

ORIGINAL ARTICLE

Prediction of thrombosis in polycythemia vera: Development and validation of a multiple factor-based prognostic score system

Wenjing Gu MD^{1,2} | Yuhui Zhang MD³ | Ting Sun MD^{1,2} | Mankai Ju MD^{1,2} |
 Xiaofan Liu MD^{1,2} | Feng Xue MD^{1,2} | Yunfei Chen MD^{1,2} | Wei Liu MD^{1,2} |
 Huiyuan Li PhD^{1,2} | Wentian Wang PhD^{1,2} | Ying Chi PhD^{1,2} | Renchi Yang MD^{1,2} |
 Rongfeng Fu MD^{1,2} | Jie Bai MD³ | Lei Zhang MD^{1,2}

¹State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin Key Laboratory of Gene Therapy for Blood Diseases, CAMS Key Laboratory of Gene Therapy for Blood Diseases, Tianjin, China

²Tianjin Institutes of Health Science, Tianjin, China

³Department of Hematology, The Second Hospital of Tianjin Medical University, Tianjin, China

Correspondence

Lei Zhang and Rongfeng Fu, State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin, 300020, China.
 Email: furongfeng@ihcams.ac.cn and zhanglei1@ihcams.ac.cn
 Jie Bai, Department of Hematology, The Second Hospital of Tianjin Medical University, Tianjin, 300211, China.
 Email: janebai86@hotmail.com

Handling Editor: Dr Vania Morelli

Abstract

Background: Thrombosis is an important cause of death in patients with polycythemia vera (PV). The conventional stratification of thrombosis may ignore some potential risk factors.

Objectives: This study aimed to develop and validate a multiple factor-based prediction model of thrombosis for the 2016 World Health Organization-defined PV.

Methods: Clinical and next-generation sequencing data from 2 cohorts of patients with PV were analyzed. Multivariable Cox regression analyses were conducted for the identification of thrombotic risk factors and model development.

Results: The study involved 372 patients in the training cohort and another 195 patients in the external validation cohort. Multivariable analyses indicated that age ≥ 60 years (hazard ratio [HR] 2.56, 95% CI 1.51-4.35, $P < .001$), cardiovascular risk factors (HR 4.22, 95% CI 2.00-8.92, $P < .001$), at least 1 high-risk mutation for thrombosis (mutations in *DNMT3A*, *ASXL1*, or *BCOR/BCORL1*) (HR 4.35, 95% CI 2.62-7.21, $P < .001$), and previous thrombosis (HR 5.93, 95% CI 3.29-10.68, $P < .001$) were independent risk factors of thrombosis. After assigning coefficient-weighted scores to each risk factor mentioned above, a multiple factor-based prognostic score system of thrombosis (MFPS-PV) was developed, classifying patients into low-risk, intermediate-risk, and high-risk groups. Patients in the 3 groups had notably different thrombosis-free survival rates ($P < .001$). The MFPS-PV outperformed the conventional model in discrimination power (C-statistic: 0.87 [95% CI 0.83-0.91] vs 0.80 [95% CI 0.74-0.86]). The MFPS-PV was well calibrated and remained consistent during external validation.

Conclusion: The MFPS-PV, integrating genetic and clinical characteristics for the first time, shows excellent accuracy and utility for thrombosis prediction in WHO-defined PV.

KEYWORDS

mutation, next-generation sequencing, polycythemia vera, risk factors, thrombosis

Essentials

- Thrombosis is an important cause of death in patients with polycythemia vera.
- We developed and validated a multiple factor-based prediction model of thrombosis in polycythemia vera.
- The model included age, cardiovascular risk factors, high-risk mutations, and previous thrombosis.
- The model showed excellent ability for discrimination and calibration in predicting thrombosis.

1 | INTRODUCTION

Polycythemia vera (PV) is a subtype of classical Philadelphia chromosome-negative (Ph⁻) myeloproliferative neoplasms (MPNs), characterized by an elevated red blood cell mass, sometimes accompanied by an increase in platelets and white blood cells. Although patients with PV have relatively long survival (median 15 years) and low incidence of myelofibrosis or leukemia transformation (4.9%-6% and 2.3%-14.4% at 10 years, respectively), some of them have a shorter life expectancy than the age- and sex-matched general populations [1-4]. Thrombosis is an important cause of death and decline in patients' quality of life. In a study of 665 patients with PV from the Mayo Clinic, the incidence of thrombosis was 25% before diagnosis and 24% after diagnosis [4]. The large cohorts of the European Collaboration on Low-Dose Aspirin in Polycythemia Vera (ECLAP) and the International Working Group for MPN Research and Treatment (IWG-MRT) studies have reported a cumulative rate of 5.5 and 2.62 per 100 person-years of thrombotic events, respectively, in patients with PV, and mortality due to thrombosis contributed to 45% of all-cause deaths in the ECLAP trial [5,6]. To reduce the incidence of life-threatening thrombosis in PV, it is necessary to accurately identify thrombotic risk factors and make appropriate risk stratification.

The main goal of treatment for patients with PV is to prevent thrombotic complications. Currently, the recommended treatment strategy for PV is on the basis of a conventional two-tiered risk stratification that classifies patients into 2 risk groups: "low risk," age <60 years and no previous thrombosis, and "high risk," age ≥60 years and/or with previous thrombosis [1,7,8]. However, conventional risk stratification is on the basis of Polycythemia Vera Study Group (PVSG) criteria [9,10]. In addition, conventional risk stratification is specified when next-generation sequencing (NGS) technology is not widely used in clinical practice, and in fact, recent studies have highlighted the role of clonal hematopoiesis and specific mutations in thrombosis of MPNs [11-13]. Moreover, additional risk factors for thrombosis, such as cardiovascular risk factors (CVF), higher *JAK2 V617F* allele burden, leukocytosis, and elevated hematocrit (Hct) levels have also been identified [14-21]. Therefore, the two-tiered stratification may ignore some potential risk factors for thrombosis in the 2016 World Health Organization (WHO)-defined PV.

In this study, we developed and validated a new thrombosis prediction model on the basis of genetic and clinical information, with the aim to improve the prediction power of thrombosis in the 2016

WHO-defined PV and provide valuable information for the practical management of thrombosis.

2 | METHODS

2.1 | Patients and samples

The study was approved by the Institutional Ethics Committee of the Institute of Hematology and Blood Diseases Hospital (Tianjin, China) and the Second Hospital of Tianjin Medical University (Tianjin, China) in accordance with the Declaration of Helsinki. Patients diagnosed at the 2 institutions from August 30, 1984 to March 30, 2022, were eligible for inclusion if they were 18 years or older with a confirmed diagnosis of PV according to the 2016 WHO criteria [22]. Written informed consent was obtained from each patient. Clinical and laboratory data at the initial diagnosis were retrospectively collected from institutional databases. Treatment and thrombosis information during follow-up was obtained by direct contact with patients or their families. Bone marrow mononuclear cells were collected from the included patients and sent for NGS. Data from the Institute of Hematology and Blood Diseases Hospital were initially analyzed for model development, whereas the cohort from the Second Hospital of Tianjin Medical University was subsequently used for external validation.

2.2 | Next-generation sequencing

NGS was performed on 1 µg of genomic DNA extracted from the patients' bone marrow mononuclear cells. Sequencing was performed using whole-genome sequencing platform (NovaSeq 6000 platform, Illumina) with a targeted 267-gene panel. A list of 267 candidate genes is provided in [Supplementary Table 1](#). After filtering out low-quality sequencing reads, clean reads were mapped to the human reference genome (hg19) using the mapping tool (Burrows-Wheeler Aligner, sourceforge). Single-nucleotide polymorphisms and short insertion/deletions were called and annotated using 1000 Genomes, ESP6500, SNPeff, Inhouse, PolyPhen-2, SIFT, and COSMIC to determine the potential functional impacts of the variants. The identified pathogenic mutations were validated by Sanger sequencing. Details of the

sequencing and bioinformatics analysis are provided in Supplemental Methods.

2.3 | Statistical analysis

Categorical variables were compared using the chi-squared test, whereas continuous variables were compared using the Mann–Whitney U-test or Kruskal–Wallis tests. Receiver operating characteristic (ROC) plots were used to define cutoff levels for continuous variables that were significant in the univariable analysis. A multivariable Cox proportional hazard regression analysis was conducted to identify independent risk factors for thrombosis-free survival (TFS). TFS was considered from the date of diagnosis to the date of the first episode of thrombosis after diagnosis (uncensored) or the last contact (censored). Regression coefficient (β), hazard ratio (HR) with 95% confidence intervals (CI), and corresponding *P* values were calculated. Statistics, including number needed to harm and population attributable fraction, were also calculated for comprehensive interpretation of each predictor [23]. To decrease bias and increase statistical accuracy, multiple imputation was used to handle missing data in baseline variables. Competing risk (CR) analysis was conducted considering death without thrombosis as a CR. The cumulative incidence of thrombosis between patients with and without each risk factor was compared using both log-rank test by Kaplan–Meier (KM) method and Gray test by CR method [24,25].

A risk score system was developed on the basis of the β values from the multivariable Cox regression analysis. Discrimination was evaluated by calculating the area under the ROC curve (AUC) and Harrell C-statistic, with larger values representing better discrimination. The accuracy and utility of the model were assessed using a calibration plot, which indicates the agreement between the predicted and the actual observed probabilities of thrombosis [26]. A nonsignificant *P* value in the Goodness-of-Fit test represents good calibration. Statistical analyses were performed using R software (version 4.0.2). A *P* value <.05 was considered significant.

3 | RESULTS

3.1 | Patient characteristics and details of thrombosis

A total of 372 NGS-annotated patients with PV from the Institute of Hematology and Blood Diseases Hospital were included in the training cohort, with a median follow-up of 43 months (range 3–454). We had a complete record of outcome data for all included patients with no missing data. For baseline characteristics, missing data occurred in 6 variables (Supplementary Figure 1). The median age at diagnosis was 53 years (range 18–86), and 59.4% were men. Of the patients, 242 (65.1%) had at least one CVF (including hypertension, diabetes, hyperlipidemia, obesity, and smoking). All patients harbored clearly elevated Hct (median 58.2%; range 46.4%–74.3%) or hemoglobin

(median 187 g/L; range 160–290) levels, and most of them (*n* = 301, 80.9%) had suppressed erythropoietin concentration (median 1.39 IU/L; range 0.13–17.73). Forty-nine patients (13.2%) had elevated C-reactive protein levels (median 1.97 mg/L; range 1.00–69.80), and the median value of the neutrophil-to-lymphocyte ratio (NLR) was 5.48 (range 1.03–25.03). Abnormal karyotype and palpable splenomegaly were documented in 10.5% and 41.1% of the patients, respectively (Table 1).

Altogether, 216 thrombotic events were recorded in 136 patients (36.6%), including 122 patients (32.8%) with only arterial thrombosis, 7 patients (1.9%) with only venous thrombosis, and 7 patients (1.9%) with both arterial and venous thrombosis. Additionally, 72 patients (19.4%) had only one thrombotic event, 51 patients (13.7%) had 2 thrombotic events, and 13 patients (3.5%) had 3 or more thrombotic events. Regarding the time of thrombosis occurrence, 68 patients (18.3%) had only thrombosis before or at diagnosis, and another 68 patients (18.3%) had thrombosis after diagnosis, corresponding to an incidence rate of 3.6/100 patient-years during follow-up (Supplementary Table 2).

3.2 | Mutation landscapes and identification of high-risk mutations for thrombosis

The driver mutation distribution was *JAK2* V617F in 338 (90.9%) and *JAK2* exon12 in 14 (3.8%) patients. Two (0.5%) patients harbored both *JAK2* V617F and *JAK2* exon12 mutations. Mutations other than *JAK2* V617F or *JAK2* exon12 were detected in 132 (35.5%) patients, with the most frequently mutated genes being involved in epigenetic regulation, such as *TET2*, *ASXL1*, and *DNMT3A* (Table 1, Figure 1, and Supplementary Table 3). No association was observed between thrombosis and *JAK2* V617F (*P* = .572) or *JAK2* exon12 mutations (*P* = .967), whereas mutations in genes encoding for epigenetic modifiers were more frequent in patients with thrombosis than in those without thrombosis after diagnosis (45.6% vs 20.1%, *P* < .001). Specifically, significant differences were noted in the frequencies of *ASXL1* (16.2% vs 5.9%, *P* = .004), *DNMT3A* (25.0% vs 4.6%, *P* < .001), and *BCOR/BCORL1* (13.2% vs 4.6%, *P* = .017) mutations between patients with and without thrombosis after diagnosis. The variant allele frequencies of *JAK2* V617F and *JAK2* exon12 mutations were also analyzed, and no significant difference was identified (*P* = .055 and *P* = .126, respectively) (Table 1).

3.3 | Risk factors for thrombosis after diagnosis

We performed univariable analysis on the basis of total thrombosis. Patients with thrombosis were older (*P* < .001) and more likely to have CVF (*P* < .001) and splenomegaly (*P* = .010). They also had higher platelet counts (*P* = .036), higher NLR (*P* = .035), and higher frequencies of epigenetic mutations, such as *ASXL1* (*P* = .003) and *DNMT3A* (*P* < .001), than those without thrombosis. For those factors included in multivariable analysis, only older age (*P* < .001), CVF (*P* <

TABLE 1 Comparison of clinical and mutation information of 372 patients with PV with or without thrombosis after diagnosis.

	All patients (n = 372)	Patients with thrombosis after diagnosis (n = 68)	Patients without thrombosis after diagnosis (n = 304)	P
Clinical characteristics				
Age, median (range), y	53 (18-86)	60 (21-81)	52 (18-86)	<.001
Sex, male, n (%)	221 (59.4)	37 (54.4)	184 (60.5)	0.353
CVF, ^a n (%)	242 (65.1)	59 (86.8)	183 (60.2)	<.001
Previous thrombosis, n (%)	113 (30.4)	45 (66.2)	68 (22.4)	<.001
Ethnicity	Han Chinese	Han Chinese	Han Chinese	-
Laboratory characteristics				
Hemoglobin, median (range), g/L	187 (160-290)	193 (163-232)	186 (160-290)	.055
Hematocrit, median (range), %	58.2 (46.4-74.3)	59.1 (47.8-73.4)	57.4 (46.4-74.3)	.264
Leukocytes, median (range), $\times 10^9/L$	11.6 (4.3-50.0)	10.7 (6.2-36.5)	11.6 (4.3-50.0)	.991
Platelets, median (range), $\times 10^9/L$	493 (109-1609)	471 (115-1241)	498 (109-1609)	.930
NLR, median (range)	5.48 (1.03-25.03)	5.99 (2.02-17.69)	5.33 (1.03-25.03)	.151
CRP, median (range), mg/L	1.97 (1.00-69.80)	1.80 (1.00-56.90)	1.97 (1.00-69.80)	.594
Erythropoietin, median (range), IU/L	1.39 (0.13-17.73)	1.57 (0.19-16.46)	1.36 (0.13-17.73)	.215
Splenomegaly, n (%)	285 (76.6)	50 (73.5)	235 (77.3)	.506
Palpable splenomegaly, n (%)	153 (41.1)	27 (39.7)	126 (41.4)	.792
Abnormal karyotype, n (%)	39 (10.5)	11 (16.2)	28 (9.2)	.090
Driver mutations, n (%)				
JAK2 V617F	338 (90.9)	63 (92.7)	275 (90.5)	.572
JAK2 exon12	14 (3.8)	2 (2.9)	12 (3.9)	.967
JAK2 V617F & JAK2 exon12	2 (0.5)	0	2 (0.7)	1.000
JAK2 negative	18 (4.8)	3 (4.4)	15 (4.9)	1.000
Mutation VAF, median (range), %				
JAK2 V617F	50.8 (1.1-94.7)	58.8 (6.1-93.5)	49.4 (1.1-94.7)	.055
JAK2 exon12	21.0 (9.0-76.0)	37.7 (29.6-45.7)	20.5 (9.0-76.0)	.126
Additional mutations, n (%)	132 (35.5)	41 (60.3)	91 (31.9)	<.001
Other mutations,^b n (%)				
TET2	46 (12.4)	10 (14.7)	36 (11.8)	.517
ASXL1	29 (7.8)	11 (16.2)	18 (5.9)	.004
DNMT3A	31 (10.3)	17 (25.0)	14 (4.6)	<.001
BCOR/BCORL1	23 (6.2)	9 (13.2)	14 (4.6)	.017
TP53	10 (2.7)	2 (2.9)	8 (2.6)	1.000
SF3B1	4 (1.1)	0	4 (1.3)	1.000
NFE2	4 (1.1)	0	4 (1.3)	1.000
CBL	2 (0.5)	0	2 (0.7)	1.000
SETBP1	2 (0.5)	0	2 (0.7)	1.000
IDH2	2 (0.5)	0	2 (0.7)	1.000
EZH2	2 (0.5)	0	2 (0.7)	1.000
CUX1	2 (0.5)	0	2 (0.7)	1.000
JAK3	2 (0.5)	0	2 (0.7)	1.000
PPM1D	2 (0.5)	1 (1.5)	1(0.3)	.805

(Continues)

TABLE 1 (Continued)

	All patients (n = 372)	Patients with thrombosis after diagnosis (n = 68)	Patients without thrombosis after diagnosis (n = 304)	P
Mutation category, n (%)				
Epigenetic regulation	92 (24.7)	31 (45.6)	61 (20.1)	<.001
mRNA splicing	6 (1.6)	0	6 (2.0)	.597
Signaling pathways	7 (1.9)	2 (2.9)	5 (1.6)	.828
Transcription regulation	9 (2.4)	0	9 (3.0)	.375
Cell cycle/apoptosis	33 (8.9)	12 (17.6)	21 (6.9)	.005
Follow-up, median (range), mos	43 (3-454)	53 (9-304)	41 (3-454)	.063

Bold indicates statistically significant values.

CRP, C-reactive protein; NLR, neutrophil-to-lymphocyte ratio; PV, polycythemia vera; VAF, variant allele frequency.

^aCVF, cardiovascular risk factors, including hypertension, diabetes, hyperlipidemia, obesity, or smoking.

^bGenes with mutations in at least 2 patients in the training cohort are displayed and compared by chi-squared test.

.001), ASXL1 ($P = .039$), and DNMT3A ($P = .004$) mutations remained notable (Supplementary Table 4).

Similarly, risk factors for thrombosis after diagnosis were older age ($P < .001$), CVF ($P < .001$), previous thrombosis ($P < .001$), presence of additional non-driver mutations ($P < .001$), mutations involved in epigenetic regulation ($P < .001$) and cell cycle/apoptosis ($P = .005$), and specific high-risk mutations for thrombosis (ASXL1, DNMT3A, and BCOR/BCORL1, $P < .05$ for each mutation) (Table 1). A ROC plot was used to define the optimal cutoff level for age, and the

best discriminant value was 60 years (Supplementary Figure 2). The representative TFS curves are displayed in Supplementary Figure 3, confirming the contribution of these factors to reduced TFS ($P < .001$). As death without thrombosis will prevent the occurrence of thrombosis during follow-up, we performed a CR analysis to exclude the influence of death on the incidence of thrombosis. The cumulative incidence of thrombosis observed using the KM and CR methods is similar within the 10-year follow-up but slightly higher in the KM method after the 10-year follow-up (Supplementary Figure 4).

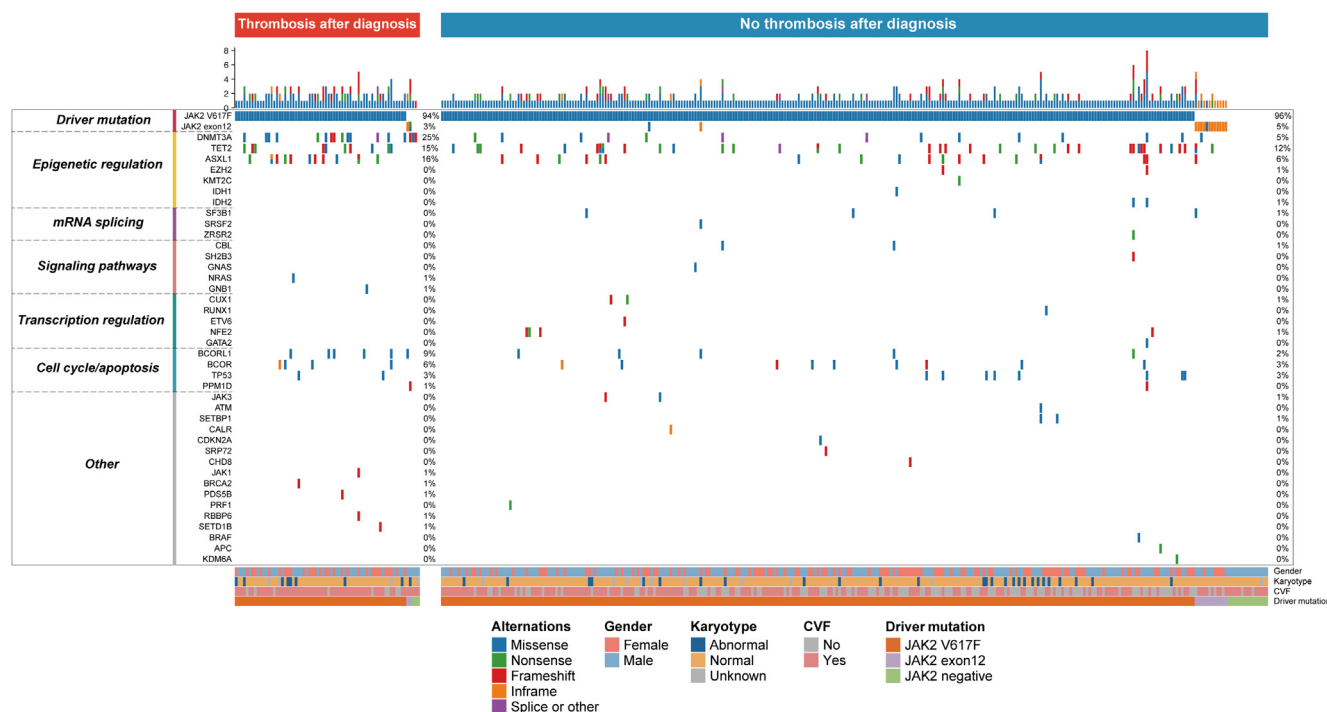


FIGURE 1 Genomic landscape of 372 patients with PV with or without thrombosis after diagnosis. Mutated genes are spread along the y-axis, and each column corresponds to a patient. Patients are grouped according to the presence (left side) or absence (right side) of thrombosis after diagnosis. Color code represents mutation status of the gene as indicated. Annotation rows at bottom include sex, karyotype, CVF, and driver mutation. Events where information is unknown are depicted in light gray. PV, polycythemia vera; CVF, cardiovascular risk factors.

TABLE 2 Multivariable Cox regression analysis of risk factors that are predictive of thrombosis after diagnosis.

	β -coefficient (95% CI)	HR (95% CI)	P	Score ^b
Age ≥ 60 ys	0.94 (0.41-1.47)	2.56 (1.51-4.35)	<.001	1
CVF	1.44 (0.69-2.19)	4.22 (2.00-8.92)	<.001	1.5
High-risk mutation for thrombosis ^a	1.47 (0.97-1.98)	4.35 (2.62-7.21)	<.001	1.5
Previous thrombosis	1.78 (1.19-2.37)	5.93 (3.29-10.68)	<.001	2

CVF, cardiovascular risk factor; HR, hazard ratio.

^aAt least 1 high-risk mutation for thrombosis, including *ASXL1*, *DNMT3A*, and *BCOR/BCORL1*.

^bTo assign a score for each predictor, we rounded the β -coefficient to the nearest half or whole number.

Comparing the cumulative incidence of thrombosis for patients with and without each risk factor, both log-rank tests and Gray tests showed significant difference ($P < .001$), indicating that these risk factors remained significant contributors to thrombosis after diagnosis, after accounting for the CR of death (Supplementary Figure 5 and Supplementary Table 5).

As shown in Supplementary Table 6, significant associations were also noted regarding arterial and venous events. Patients with older age ($P < .001$), CVF ($P < .001$), previous thrombosis ($P < .001$), and specific high-risk mutations for thrombosis (*ASXL1*, $P = .027$; *DNMT3A*, $P < .001$; *BCOR/BCORL1*, $P = .002$) experienced higher rates of arterial thrombosis after diagnosis. By contrast, patients with higher hemoglobin levels ($P = .006$), higher NLR ($P = .017$), and *DNMT3A* mutations ($P = .033$) were more likely to experience venous thrombosis after diagnosis.

3.4 | Development of a multiple factor-based prognostic score system for thrombosis in PV (MFPS-PV)

In the multivariable Cox regression analysis, we identified 4 independent risk factors for thrombosis after diagnosis, including age ≥ 60 years (HR 2.56, 95% CI, 1.51-4.35, $P < .001$), CVF (HR 4.22, 95% CI, 2.00-8.92, $P < .001$), at least one high-risk mutation for thrombosis (HR 4.35, 95% CI, 2.62-7.21, $P < .001$), and previous thrombosis (HR 5.93, 95% CI, 3.29-10.68, $P < .001$) (Table 2). For a complete interpretation of the predictors noted above, we calculated multiple statistics (Supplementary Table 7). The 5-year number needed to harm values were 9.21, 7.82, 5.00, and 4.53 for age ≥ 60 years, CVF, high-risk mutation for thrombosis, and previous thrombosis, respectively, demonstrating that previous thrombosis was the most detrimental factor, whereas age ≥ 60 years was less harmful. The population attributable fraction was 0.15, 0.44, 0.28, and 0.41 for those factors, respectively, indicating that a considerable fraction of thrombosis occurrence can be attributed to CVF or previous thrombosis.

To develop a multiple factor-based thrombosis scoring system, β -weighted risk points were assigned to risk factors identified in the

multivariable analysis (Table 2, age ≥ 60 years, 1 point; CVF, 1.5 points; at least one high-risk mutation for thrombosis, 1.5 points; previous thrombosis, 2 points). After summarizing the points, patients were divided into low-risk ($n = 96$, 25.8%, ≤ 1 point), intermediate-risk ($n = 146$, 39.2%, 1.5-2.5 points), and high-risk ($n = 130$, 35.0%, ≥ 3 points) groups. The incidence of thrombosis after diagnosis in the low-, intermediate-, and high-risk groups was 0.45, 1.46, and 14.00/100 patient-years, with an estimated 10-year TFS of 95.7%, 85.3%, and 18.4%, respectively (Supplementary Table 8). Significantly different TFS curves were observed among the 3 risk groups by the Kaplan-Meier analysis ($P < .001$) (Figure 2A).

3.5 | Comparison of predictive performance between MFPS-PV and conventional model

To assess the predictive performance of MFPS-PV, the risk stratification of patients in the training cohort according to the MFPS-PV and conventional model was compared. As shown in Figure 2B, 82 patients (41.4%) in the conventional low-risk group were still confirmed to be at low-risk by MFPS-PV, whereas 100 (50.5%) and 16 (8.1%) patients were regrouped as intermediate and high risk, respectively. Among the 16 patients who were regrouped as high risk, the actual thrombosis rate was high at 7.10/100 patient-years. Additionally, 114 patients (65.5%) in the conventional high-risk group were also considered to be at high risk according to the MFPS-PV, whereas 14 patients (8.1%) were regarded as low risk and 46 patients (26.4%) as an intermediate-risk category. Among the 14 patients who were regrouped as low risk, only one experienced thrombosis during follow-up, with a thrombosis rate of 1.89/100 patient-years. Therefore, MFPS-PV can identify high-risk patients ignored by the conventional model and non-high-risk patients over-evaluated by the conventional model, so as to avoid insufficient or excessive treatment.

The MFPS-PV outperformed the conventional model with higher C-statistic (0.87 [95% CI 0.83-0.91] vs 0.80 [95% CI 0.74-0.86]) and AUC (0.90 [95% CI 0.86-0.95] vs 0.82 [95% CI 0.76-0.89]) (Figure 2C). The calibration plot of MFPS-PV showed good agreement between the predictive and observed probability of thrombosis (Figure 2D). The Goodness-of-Fit test results were nonsignificant ($P = 0.204$), suggesting that the model was well calibrated. Therefore, MFPS-PV better predicted the incidence of thrombosis than the conventional model.

3.6 | External validation of MFPS-PV

A total of 195 WHO (2016)-defined patients with PV from the Second Hospital of Tianjin Medical University were included as an external validation cohort. The median age was 55 years (range 21-85) at diagnosis, and the sex distribution was close to 1:1 (male, 49.2%) (Supplementary Table 9). During a median follow-up of 96 months (range 12-576), 88 (45.1%) patients experienced thrombosis after diagnosis with an incidence rate of 6.7/100 patient-years. The MFPS-PV model was applied to the external cohort and divided patients into low-risk ($n = 52$), intermediate-risk ($n = 76$), and high-risk ($n = 67$) groups, with corresponding 10-year TFS rates of 77.1%, 66.4%, and 23.9%, respectively

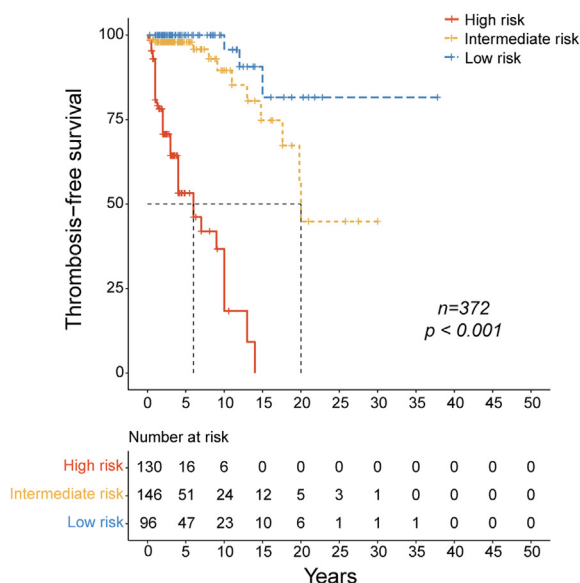
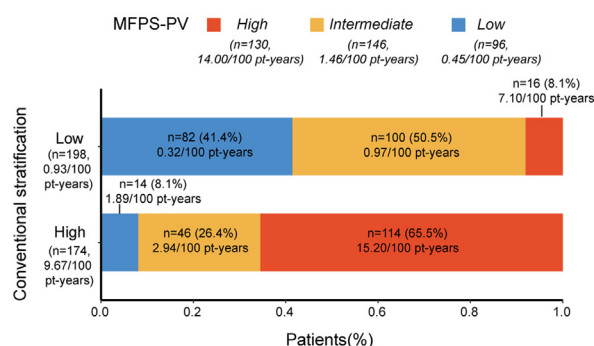
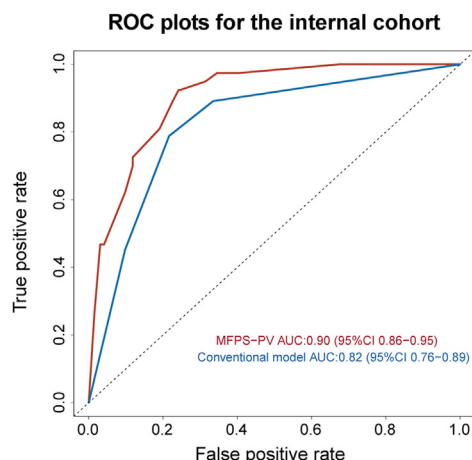
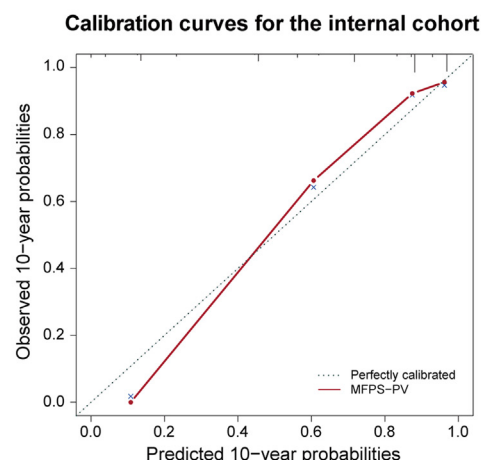
A MFPS-PV**B****C****D**

FIGURE 2 (A) Thrombosis-free survival of 372 patients with PV in the internal cohort stratified by MFPS-PV. Patients among different risk groups were compared by the log-rank test. (B) Risk distribution of 372 patients with PV in the internal cohort and their thrombosis rates according to the conventional model and MFPS-PV. Colored bars represent distribution of patients according to the MFPS-PV in the context of conventional low-risk or high-risk groups, respectively. The incidence of thrombosis for each category is shown. Conventional model, high risk, age ≥ 60 years, or/and thrombosis history; low risk, absence of both factors. Hundred patient-years, number of patients suffered from thrombosis after diagnosis per 100 patient-years. (C) ROC curves of MFPS-PV and conventional model in the internal cohort. ROC curves indicate that the discrimination of MFPS-PV (AUC 0.90, 95% CI 0.86-0.95) in predicting thrombosis is better than that of conventional model (AUC 0.82, 95% CI 0.76-0.89). False positive rate represents 1-specificity, and true positive rate represents sensitivity. ROC, Receiver-operating characteristic; AUC, area under the ROC curve. (D) Calibration curve of MFPS-PV in the internal cohort. Calibration curve indicates the agreement between the predicted and observed probabilities of thrombosis. The x-axis represents the predicted 10-year probabilities of thrombosis. The y-axis represents the observed 10-year probabilities of thrombosis. The diagonal-dashed line represents a perfectly calibrated prediction model. The red solid line represents the performance of MFPS-PV. A closer fit to the diagonal-dashed line indicates a better prediction.

(Supplementary Table 10). Kaplan-Meier curves confirmed the significant difference in TFS among the 3 risk groups in the external cohort ($n = 195$, $P < .001$) and both cohorts ($n = 567$, $P < .001$) (Figure 3A, B).

For prediction of thrombosis in the external cohort, the C-statistic was 0.79 (95% CI 0.74-0.84) for MFPS-PV and 0.76 (95% CI 0.71-0.82) for the conventional model. The AUC was 0.82 (95% CI 0.75-0.89) for

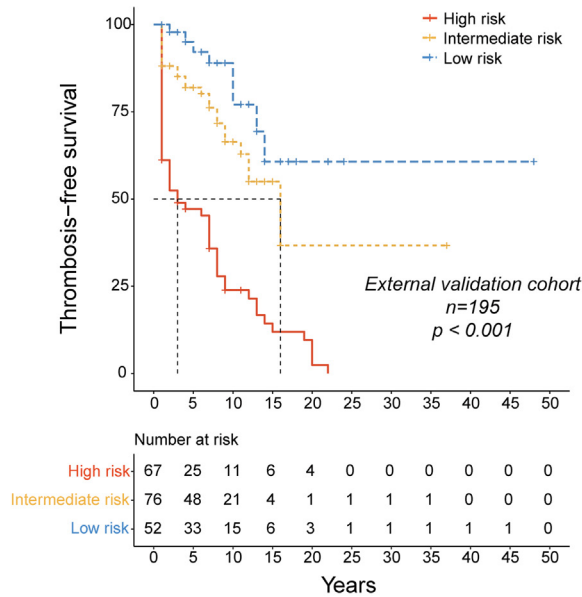
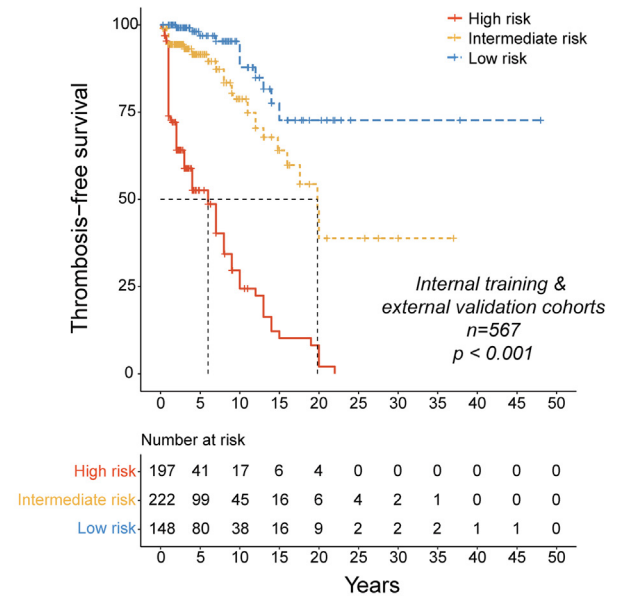
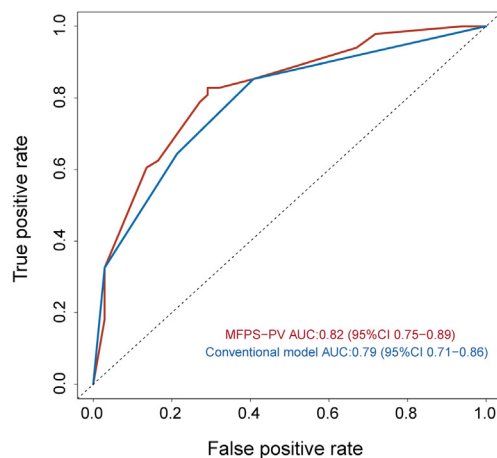
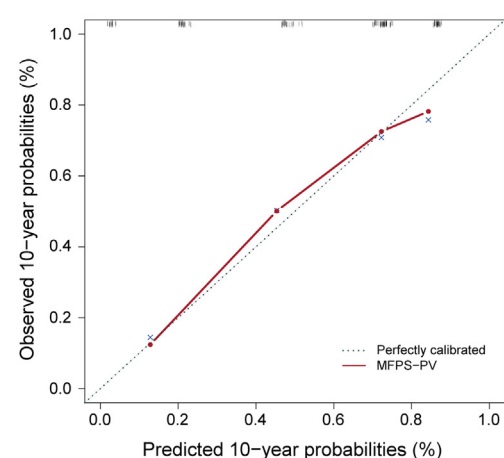
A MFPS-PV**B MFPS-PV****C****ROC plots for the external cohort****D****Calibration curves for the external cohort**

FIGURE 3 (A) Thrombosis-free survival of 195 patients with PV in the external validation cohort stratified by MFPS-PV. Patients among different risk groups were compared by the log-rank test. (B) Thrombosis-free survival of 567 patients with PV in both internal and external cohorts stratified by MFPS-PV. Patients among different risk groups were compared by log-rank test. (C) ROC curves of MFPV-PV and conventional model in the external validation cohort. ROC curves indicate that the discrimination of MFPS-PV (AUC 0.82, 95% CI 0.75-0.89) in predicting thrombosis is better than that of the conventional model (AUC 0.79, 95% CI 0.71-0.86). False positive rate represents 1-specificity, and true positive rate represents sensitivity. ROC, Receiver-operating characteristic; AUC, area under the ROC curve. (D) Calibration curve of MFPV-PV in the external validation cohort. Calibration curve indicates the agreement between the predicted and observed probabilities of thrombosis. The x-axis represents the predicted 10-year probabilities of thrombosis. The y-axis represents the observed 10-year probabilities of thrombosis. The diagonal-dashed line represents a perfectly calibrated prediction model. The red solid line represents the performance of MFPS-PV. A closer fit to the diagonal-dashed line indicates a better prediction.

MFPS-PV and 0.79 (95% CI 0.71-0.86) for the conventional model (Figure 3C). For the calibration plot, the actual observed probabilities of thrombosis were very close to the probabilities of thrombosis predicted by MFPS-PV (Figure 3D). The Goodness-of-Fit test showed a

nonsignificant statistic ($P = .632$). The results suggested that the MFPS-PV had a good performance of discrimination and calibration in the external validation set, which has similar basic characteristics but a much longer follow-up than the training cohort.

3.7 | Treatment patterns of patients in the training cohort

We analyzed the treatment patterns of 372 patients in the training cohort. Among the 372 patients, 208 patients (55.9%) received cytoreductive therapy, and 266 patients (71.5%) received antiplatelet therapy. Furthermore, 13 (3.5%) patients received oral anti-coagulants, including 8 patients (2.2%) with warfarin and 5 patients (1.3%) with rivaroxaban. In addition to drug administration, 110 (29.6%) patients underwent regular therapeutic phlebotomy (TP) for at least 3 times a year. The proportion of patients receiving cytoreductive therapy, antiplatelet therapy, or regular TP is similar among the low-, intermediate-, and high-risk groups of MFPS-PV. The MFPS-PV applied to patients in each treatment group all showed good discrimination with high C-statistics and good calibration with nonsignificant *P* values of the Goodness-of-Fit test (Supplementary Table 11).

4 | DISCUSSION

In this study, we analyzed clinical information and NGS data from a large cohort of patients with PV to reveal risk factors of thrombosis. After multivariable analysis, we identified age ≥ 60 years, CVF, previous thrombosis, and high-risk mutations (eg, *DNMT3A*, *ASXL1*, and *BCOR/BCORL1* mutations) as risk factors of thrombosis after diagnosis and then established a multiple factor-based prediction model classifying patients into low-risk, intermediate-risk, and high-risk groups. This new model incorporated genetic information into thrombosis prediction and successfully stratified patients at the first diagnosis according to their risk of thrombosis.

Compared with the conventional model, the new model has some differences and potential advantages. First, the MFPS-PV was developed on the basis of the 2016 WHO-defined PV [22], whereas the conventional model was on the basis of PVSG-defined PV [9,10]. The 2016 WHO criteria recognize the importance of bone marrow morphology; thus, patients previously considered to be “masked” or “prodromic” PV were included according to the new criteria [27,28]. In fact, “masked” or “prodromic” PV displays a higher risk of thrombosis in younger patients probably because of the lower intensity of treatment [29]. Hence, MFPS-PV is more suitable for patients diagnosed using the current diagnostic standard than the traditional model.

Second, the MFPS-PV model highlights the significance of CVF. The current guideline recommendations from the National Comprehensive Cancer Network clearly emphasize that CVF in patients with PV needs to be controlled [7]. Previous studies have also reported that CVF may increase the risk of thrombosis in patients with PV [14,15]. Moreover, some experts commonly provide different treatment suggestions according to the presence or absence of CVF because it plays an important role in the management of thrombosis [1].

Third and notably, we have incorporated genetic aberrations into the prognostic stratification system for the first time. The wide application of NGS in a large cohort of patients with PV allows us to

evaluate the impact of specific mutations on thrombosis, which has not been mentioned in most previous studies. Several thrombogenic mutations have been proposed in our study, including mutations in *DNMT3A*, *ASXL1*, and *BCOR/BCORL1*. Interestingly, those epigenetic regulators *DNMT3A* and *ASXL1* accounted for more than half of the clonal hematopoiesis of indeterminate potential (CHIP) [30]. Recent studies have revealed that CHIP is associated with an increased risk of cardiovascular diseases and atherosclerosis, mainly because of the upregulation of inflammatory signaling by the innate and adaptive immune systems [31–33]. The effect of CHIP on thrombosis occurrence is as great as or even greater than the effect of those commonly recognized cardiovascular risk factors, such as hypertension, diabetes, hyperlipidemia, obesity, and smoking [30]. In addition, most CHIP-associated mutations are drivers of hematological malignancy; eg, *JAK2* mutations occur in more than 60% of patients with MPN and are important MPN-driver mutations [34]. *DNMT3A* and *ASXL1* are commonly mutated in MPNs (5%–10%) and have proved to be associated with MPN initiation and progression [34–36]. Therefore, we can predict that individuals with CHIP-associated mutations would develop thrombotic events or hematological malignancies at a rate above the background. For the risk of thrombotic events, the HR associated with CHIP in patients with PV (HR 4.4) in our study is higher than that in the general population (HR 1.8) [37], indicating that the influence of CHIP on thrombosis may be amplified by a PV background. A previous study has demonstrated that mutations in *DNMT3A*, *TET2*, and *ASXL1* are strongly correlated with thrombosis occurrence in patients with PV, even if they evaluated a limited number of patients [11]. These studies support our findings that *DNMT3A* and *ASXL1* mutations are important thrombogenic mutations in patients with PV.

BCOR (BCL6 Corepressor) and *BCORL1* (BCL6 Corepressor-like 1) are 2 homologous genes located on chromosome X that have been described in approximately 4%–6% of myelodysplastic syndrome cases and 16% of blastic phase chronic myeloid leukemia cases. Mutations in *BCOR/BCORL1* are associated with shortened survival in myeloid malignancies [38–41]. In aplastic anemia, patients with *BCOR* and *BCORL1* mutations have an improved response to immunosuppression [42]. Although reports of *BCOR/BCORL1* mutations involved in Ph⁺ MPNs are rare, there is still evidence supporting their role in treatment resistance and poor prognosis [43–45]. To the best of our knowledge, this is the first study to reveal that *BCOR/BCORL1* mutations may increase the risk of thrombosis in patients with PV.

Fourth, the MFPS-PV displayed better performance in terms of discrimination and calibration compared with the conventional model. When regrouping according to the MFPS-PV, nearly 10% of patients in the conventional low-risk group were regrouped as high risk with a high incidence of thrombosis. Meanwhile, more than one-third of patients in the conventional high-risk group were regrouped as having low or intermediate risk. The actual thrombosis rates during follow-up demonstrated that MFPS-PV better stratified patients than the conventional model and had the potential to avoid under- or over-therapy in some patients. Meanwhile, the higher C-statistic of MFPS-PV in both the derivation and validation cohorts confirmed that

incorporating genetic information and CVF may increase the accuracy of thrombosis prediction. A well-fitted calibration plot in the external validation confirmed the general applicability of MFPS-PV in different patient cohorts.

In addition to the above advantages, there are some limitations regarding MFPS-PV. First, the number of thrombotic events analyzed in our study is limited, and 93% are arterial. Although this frequency of arterial thrombosis is higher than those reported in patients of European descent (50%-65%) [5,6], it is consistent with previous studies conducted in China (91%-96%) [46–48]. Inevitably, the role of venous thrombosis in model development may be diminished. Therefore, a larger sample size and longer follow-ups are needed in our future studies to allow more events to be analyzed. Second, we found no significance of *JAK2 V617F* mutation status but the borderline significance of higher *JAK2 V617F* allele burden in association with thrombosis in our cohort. Previous studies have reported different results regarding the contribution of *JAK2 V617F* mutation status to thrombosis development [14,17,49–52], whereas most studies confirmed that patients with higher *JAK2 V617F* allele burden were at higher risk of venous, but not of arterial thrombosis [16–18]; therefore, the borderline significance of higher *JAK2 V617F* allele burden in thrombosis can be explained by the dominance of arterial events in our cohort. We proposed that MFPS-PV was more suitable for patients in China who developed mainly arterial thrombosis. We further distinguished thrombotic events according to their location but failed to find an association between *JAK2 V617F* allele burden and venous thrombosis because of the limited number of venous events in our cohort. Third, because the goal of our prediction model is to stratify patients at first diagnosis, we did not include treatment in the model. A well-designed prospective study, instead of a retrospective study, is needed to develop a dynamic prediction model that includes treatment. However, we found similar treatment patterns among patients in the 3 risk groups defined by MFPS-PV so that the effect of treatment on different thrombosis rates among the 3 risk groups can be minimized. The good discrimination and calibration of the model for patients in each treatment group also confirmed the robustness of the model.

5 | CONCLUSIONS

By integrating basic clinical data together with thrombotic high-risk mutations, we presented a multiple factor-based, 3-tiered, and 4-factor thrombosis prediction model for the 2016 WHO-defined PV. This model improves the prediction power of thrombosis and is valuable for practical thrombosis management. Modified treatment selection on the basis of the model needs to be assessed by future studies.

ACKNOWLEDGMENTS

We thank all members of our team for their technical support and suggestions.

FUNDING

This project was supported by grants from the National Natural Science Foundation of China (82270152, 81970121, 82000136), CAMS Innovation Fund for Medical Sciences (2021-I2M-1-073, 2022-I2M-2-003, 2021-I2M-1-003, 2020-I2M-C&T-B-086), Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (2020-PT310-011), National Key Research and Development Program of China (2019YFA0110802), Clinical Research Fund of National Center for Clinical Medical Research of Hematology Diseases (2023NCRCA0109), and Haihe Laboratory of Cell Ecosystem Innovation Fund (22HHXBSS00022).

ETHICS STATEMENT

The study was approved by the Institutional Ethics Committee of the Institute of Hematology and Blood Diseases Hospital (Tianjin, China) and the Second Hospital of Tianjin Medical University (Tianjin, China) in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

W.J.G. collected data, analyzed data and wrote the paper. L.Z., R.F.F., and J.B. designed the study, analyzed data and revised the manuscript. Y.H.Z., T.S., and M.K.J. collected data and analyzed data. X.F.L., F.X., Y.F.C., W.L., H.Y.L., W.T.W., Y.C., and R.C.Y. helped with data collection.

RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

DATA AVAILABILITY

Additional information is provided in the Supplementary file.

REFERENCES

- [1] Tefferi A, Barbui T. Polycythemia vera and essential thrombocythemia: 2021 update on diagnosis, risk-stratification and management. *Am J Hematol*. 2020;95:1599–613.
- [2] Cerquozzi S, Tefferi A. Blast transformation and fibrotic progression in polycythemia vera and essential thrombocythemia: a literature review of incidence and risk factors. *Blood Cancer J*. 2015;5:e366.
- [3] Tefferi A, Guglielmelli P, Larson DR, Finke C, Wassie EA, Pieri L, et al. Long-term survival and blast transformation in molecularly annotated essential thrombocythemia, polycythemia vera, and myelofibrosis. *Blood*. 2014;124:2507–13.
- [4] Szuber N, Mudireddy M, Nicolosi M, Penna D, Vallapureddy RR, Lasho TL, et al. 3023 Mayo Clinic Patients With Myeloproliferative Neoplasms: Risk-Stratified Comparison of Survival and Outcomes Data Among Disease Subgroups. *Mayo Clin Proc*. 2019;94:599–610.
- [5] Marchioli R, Finazzi G, Landolfi R, Kutti J, Gisslinger H, Patrono C, et al. Vascular and neoplastic risk in a large cohort of patients with polycythemia vera. *J Clin Oncol*. 2005;23:2224–32.
- [6] Barbui T, Carobbio A, Rumi E, Finazzi G, Gisslinger H, Rodeghiero F, et al. In contemporary patients with polycythemia vera, rates of thrombosis and risk factors delineate a new clinical epidemiology. *Blood*. 2014;124:3021–3.

- [7] Mesa RA, Jamieson C, Bhatia R, Deininger MW, Fletcher CD, Gerds AT, et al. NCCN Guidelines insights: myeloproliferative neoplasms, version 2.2018. *J Natl Compr Canc Netw*. 2017;15:1193–207.
- [8] Barbui T, Tefferi A, Vannucchi AM, Passamonti F, Silver RT, Hoffman R, et al. Philadelphia chromosome-negative classical myeloproliferative neoplasms: revised management recommendations from European LeukemiaNet. *Leukemia*. 2018;32:1057–69.
- [9] Berk PD, Wasserman LR, Fruchtmann SM, Goldberg JD. Treatment of polycythemia vera: a summary of clinical trials conducted by the polycythemia vera study group. In: Wasserman LR, Berk PD, Berlin NI, eds. *Polycythemia Vera and the Myeloproliferative Disorders*. WB Saunders; 1995, p. 166–194.
- [10] Pearson TC, Messiney M, Westwood N, Green AR, Bench AJ, Green AR, et al. A polycythemia vera updated: diagnosis, pathobiology, and treatment. *Hematology Am Soc Hematol Educ Program*. 2000:51–68.
- [11] Segura-Díaz A, Stuckey R, Florido Y, González-Martín JM, López-Rodríguez JF, Sánchez-Sosa S, et al. Thrombotic risk detection in patients with polycythemia vera: the predictive role of DNMT3A/TET2/ASXL1 mutations. *Cancers (Basel)*. 2020;12:934.
- [12] Tefferi A, Guglielmelli P, Lasho TL, Coltro G, Finke CM, Loscocco GG, et al. Mutation-enhanced international prognostic systems for essential thrombocythaemia and polycythaemia vera. *Br J Haematol*. 2020;189:291–302.
- [13] Knudsen TA, Skov V, Stevenson K, Werner L, Duke W, Laurore C, et al. Genomic profiling of a randomized trial of interferon-alpha vs hydroxyurea in MPN reveals mutation-specific responses. *Blood Adv*. 2022;6:2107–19.
- [14] Cerquozzi S, Barraco D, Lasho T, Finke C, Hanson CA, Ketterling RP, et al. Risk factors for arterial versus venous thrombosis in polycythemia vera: a single center experience in 587 patients. *Blood Cancer J*. 2017;7:662.
- [15] Barbui T, Vannucchi AM, Carobbio A, Rumi E, Finazzi G, Gisslinger H, et al. The effect of arterial hypertension on thrombosis in low-risk polycythemia vera. *Am J Hematol*. 2017;92:E5–6.
- [16] Alvarez-Larrán A, Bellosillo B, Pereira A, Kerguelen A, Hernández-Boluda JC, Martínez-Avilés L, et al. JAK2V617F monitoring in polycythemia vera and essential thrombocythemia: clinical usefulness for predicting myelofibrotic transformation and thrombotic events. *Am J Hematol*. 2014;89:517–23.
- [17] Borowczyk M, Wojtaszewska M, Lewandowski K, Gil L, Lewandowska M, Lehmann-Kopydłowska A, et al. The JAK2 V617F mutational status and allele burden may be related with the risk of venous thromboembolic events in patients with Philadelphia-negative myeloproliferative neoplasms. *Thromb Res*. 2015;135:272–80.
- [18] Guglielmelli P, Loscocco GG, Mannarelli C, Rossi E, Mannelli F, Ramundo F, et al. JAK2V617F variant allele frequency >50% identifies patients with polycythemia vera at high risk for venous thrombosis. *Blood Cancer J*. 2021;11:199.
- [19] De Stefano V, Za T, Rossi E, Vannucchi AM, Ruggeri M, Elli E, et al. Leukocytosis is a risk factor for recurrent arterial thrombosis in young patients with polycythemia vera and essential thrombocythemia. *Am J Hematol*. 2010;85:97–100.
- [20] Landolfi R, Di Gennaro L, Barbui T, De Stefano V, Finazzi G, Marfisi R, et al. Leukocytosis as a major thrombotic risk factor in patients with polycythemia vera. *Blood*. 2007;109:2446–52.
- [21] Parasuraman S, Yu J, Paranagama D, Shrestha S, Wang L, Baser O, et al. Hematocrit levels and thrombotic events in patients with polycythemia vera: an analysis of Veterans Health Administration data. *Ann Hematol*. 2019;98:2533–9.
- [22] Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127:2391–405.
- [23] Siegerink B, Rohmann JL. Impact of your results: beyond the relative risk. *Res Pract Thromb Haemost*. 2018;2:653–7.
- [24] Verduijn M, Grootendorst DC, Dekker FW, Jager KJ, le Cessie S. The analysis of competing events like cause-specific mortality—beware of the Kaplan-Meier method. *Nephrol Dial Transplant*. 2011;26:56–61.
- [25] Ay C, Posch F, Kaider A, Zielinski C, Pabinger I. Estimating risk of venous thromboembolism in patients with cancer in the presence of competing mortality. *J Thromb Haemost*. 2015;13:390–7.
- [26] Alba AC, Agoritsas T, Walsh M, Hanna S, Iorio A, Devereaux PJ, et al. Discrimination and calibration of clinical prediction models: users' guides to the medical literature. *JAMA*. 2017;318:1377–84.
- [27] Barbui T, Thiele J, Gisslinger H, Finazzi G, Carobbio A, Rumi E, et al. Masked polycythemia vera (mPV): results of an international study. *Am J Hematol*. 2014;89:52–4.
- [28] Passamonti F, Maffioli M. Update from the latest WHO classification of MPNs: a user's manual. *Hematology Am Soc Hematol Educ Program*. 2016;2016:534–42.
- [29] Lussana F, Carobbio A, Randi ML, Elena C, Rumi E, Finazzi G, et al. A lower intensity of treatment may underlie the increased risk of thrombosis in young patients with masked polycythaemia vera. *Br J Haematol*. 2014;167:541–6.
- [30] Jaiswal S, Ebert BL. Clonal hematopoiesis in human aging and disease. *Science*. 2019;366:eaan4673.
- [31] Fuster JJ, MacLauchlan S, Zuriaga MA, Polackal MN, Ostriker AC, Chakraborty R, et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science*. 2017;355:842–7.
- [32] Sano S, Oshima K, Wang Y, Katanasaka Y, Sano M, Walsh K. CRISPR-mediated gene editing to assess the roles of Tet2 and Dnmt3a in clonal hematopoiesis and cardiovascular disease. *Circ Res*. 2018;123:335–41.
- [33] Frick M, Chan W, Arends CM, Habesreiter R, Halik A, Heuser M, et al. Role of donor clonal hematopoiesis in allogeneic hematopoietic stem-cell transplantation. *J Clin Oncol*. 2019;37:375–85.
- [34] Grinfeld J, Nangalia J, Baxter EJ, Wedge DC, Angelopoulos N, Cantrill R, et al. Classification and personalized prognosis in myeloproliferative neoplasms. *N Engl J Med*. 2018;379:1416–30.
- [35] Jacquelin S, Straube J, Cooper L, Vu T, Song A, Bywater M, et al. Jak2V617F and Dnmt3a loss cooperate to induce myelofibrosis through activated enhancer-driven inflammation. *Blood*. 2018;132:2707–21.
- [36] Andréasson B, Pettersson H, Wasslavik C, Johansson P, Palmqvist L, Asp J. ASXL1 mutations, previous vascular complications and age at diagnosis predict survival in 85 WHO-defined polycythaemia vera patients. *Br J Haematol*. 2020;189:913–9.
- [37] Jaiswal S, Natarajan P, Silver AJ, Gibson CJ, Bick AG, Shvartz E, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. *N Engl J Med*. 2017;377:111–21.
- [38] Adnan Awad S, Kankainen M, Ojala T, Koskenvesa P, Eldfors S, Ghimire B, et al. Mutation accumulation in cancer genes relates to nonoptimal outcome in chronic myeloid leukemia. *Blood Adv*. 2020;4:546–59.
- [39] Loy K, Zenger M, Meggendorfer M, Hutter S, Kern W, Haeflrich T, et al. Analysis of mechanisms of blast crisis in chronic myeloid leukemia by whole genome sequencing. *Blood*. 2020;136:19–19.
- [40] Gangat N, Patnaik MM, Tefferi A. Myelodysplastic syndromes: contemporary review and how we treat. *Am J Hematol*. 2016;91:76–89.
- [41] Damm F, Chesnais V, Nagata Y, Yoshida K, Scourzac L, Okuno Y, et al. BCOR and BCORL1 mutations in myelodysplastic syndromes and related disorders. *Blood*. 2013;122:3169–77.
- [42] Yoshizato T, Dumitriu B, Hosokawa K, Makishima H, Yoshida K, Townsley D, et al. Somatic mutations and clonal hematopoiesis in aplastic anemia. *N Engl J Med*. 2015;373:35–47.

- [43] Zaidi U, Shahid S, Fatima N, Ahmed S, Sufaida G, Nadeem M, et al. Genomic profile of a patient with triple negative essential thrombocythemia, unresponsive to therapy: A case report and literature review. *J Adv Res.* 2017;8:375–8.
- [44] Luque Paz D, Jouanneau-Courville R, Riou J, Ianotto JC, Boyer F, Chauveau A, et al. Leukemic evolution of polycythemia vera and essential thrombocythemia: genomic profiles predict time to transformation. *Blood Adv.* 2020;4:4887–97.
- [45] Sportoletti P, Sorcini D, Falini B. BCOR gene alterations in hematologic diseases. *Blood.* 2021;138:2455–68.
- [46] Liu D, Li B, Xu Z, Zhang P, Qin T, Qu S, et al. RBC distribution width predicts thrombosis risk in polycythemia vera. *Leukemia.* 2022;36:566–8.
- [47] Zhang Y, Zhou Y, Wang Y, Teng G, Li D, Wang Y, et al. Thrombosis among 1537 patients with JAK2V617F-mutated myeloproliferative neoplasms: risk factors and development of a predictive model. *Cancer Med.* 2020;9:2096–105.
- [48] Bai J, Xue Y, Ye L, Yao J, Zhou C, Shao Z, et al. Risk factors of long-term incidences of thrombosis, myelofibrosis and evolution into malignance in polycythemia vera: a single center experience from China. *Int J Hematol.* 2008;88:530–5.
- [49] Yesilova AM, Yavuzer S, Yavuzer H, Cengiz M, Toprak ID, Hanedar E, et al. Analysis of thrombosis and bleeding complications in patients with polycythemia vera: a Turkish retrospective study. *Int J Hematol.* 2017;105:70–8.
- [50] Kaifé A, Kirschner M, Wolf D, Maintz C, Hänel M, Gattermann N, et al. Bleeding, thrombosis, and anticoagulation in myeloproliferative neoplasms (MPN): analysis from the German SAL-MPN-registry. *J Hematol Oncol.* 2016;9:18.
- [51] Koren-Michowitz M, Landman J, Cohen Y, Rahimi-Levene N, Salomon O, Michael M, et al. JAK2V617F allele burden is associated with transformation to myelofibrosis. *Leuk Lymphoma.* 2012;53:2210–3.
- [52] Ohyashiki K, Akahane D, Gotoh A, Ito Y, Tauchi T, Miyazawa K, et al. Uncontrolled thrombocytosis in polycythemia vera is a risk for thrombosis, regardless of JAK2(V617F) mutational status. *Leukemia.* 2007;21:2544–5.

SUPPLEMENTARY MATERIAL

The online version contains supplementary material available at <https://doi.org/10.1016/j.rpth.2023.100132>