospital. Written informed consent was obtained from all participants before enrolment, and patient confidentiality was maintained throughout the study.

Results

Study Cohort and JAK2 V617F Allele Burden Distribution

A total of 120 patients with MPNs were included in the analysis. The cohort comprised PV, ET, and PMF cases, with baseline clinical and hematological characteristics summarized in Table 1. JAK2 V617F mutation was detected in 94 (78.3%) patients. Allele burden differed significantly across MPN subtypes, with higher values observed in PV and PMF than in ET [Figure 1].

Association of JAK2 V617F Allele Burden with Thrombotic Risk and Events

The relationship between allele burden category and thrombotic outcomes is summarized in Table 2 and illustrated in Figure 2. Higher allele burden was accompanied by a greater frequency of leukocytosis, splenomegaly, high thrombotic risk classification, prior thrombosis, venous thrombosis, and overall thrombotic events. Follow-up thrombotic events were infrequent and did not differ significantly across allele burden categories.

Correlation of Allele Burden with Clinical and Hematological Parameters

Correlation analyses between JAK2 V617F allele burden and clinical or hematological parameters are presented in Table 3. Allele burden was positively related to erythrocytosis, leukocytosis, LDH level, and thrombotic risk score, while platelet count showed an inverse association. Patients with splenomegaly or leukocytosis had higher allele burden than those without these features.

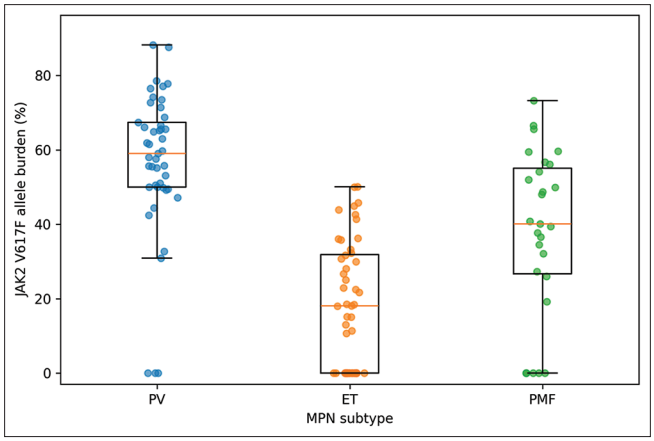

Figure 1: Janus Kinase 2 (JAK2) V617F allele burden by myeloproliferative neoplasms subtype. Box-and-point plot showing the distribution of JAK2 V617F allele burden across polycythemia vera, essential thrombocythemia, and primary myelofibrosis

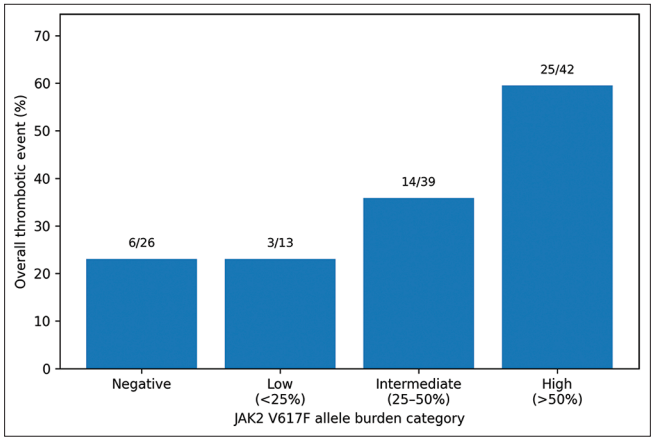

Figure 2: Thrombotic events according to Janus Kinase 2 (JAK2) V617F allele burden category. Bar chart showing the proportion of patients with overall thrombotic events across increasing JAK2 V617F allele burden categories. Labels above bars indicate event counts over category totals

Table 1: Baseline clinical and hematological characteristics according to MPN subtype

Characteristic	*Overall n=120*	*PV n=45*	*ET n=48*	*PMF n=27*	*Test statistic*	*P-value*
Age, years	58.9±10.7	59.3±10.4	55.7±10.9	63.9±8.9	F=5.50	0.005
Male sex, *n* (%)	62 (51.7)	28 (62.2)	21 (43.8)	13 (48.1)	χ^2=3.35	0.188
BMI, kg/m^2	25.2±3.6	25.7±3.4	24.5±3.7	25.5±3.6	F=1.34	0.266
Disease duration, months	18 (7–31)	13 (6–34)	18 (7–30)	21 (10–30)	H=0.60	0.739
JAK2 V617F positive, *n* (%)	94 (78.3)	42 (93.3)	30 (62.5)	22 (81.5)	χ^2=13.21	0.001
JAK2 V617F allele burden, %, median (IQR)	40.5 (15.2–56.9)	59.0 (50.0–67.4)	18.1 (0.0–31.8)	40.1 (26.6–55.1)	H=55.53	<0.001
High allele burden >50%, *n* (%)	42 (35.0)	32 (71.1)	1 (2.1)	9 (33.3)	χ^2=48.69	<0.001
Hemoglobin, g/dL	13.8±2.5	16.4±1.3	12.9±1.2	11.2±1.5	F=148.22	<0.001
Total leukocyte count, ×10^9/L	10.3±3.6	11.3±3.0	8.3±2.5	12.3±4.4	F=17.44	<0.001
Leukocytosis >11×10^9/L, n (%)	48 (40.0)	28 (62.2)	4 (8.3)	16 (59.3)	χ^2=33.49	<0.001
Platelet count, ×10^9/L	577.0±231.1	488.8±190.1	753.4±187.1	410.4±142.8	F=40.27	<0.001
LDH, U/L, median (IQR)	340 (266–456)	399 (312–453)	250 (213–311)	516 (448–618)	H=62.19	<0.001
Splenomegaly, *n* (%)	48 (40.0)	22 (48.9)	5 (10.4)	21 (77.8)	χ^2=35.04	<0.001
Prior thrombosis, *n* (%)	40 (33.3)	23 (51.1)	6 (12.5)	11 (40.7)	χ^2=16.44	<0.001
Overall thrombotic event, *n* (%)	48 (40.0)	28 (62.2)	8 (16.7)	12 (44.4)	χ^2=20.37	<0.001
Cytoreductive therapy, *n* (%)	70 (58.3)	30 (66.7)	19 (39.6)	21 (77.8)	χ^2=12.43	0.002

Values are presented as mean±SD, median (IQR), or *n* (%), as appropriate. PV: Polycythemia vera, ET: Essential thrombocythemia, PMF: Primary myelofibrosis, SD: Standard deviation, BMI: Body mass index, MPN: Myeloproliferative neoplasm, JAK2: Janus Kinase 2, IQR: Interquartile range, LDH: Lactate dehydrogenase. Comparisons used one-way analysis of variance, Kruskal–Wallis test, or Chi-square test, as appropriate

Table 2: Clinical and thrombotic outcomes according to JAK2 V617F allele burden category

Characteristic	*Negative n=26*	*Low<25% n=13*	*Intermediate 25–50% n=39*	*High>50% n=42*	*Test statistic*	*P-value*
Age, years	59.0±10.1	49.7±9.2	60.4±10.3	60.2±10.7	F=3.96	0.010
Total leukocyte count, ×10^9/L	8.2±3.3	8.5±2.7	10.8±3.7	11.7±3.2	F=7.39	<0.001
Leukocytosis>11 ×10^9/L, *n* (%)	3 (11.5)	2 (15.4)	17 (43.6)	26 (61.9)	χ^2=20.66	<0.001
Splenomegaly, *n* (%)	10 (38.5)	0 (0.0)	14 (35.9)	24 (57.1)	χ^2=14.11	0.003
High thrombotic risk category, *n* (%)	4 (15.4)	3 (23.1)	17 (43.6)	26 (61.9)	χ^2=16.37	<0.001
Prior thrombosis, *n* (%)	4 (15.4)	3 (23.1)	12 (30.8)	21 (50.0)	χ^2=9.75	0.021
Follow-up thrombotic event, *n* (%)	3 (11.5)	1 (7.7)	3 (7.7)	4 (9.5)	χ^2=0.32	0.957
Arterial thrombosis ever, *n* (%)	6 (23.1)	2 (15.4)	13 (33.3)	14 (33.3)	χ^2=2.34	0.504
Venous thrombosis ever, *n* (%)	1 (3.8)	2 (15.4)	2 (5.1)	16 (38.1)	χ^2=19.87	<0.001
Overall thrombotic event, *n* (%)	6 (23.1)	3 (23.1)	14 (35.9)	25 (59.5)	χ^2=11.60	0.009

JAK2: Janus Kinase 2. Values are presented as mean±standard deviation or *n* (%). Between-category comparisons used one-way analysis of variance for continuous variables and Chi-square test for categorical variables

Table 3: Correlation of JAK2 V617F allele burden with clinical and hematological parameters

Analysis	*Estimate*	*Test statistic*	*P-value*
Allele burden versus Age, years	Spearman ρ=0.110	*t*=1.21	0.230
Allele burden versus Hemoglobin, g/dL	Spearman ρ=0.387	*t*=4.56	<0.001
Allele burden versus Hematocrit, %	Spearman ρ=0.393	*t*=4.65	<0.001
Allele burden versus Total leukocyte count, ×10^9/L	Spearman ρ=0.384	*t*=4.51	<0.001
Allele burden versus Platelet count, ×10^9/L	Spearman ρ=-0.331	*t*=−3.81	<0.001
Allele burden versus LDH, U/L	Spearman ρ=0.373	*t*=4.37	<0.001
Allele burden versus Thrombotic risk score	Spearman ρ=0.447	*t*=5.43	<0.001
Allele burden versus Cardiovascular risk factor count	Spearman ρ=0.077	*t*=0.83	0.406
Allele burden in splenomegaly present versus absent	49.9 (31.8–63.5) versus 32.0 (12.6–50.0)	U=2172.0	0.017
Allele burden in leukocytosis present versus absent	53.6 (37.4–65.6) versus 28.4 (0.0–49.9)	U=2577.5	<0.001

JAK2: Janus Kinase 2, LDH: Lactate dehydrogenase. Spearman correlation was used for continuous variables. Mann–Whitney U test was used for median allele burden comparisons between binary clinical groups

Predictors of Thrombotic Risk and Thrombotic Events

Multivariable logistic regression models are shown in Table 4. Higher JAK2 V617F allele burden remained associated with high thrombotic risk classification and overall thrombotic events after adjustment for age, leukocytosis, and cardiovascular risk factor burden.

Discussion

JAK2 V617F mutation was found in 78.3% of the 120 patients with MPNs, with the highest prevalence in PV (93.3%), followed by PMF (81.5%) and ET (62.5%). Median allele burden was also highest in PV (59.0%), followed by PMF (40.1%) and ET (18.1%). High allele burden (>50%) was observed in 35.0% of the cohort and was especially common in PV (71.1%). Increasing allele burden was associated with leukocytosis, splenomegaly, high thrombotic risk category, prior thrombosis, venous thrombosis, and overall thrombotic events. JAK2 allele burden was independently associated with high thrombotic risk category and overall thrombotic events in multivariable analysis with adjusted ORs of 1.38 and 1.34/10% increase, respectively.

The distribution of high allele burden in the current study is similar to the homozygous JAK2 V617F phenotype reported by Vannucchi *et al*. In their multicenter study, 118 patients (104

Table 4: Multivariable logistic regression models for thrombotic risk and overall thrombotic events

Outcome	*Predictor*	*Adjusted OR (95% CI)*	*Test statistic*	*P-value*
High thrombotic risk category	JAK2 allele burden per 10%	1.38 (1.13–1.68)	z=3.13	0.002
High thrombotic risk category	Age≥60 years	8.21 (2.82–23.91)	z=3.86	<0.001
High thrombotic risk category	Leukocytosis>11×10^9/L	4.43 (1.60–12.28)	z=2.86	0.004
High thrombotic risk category	CV risk factor count	0.92 (0.51–1.66)	z=−0.28	0.779
Overall thrombotic event	JAK2 allele burden per 10%	1.34 (1.12–1.61)	z=3.17	0.002
Overall thrombotic event	Age≥60 years	1.66 (0.70–3.91)	z=1.16	0.246
Overall thrombotic event	Leukocytosis>11×10^9/L	1.56 (0.66–3.69)	z=1.02	0.307
Overall thrombotic event	CV risk factor count	1.05 (0.62–1.76)	z=0.18	0.859

CI: Confidence intervals, OR: Odds ratio, CV: Cardiovascular, JAK2: Janus Kinase 2, LDH: Lactate dehydrogenase, Adjusted odds ratios were estimated using logistic regression. JAK2 allele burden was modeled per 10% increase

PV and 14 ET) were identified as homozygous mutation and compared with 587 heterozygous and 257 wild-type patients.[10] Homozygous patients were older and had higher leukocyte count, higher hematocrit, and larger spleen volume, which are all markers of a more proliferative phenotype. Importantly, homozygous ET patients had a significantly increased risk of cardiovascular events after adjustment for sex, age, leukocyte count, and previous thrombosis (hazard ratio [HR] 3.97; 95% CI 1.34–11.7), but not PV.[10] This is consistent with our observation that increased allele burden is associated with a more clinically active MPN phenotype, with the degree of association with thrombotic events differing between MPN subtypes.

Passamonti *et al*. provide further PV-specific evidence. In their prospective study of 338 PV patients, 320 (94.7%) were JAK2 V617F positive, which is similar to the high PV mutation frequency observed in our cohort.[11] They identified direct relationships between allele burden and hemoglobin, leukocytes, spleen size, and bone marrow cellularity and an inverse relationship with platelet count.[11] This was also true for our cohort, in which allele burden was positively associated with hemoglobin, hematocrit, leukocytes, LDH, and splenomegaly, and negatively associated with platelet count. However, Passamonti *et al*. found allele burden >50% to be a predictor of post-PV myelofibrosis rather than thrombosis, as all eight patients who progressed to post-PV myelofibrosis had >50% mutant alleles.[11] This discrepancy could be due to our mixed MPN population and the fact that we included overall thrombotic history and not just prospective PV events.

Silver *et al*. also showed a high correlation between allele burden and disease phenotype in PV. The mean allele burden in their study of 105 PV patients was 46.0 ± 29.7%, which is very similar to the overall median allele burden in our cohort of 40.5%. Their allele burden was progressively higher with disease features: Mean WBC was 6.6 ×10^9/L in the 0–20% allele burden group and 18.3 ×10^9/L in the 81–100% group, and mean allele burden was 36.3% in patients without splenomegaly and 81.8% in those with spleen size >9 cm. They also exhibited a progressive median allele burden from 30.9% in grade 1 to 90.0% in grade 4. These findings are very similar to our results, in which high allele burden correlated with leukocytosis and splenomegaly, and thus support the use of allele burden as a measure of clonal expansion and disease activity in the proliferative phase.

The thrombotic results in Silver *et al*. were less dramatic than those in the current study. They found thrombosis in 25.7% of PV patients, with 15.2% having arterial thrombosis, 8.6% having venous thrombosis, and 1.9% having both arterial and venous thrombosis. They did not identify a statistically significant high-risk thrombotic subpopulation, but they did find that the mean allele burden was higher in venous thrombosis than in arterial thrombosis (47.7% vs. 37.4%), although this was not significant. In the current study, the difference was more marked: Venous thrombosis was more common in the high allele burden group (38.1%) than in the other groups, but there was no significant difference in the prevalence of arterial thrombosis between the different allele burden groups. This suggests that JAK2 allele burden may be more closely associated with venous than arterial thrombosis.

De Stefano *et al*. also correlated the JAK2 mutation load with thrombosis in ET. In the 132 ET patients in their cohort, 63% had JAK2 V617F and 29% had thrombosis.[13] JAK2-mutated patients younger than 60 years had higher thrombotic risk, and homozygous patients were more prone to thrombosis during follow-up.[13] Our study involved PV, ET, and PMF, but the trend of increasing prior thrombosis and overall thrombosis events with increasing allele burden is directionally consistent with this finding.

Borowczyk *et al*. reported that JAK2 V617F mutation status and allele burden were particularly related to venous thromboembolic events in Philadelphia-negative MPNs.[14] In their study, they reported arterial thrombosis in 20.4%, venous thromboembolism in 11.3%, and bleeding in 24.7% of patients.[14] The same event-type distinction was observed in the present cohort: high allele burden was strongly associated with venous thrombosis, but not with arterial thrombosis. This distinction is clinically relevant as arterial events might be more dependent on age, hypertension, diabetes, smoking, and other cardiovascular risk factors, and venous events might more directly reflect the clonal and inflammatory biology of the MPN.

This venous predominance is further supported by Soudet *et al*., who found that JAK2 allele burden was associated with venous thromboembolism but not arterial cardiovascular events.[15] In our study, high allele burden (>50%) was associated with venous thrombosis ever, but not with arterial thrombosis, which did not differ significantly between allele burden categories. These results indicate that quantitative allele burden may be more useful for venous thrombotic risk stratification than for prediction of arterial events.

The present results are also corroborated by recent real-world data from Brown *et al*. JAK2 V617F VAF was higher in PV and PMF than in ET, and higher VAF was associated with thrombotic events, especially post-diagnosis events in 159 MPN patients.[16] We also found that our cohort had a higher allele burden in PV and PMF than in ET and that allele burden was independently associated with overall thrombotic events. In our cohort, however, follow-up thrombotic events were rare, which may account for the stronger cross-sectional "ever thrombosis" association compared to the prospective follow-up event comparison.

Lee *et al*. offer helpful comparator data for PV and ET by subtype. In their retrospective study of 127 JAK2 V617F-positive patients, comprising 61 PV and 66 ET patients, the median JAK2 V617F allele burden was 58% in PV and 30% in ET.[17] This is similar to the current study, which found a higher allele burden in PV than ET. Lee *et al*. also observed a positive correlation between allele burden and white blood cell count, hemoglobin, LDH, and platelet count, which further suggests a relationship between allele burden and proliferative disease activity. Importantly, in ET, allele burden ≥30%, older age, and higher hemoglobin were risk factors for thrombotic events, while in PV, only older age was a thrombotic risk factor.[17] In the current cohort, allele burden was higher in each thrombotic risk category and was independently associated with overall thrombotic events, indicating that quantitative allele burden may provide clinical utility, especially when combined with age, erythrocytosis, and leukocytosis.

Landolfi *et al*. studied 1638 patients with PV in the European Collaboration on Low-Dose Aspirin in Polycythemia Vera observational database for a mean follow-up of 2.7 ± 1.3 years[18] and found that leukocytosis was a thrombotic cofactor. During follow-up, 205 thrombotic events occurred. In time-dependent analysis, patients with white blood cell count >15 $\times 10^9$/L had a significantly higher risk of thrombosis compared with those with white blood cell count <10 $\times 10^9$/L (HR 1.71; 95% CI 1.10–2.65; $P = 0.017$), with a particularly increased risk of myocardial infarction (HR 2.84; 95% CI 1.25–6.46; $P = 0.013$).[18] In the current study, the percentage of leukocytosis was found to be progressively higher with increasing JAK2 allele burden (11.5% in JAK2-negative patients, 61.9% in the high allele burden group), and there was a significant correlation between allele burden and leukocyte count. However, JAK2 allele burden was still independently associated with thrombotic events after accounting for leukocytosis, suggesting that allele burden may reflect other aspects of thrombotic biology beyond leukocytosis.

These results indicate that JAK2 V617F allele burden, leukocytosis, erythrocytosis, and age are clinically relevant determinants of thrombotic risk that act in concert, although the relative importance of each may vary in PV, ET, and PMF.

The present study has important clinical implications. First, JAK2 V617F allele burden offers quantitative data in addition to mutation positivity. Second, high allele burden is associated with a proliferative phenotype, such as elevated hemoglobin/hematocrit, leukocytosis, LDH elevation, and splenomegaly. Third, the most significant thrombotic association was with venous thrombosis, indicating that allele burden may be especially important for venous risk assessment. Finally, the independent association of allele burden with overall thrombotic events suggests that it may be useful to include in thrombotic risk stratification models.

Conclusion

Higher JAK2 V617F allele burden was significantly associated with proliferative disease features, including higher hemoglobin, hematocrit, leukocytosis, LDH, and splenomegaly and with increased thrombotic risk in MPNs. The association was strongest for venous and overall thrombotic events. Quantitative assessment of JAK2 V617F allele burden may therefore serve as a useful adjunct to conventional clinical and hematological risk factors for thrombotic risk stratification in MPN patients.

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