Molecular and Clinical Risk Factors Associated with Thrombosis and Bleeding in Myelofibrosis Patients

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Abstract

Background The risk of thrombosis and bleeding in myelofibrosis (MF) has been historically underappreciated. We sought to investigate potential molecular and clinical risk factors for venous (VTE) and arterial (ATE) thrombotic events as well as bleeding episodes.

Methods Data from 246 consecutive MF patients were analyzed. Driver mutations were tested in 191 patients.

Results In total, 181 mutations were found in 177 MF patients: 118 (61.8%) patients showed *JAK2-V617F*, 50 patients (26.2%) showed *CALR*, and 6 patients (3.1%) showed *MPL* mutations. Two patients were *JAK2-V617F* and *MPL* positive and one patient was positive for all three genes. Fourteen (7.3%) patients were triple negative. The *JAK2-V617F* allele burden was assessed in 63 *JAK2-V617F*-mutated patients, revealing a median of 35.6% (range: 5.0–96.0). At the time of MF diagnosis and during follow-up, 84 thrombotic events (52 VTEs and 32 ATEs) were observed, corresponding to 6.6% of patients per year. A significant association was found between *JAK2-V617F* mutation (OR: 2.5, 95% CI: 1.1–5.6) and prior VTE (OR: 7.6, 95% CI: 2.1–27.1) with an increased risk of VTE. Patients with prefibrotic MF had a higher rate of ATE than patients with overt MF. Hemorrhagic events occurred in 34 (13.8%) patients, corresponding to 3.8% of patients per year. Fibrosis grade 3 was associated with bleeding risk (OR: 3.4, 95% CI: 1.2–9.2, $p = 0.02$).

Conclusions The presence of the *JAK2-V617F* mutation, regardless of allele burden, and prior thrombosis were strongly associated with an increased risk of VTE. Patients with prefibrotic MF might be considered at high risk for developing ATE.

Keywords
- venous thromboembolism
- arterial thromboembolism
- bleeding
- myelofibrosis

Introduction

Myelofibrosis (MF) can manifest as a de novo disease (primary myelofibrosis [PMF]) or following a previous diagnosis of polycythemia vera or essential thrombocythemia (post-PV or post-ET MF).[1] Transformation to acute myeloid leukemia (AML), both venous (VTE) and arterial (ATE) thrombotic events, and infection are the most common causes of death in both PMF and secondary MF patients.[2] Compared with patients with PV and ET, the risk of thrombosis and bleeding

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in MF patients has been historically underappreciated. In the WHO 2016 classification,[3] prefibrotic MF (pre-MF) was defined as a distinct entity for the first time. Pre-MF at diagnosis typically mimics ET with isolated thrombocytosis and often presents asymptomatically, unlike classic "overt fibrotic" MF. However, pre-PMF can become highly symptomatic and has a worse outcome compared with ET.[4] Although thrombotic and bleeding events in pre-MF patients may be more common than in PV or ET[5–7] and even distinct from other MF subtypes,[8–10] no score has been developed and validated to assess thrombotic and bleeding risk in this patient group.

In the last decade, the role of older age, medical history, cardiovascular risk factors, *JAK2-V617F* and non-driver mutations (*TET2* and *DNMT3A*), blood cell counts, and their dysfunctions have been described as associated with an increased thrombotic risk in MPN patients.[11–27] Thrombocytopenia secondary to marrow failure, platelet dysfunction, acquired von Willebrand syndrome (AvWS), antithrombotic drug use, *CALR* and *ASXL1* mutations, and varices caused by portal hypertension have been mentioned as possible causes of bleeding.[8,10,28–30] Unfortunately, there is a notable scarcity of research specifically addressing patients with MF, with existing studies often limited by small sample sizes.

A comprehensive understanding of the pathophysiology is necessary for risk prediction, improving thromboprophylaxis in daily practice, developing targeted therapy, reduction of mortality, and healthcare costs. The aim of this study was to explore potential molecular and clinical risk factors for VTE and ATE, as well as bleeding events, in a substantial cohort of MF patients.

Methods

Patients

Retrospective data of 246 MF patients treated from 2005 to 2023 at Jena University Hospital were analyzed. *JAK2-V617F*, *CALR*, and *MPL* mutations were tested in 191 patients. The *JAK2-V617F* allele burden was assessed in 63/121 *JAK2-V617F* mutated patients. Patients were divided into two groups: those with *JAK2-V617F* allele burden >50% and those with *JAK2-V617F* allele burden $\leq$ 50%.[26,31]

Clinical variables were age, sex, cardiovascular risk factors (smoking, arterial hypertension, diabetes mellitus, obesity, and hypercholesterolemia), prior thrombosis or bleeding (1 year before MF diagnosis and earlier), anticoagulation or antiplatelet drugs at baseline, spleen size, bone marrow fibrosis grade, DIPSS/DIPSS plus, and, if possible, MIPSS70 risk scores. These scores aid in predicting disease progression and guiding treatment decisions in MF patients and include age, hemoglobin level, leukocyte count, platelet count, and the presence of constitutional symptoms (DIPSS) or DIPSS criteria, as well as additional criteria such as chromosomal abnormalities and transfusion dependency (DIPSS plus). The MIPSS70 score includes, among other criteria, the presence of high-risk mutations. The current study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University Jena (Reg. No.: 2021–2094-Material).

Laboratory Parameters

Laboratory parameters were blood counts, creatinine, albumin, C-reactive protein, lactate dehydrogenase, and global clotting assays.

Mutation Analyses

After erythrocyte lysis, DNA was isolated using the MagnaPure system (Roche, Mannheim, Germany) or the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's recommendations. The hotspot *JAK2-V617F*, the *MPL* exon10 and *CALR* exon 9 sequencing, was performed on the Illumina MiSeq (San Diego, California, United States) and analyzed with the JSI module SeqNext (Kippenheim, Germany)[32] or using the amplification-refractory mutation system (*JAK2*-ARMS-PCR, Mastercycler X50, Eppendorf, Germany) according to standard techniques.[33] To analyze *CALR* and *MPL* mutations, DNA fragment analysis and Sanger sequencing were performed on the 3500 Genetic Analyser (Life Technologies), respectively.[34]

Statistics

The primary endpoint was the occurrence of a thrombotic or bleeding event, acute leukemia transformation, or death at any time. VTE includes deep vein thrombosis (DVT), pulmonary embolism (PE), splanchnic vein thrombosis (SVT), and cerebral venous sinus thrombosis (CVT). ATE includes ischemic stroke or transient ischemic attack (TIA), peripheral vascular disease (PVD), or acute coronary syndrome (ACS). Variables were reported as median with interquartile range (IQR). The medians of the two groups were compared using the Wilcoxon–Mann–Whitney test. Categorical variables were reported as counts and proportions and compared using chi-square or Fisher's exact test. Univariable and multivariable logistic regression analyses were used to identify independent predictors of events. Results were presented as odds ratio (OR) with 95% confidence interval (CI). Median follow-up and overall survival (OS) were estimated using the Kaplan–Meier method. All reported p-values are two-sided, and $p < 0.05$ was considered statistically significant. SPSS Statistics 28.0 was used for statistical calculations.

Results

In total 246 MF patients were analyzed including 39 (15.9%) patients with pre-MF, 153 (62.2%) with overt MF, and 54 (22.0%) with secondary MF. Pre-MF was diagnosed only in patients after 2016 according to the criteria outlined in the WHO 2016 classification. The median age at the time of diagnosis was 64 years (range, 26–82), with 58.1% being male. Mutation analysis was performed for 191/246 patients (77.6%): 118/191 (61.8%) patients showed *JAK2-V617F*, 50/191 (26.2%) patients showed *CALR*, 6/191 (3.1%) patients showed *MPL* mutation, 14 (7.3%) were triple negative, two patients were *JAK2-V617F* and *MPL* positive, and 1 patient

was positive for all three driver genes. The *JAK2-V617F* allele burden was assessed in 63 of 121 patients, revealing a median of 35.6% (range: 5.0–96.0), and 17 of 63 patients (26.9%) had an allele burden > 50%. Further details on molecular and clinical patient characteristics are provided in ►Table 1.

Thrombotic Events

Twenty-four (9.8%) patients had a prior history of VTE or ATE: VTE manifested as a DVT and/or PE in four ($n = 4/24$, 16.7%), and as an SVT in three ($n = 3/24$, 12.5%) patients; ATE presented as an ischemic stroke or TIA in five ($n = 5/24$, 20.8%), and as an ACS in eight ($n = 8/24$, 33.3%) patients; four ($n = 4/24$, 16.7%) patients had both thrombotic events. In total, 84 thrombotic events (52 venous and 32 arterial) in 70 patients were observed, corresponding to an incidence of 6.6% patients per year (pt/y): for VTE 4.4% pt/y and ATE 2.2% pt/y. Localizations of thromboembolic events are shown in ►Table 2. One case of splanchnic thrombosis was diagnosed following splenectomy. Among the 21 patients diagnosed with SVT, 15 (71.4%) of them had a *JAK2-V617F* mutation, two carried a *CALR* mutation, and four patients were not examined.

Risk Factors of VTE and ATE

The multivariable analysis revealed a significant association between *JAK2-V617F* mutations (OR: 2.5, 95% CI: 1.1–5.6) and prior VTE (OR: 7.6, 95% CI: 2.1–27.1) with an increased risk of developing VTE, but not ATE. At the time of MF diagnosis, for the reason of previous thrombotic events or atrial fibrillation, 37 (15.0%) patients received anticoagulation: 13 patients received vitamin K antagonists, 16 patients received low-molecular-weight heparin (of which 7 had a therapeutic dose), and 8 patients received direct oral anticoagulants. The *JAK2-V617F* allele burden did not impact the risk of VTE: allele burden ≤ 50% versus allele burden > 50% ($p = 0.5$). The multivariable analysis showed that arterial hypertension was the only significant risk factor of ATE

Table 1 Molecular and clinical characteristics of the study cohort ($n = 246$)

Variables	No. of patients (%)
Diagnosis	
– Prefibrotic MF	39 (15.9%)
– Primary overt fibrotic MF	153 (62.2%)
– Post-PV MF	32 (13%)
– Post-ET MF	22 (8.9%)
Prior history of thrombosis	**24 (9.8%)**
– Venous	7 (29.2%)
– Arterial	13 (54.2%)
– Both	4 (16.6%)
Thrombosis as diagnostic event	**18 (7.3%)**
– Venous	7 (38.9%)
– Arterial	9 (50%)
– Both	2 (11.1%)
Thrombosis during follow-up	**59 (24%)**
– Venous	38 (64.4%)
– Arterial	16 (27.1%)
– Both	5 (8.5%)
Cardiovascular risk factors	
– Smoking	32 (13.0%)
– Arterial hypertension	113 (45.7%)
– Diabetes mellitus	37 (15.0%)
– Hypercholesterolemia	32 (13.0%)
– Obesity (body mass index >30)	16 (6.5%)
Prior history of bleeding	6 (2.4%)
Bleeding during follow-up	34 (13.8%)
Major bleeding[a]	16/34 (47%)
Antiplatelet therapy at the baseline	84 (34.1%)
Anticoagulation at the baseline	37 (15.0%)
Driver mutations	
– *JAK2-V617F*	118/191 (61.8%)
– *CALR*	50/191 (26.2%)
– *MPL*	6/191 (3.1%)
– Triple negative	14/191 (7.3%)
– Other	3/191 (1.6%)[b]
Bone marrow fibrosis grade	**181**
– 0	36 (19.9%)
– 1	28 (15.5%)
– 2	54 (29.8%)
– 3	63 (34.8%)
Spleen size	**207**
– Normal	40 (19.3%)
– Enlarged	167 (80.7%)
DIPPS/DIPSS-plus score	**151**
– Low	23 (15.2%)
– Intermediate-1	38 (25.2%)
– Intermediate-2	54 (35.8%)
– High	36 (23.8%)
MIPSS70 score	**87**
– Low	26 (29.9%)
– Intermediate	26 (29.9%)
– High	35 (40.2%)

Abbreviations: ET, essential thrombocythemia; MF, myelofibrosis; PV, polycythemia vera.
[a]Symptomatic bleeding in a critical organ, or associated with a hemoglobin decrease of more than 2 mmol/L or transfusion.
[b]*JAK2-V617F* and *MPL* positive—two patients, triple positive—one patient.

Table 2 Clinical manifestation of thrombosis at the time of diagnosis and during follow-up

	Prefibrotic MF, $n = 39$	Overt MF, $n = 153$	Secondary MF, $n = 54$
Venous thromboembolism, $n = 52$	10/39 (25.6%)	31/153 (20.3%)	10/54 (18.5%)
Deep vein thrombosis	2/39 (5.2%)	7/153 (4.6%)	7/54 (12.9%)
Pulmonary embolism	1/39 (2.6%)	5/153 (3.3%)	1/54 (1.9%)
Deep vein thrombosis + pulmonary embolism	2/39 (5.2%)	2/153 (1.3%)	–
Splanchnic vein thrombosis	5/39 (12.8%)	14/153 (9.2%)	2/54 (3.7%)
Cerebral venous sinus thrombosis	–	2/153 (1.3%)	–
Ocular vein thrombosis and central line catheter thrombosis	–	2/153 (1.3%)	–
Arterial thromboembolism, $n = 32$	11/39 (28.2%)[a]	13/153 (8.5%)	8/54 (14.8%)
Acute coronary syndrome	–	4/153 (2.6%)	2/54 (3.7%)
Ischemic stroke or TIA	10/39 (25.6%)[b]	7/153 (4.6%)	6/54 (11.1%)
Acute vascular occlusion	1/39 (2.6%)	2/153 (1.3%)	–

Abbreviations: MF, myelofibrosis; TIA, transient ischemic attack.
[a]$p = 0.01$.
[b]Odds ratio: 0.2, 95% CI: 0.08–0.61, $p = 0.004$.

(OR: 3.3, 95% CI: 1.5–7.6, $p = 0.003$). At the time of the arterial event during the follow-up, 84 (34.1%) patients underwent antiplatelet therapy, all of who received acetylsalicylic acid. Patients with overt MF had a significantly lower risk of developing ATE (8.5 vs. 21.1%, $p = 0.01$), particularly ischemic stroke or TIA (OR: 0.2, 95% CI: 0.1–0.6, $p = 0.004$) than patients with pre-MF. Patients with pre-MF had higher platelet counts (610 ± 67 vs. $454 \pm 46 \times 10^9$/L, $p = 0.01$) and erythrocyte counts (4.4 ± 0.2 vs. 3.9 ± 0.10^{12}/L, $p = 0.02$) than patients with overt MF at the time of thrombotic event. Patients with pre-MF were also diagnosed younger, with a median age of 58 versus 63 years ($p = 0.05$). JAK inhibitor therapy was administered to seven (13.5%) patients who experienced a thrombotic event during follow-up and was found to be statistically associated with thrombosis ($p < 0.001$). No difference for other indicators of myeloproliferation, such as splenomegaly or bone marrow fibrosis grade, was found. Risk scores or other laboratory parameters, most particularly leucocyte count, had not been associated with a significantly higher incidence of thrombosis.

Bleeding Events

A total of 47 episodes of bleeding were documented in 34 patients (corresponding to incidence: 3.8% pt/y), including the gastrointestinal (GI) tract ($n = 25$, 9 of them had variceal bleeding caused by portal hypertension), central nervous system ($n = 4$), nose ($n = 5$), soft tissues ($n = 7$), and other locations ($n = 6$). Sixteen (47.1%) patients experienced major bleeding, and 10 of them had GI tract bleeding. Five of the 16 patients with major bleeding had at least one recurrent bleeding event. Thrombocytopenia ($< 100 \times 10^9$/L) at the time of diagnosis was observed in 21 of the 246 (8.5%) patients. AvWS was diagnosed in eight patients, two of who had bleeding events, and one patient experienced a severe event. AvWS diagnostic was performed in a small selective cohort of patients and could not be statistically interpreted. JAK inhibitors were administered to 9 of the 34 (26.5%) patients who experienced bleeding. No hemorrhages occurred as a complication of extramedullary hematopoiesis.

Risk Factors of Bleeding

The multivariable analysis demonstrated a significant association between an advanced fibrosis grade (grade 3) and an increased risk of bleeding (OR: 3.4 95% CI: 1.2–9.2, $p = 0.02$), regardless of platelet count. Thrombocytopenia at the time of diagnosis ($p = 0.27$), the use of antiplatelet drugs ($p = 0.87$), anticoagulation ($p = 0.74$), *CALR* mutation ($p = 0.81$), and treatment with JAK inhibitors ($p = 0.09$) were not independently associated with the risk of bleeding or severe bleeding.

Overall Survival and Thrombotic or Bleeding Events

The median follow-up was 11.9 (95% CI: 8.9–14.9) years. At the time of data cutoff, 73 (29.7%) patients had died. AML transformation caused 13 of 73 (17.8%) deaths, infections caused 34 of 73 (46.6%), and 1 patient died following PE. Other causes of death included cardiac decompensation ($n = 6$), acute kidney failure ($n = 4$), and unknown reasons ($n = 15$). Allogeneic hemopoietic stem cell transplantation was performed in 29 of 246 (11.8%) patients. No correlation was found between OS and the occurrence of thrombotic ($p = 0.21$) or bleeding ($p = 0.18$) events (►Figs. 1 and 2). The median time to thrombosis was 13 months (IQR: 1–55), and to bleeding was 30 months (IQR: 13–71).

Discussion

This study investigated clinical and molecular factors associated with thrombosis and bleeding in a substantial cohort

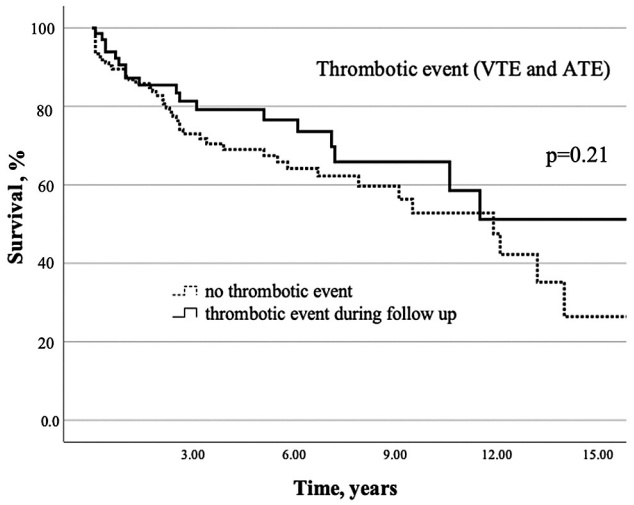

Fig. 1 Survival of patients with thrombotic versus no thrombotic event.

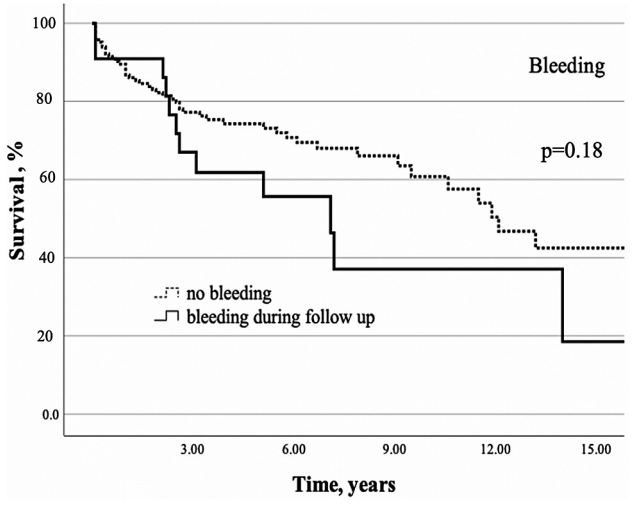

Fig. 2 Survival of patients with bleeding versus no bleeding event.

of MF patients ($n = 246$) with long-term follow-up (11.9 years). A comparable or higher incidence of VTE (4.4% pt/y) and ATE (2.2% pt/y) was observed in comparison to previously published studies.[10,12,14,16,35–39] This discrepancy, particularly with historical cohorts, could be explained by the new definition of pre-MF introduced in 2016, a lack of recommendations for primary thrombotic prophylaxis in this patient group, and one of the longest follow-up periods described in recent publications.

Prior thrombosis and the *JAK2-V617F* mutation had already been established as risk factors of thrombosis in PV and ET patients[26,29,38–42] and are routinely used to stratify patients into risk groups.[2,4,43] In the current study, the role of prior thrombosis and the *JAK2-V617F* mutation in the risk of venous, but not arterial, thrombosis in patients with MF was confirmed. It could be attributed to the activation of mutated *JAK2* blood cells, which leads to the production of inflammatory cytokines due to the JAK/STAT pathway activation, consequently causing an enormous stimulation of the plasmatic coagulation cascade.[44,45] Furthermore, *JAK2* is also expressed in splanchnic area endothelial cells,[46] and endothelial dysfunction contributes an essential role in the pathogenesis of SVT in the already predisposed splanchnic system (hepatosplenomegaly, slower blood flow, altered immunogenicity) in MF patients. This theory is supported by the high prevalence of SVT in our study (12.8% in patients with pre-MF and 9.2% with overt MF) and recent publications.[8,11]

The multivariable analysis confirmed that only arterial hypertension was an independent risk factor of ATE occurrence. Patients with pre-MF demonstrated a significantly higher risk of developing ATE, particularly cerebral events, than patients with overt MF. These findings resemble the results of recent studies.[4,25] Possible explanations include high platelet counts (610 ± 67 in pre-MF patients vs. $454 \pm 46 \times 10^9$/L in overt MF patients, $p = 0.01$), activation of their function or stimulated proinflammatory signals resulting from cardiovascular risk factors. This hypothesis is supported by recently published data indicating a high incidence of ischemic cerebral events in ET patients. The authors[47–49] proposed that ET be considered a risk factor for primarily small-vessel type stroke, and that abnormal megakaryopoiesis increases thrombotic risk beyond conventional cardiovascular risk factors. In accordance with colleagues,[14] future studies testing primary prophylaxis with low-dose acetylsalicylic acid (if not contraindicated) in a specific patient cohort are warranted. Furthermore, despite the use of acetylsalicylic acid, a high incidence of ATE during follow-up was observed. This could be due to insufficient platelet inhibition by acetylsalicylic acid, also known as "aspirin resistance"[50,51] or a consequence of excessive platelet turnover.[52] Recent studies have shown a twice-daily acetylsalicylic acid administration as more efficient than once-daily regime in reducing turnover resistance and "aspirin resistance."[53,54]

In contrast to a recent study,[10] no correlation between leucocyte count and the risk of arterial or venous thrombosis was found. Neutrophils are known to participate in the pathogenesis of thrombosis by forming neutrophil extracellular traps (NETs). Scientific interest is currently focused on the thrombotic role of NETs in MPN patients.[55–57] In the most recent study, it was discovered that acetylsalicylic acid reduced NETosis in an MPN mouse model and MPN patients. We agree that further study in this area, particularly in patients with MF, has enormous potential for improving our understanding of the pathogenesis of thrombosis.

Based on risk factors identified in the current study (*JAK2* mutation, prior VTE, and arterial hypertension) and considering the similar risk of thrombotic events for patients with ET and pre-MF, it could be proposed that the IPSET (international prognostic score for thrombosis in ET)[36] might be a convenient tool for thrombosis risk stratification in patients with pre-MF. However, IPSET and conventional risk stratification in PV do not distinguish between venous and arterial events in risk assessment. Following the recent study on a general group of MPN patients,[12] it is essential to evaluate the risks of VTE and ATE separately and establish further

distinct scoring systems for arterial and venous thrombosis in MF patients.

Published research on the increased thrombotic risk in patients with autoimmune diseases treated with JAK inhibitors yielded inconsistent results.[58–61] The current investigation demonstrated a statistically significant association between JAK inhibitor treatment and thrombosis, contrasting previous studies in MPN patients receiving ruxolitinib.[62,63] However, these findings should be interpreted with caution because four of seven patients, who developed a thrombosis during follow-up and received ruxolitinib treatment, had additional significant risk factors for thrombosis (e.g., combination therapy with pomalidomide, or the presence of another tumor).

Post-diagnosis bleeding occurred in 13.8% of patients, corresponding to a rate of 3.8% per patient-year, which aligns with findings from recent studies.[25,64,65] Interestingly, the peak of bleeding events occurred after diagnosis rather than at the time of diagnosis, consistent with findings in a German MPN registry.[9] This observation supports the theory that bleeding may be related to the progression of fibrosis grade and, consequently, the underlying disease. It could be explained by platelet dysfunction, secondary storage pool defect associated with advanced fibrosis,[66,67] or the development of AvWS even in the absence of extreme thrombocytosis.[68,69] Additionally, the high incidence of bleeding in the current study could be attributed to relatively frequent episodes of variceal bleeding caused by portal hypertension ($n = 9$, 26.5%).

In our cohort, no correlation was found between OS and the occurrence of thrombotic or bleeding events. It could be explained by a high mortality rate from infections or hematologic malignancies, particularly among younger MF patients.[70]

Conclusion

In summary, this study showed a substantial risk of both thrombotic (6.6% pt/y) and bleeding (3.8% pt/y) events, highlighting the complex coexistence of these complications in MF patients. Patients with pre-MF should be considered a distinct entity with regard to their heightened risk of thrombosis, particularly ATE. The *JAK2-V617F* mutation, regardless of allele burden, and a history of prior venous thrombosis were strongly associated with an increased risk of VTE. Patients with fibrosis grade 3 demonstrated an increased risk of bleeding. These findings should be useful for counseling patients, guiding treatment decisions in clinical practice, and defining target patient groups for prospective studies addressing thrombotic and bleeding complications in MF.

The study has significant limitations, including its retrospective single-center design and confounding bias. The follow-up was interrupted after AML transformation or transplantation of allogeneic hemopoietic cells. Only a small percentage of patients underwent the AvWS diagnostic, making it impossible to assess its correlation with this significant potential risk factor for bleeding.

What is known about this topic?

- The *JAK2* mutation and prior thrombosis have previously been identified as risk factors for thrombosis in patients with polycythemia vera or essential thrombocythemia.
- In a general population of MPN, the *JAK2* allele burden is linked to an increased risk of thrombosis (frequently, only a small number of MF patients were included and they were not analyzed separately).

What does this paper add?

- High incidence of thrombosis in a substantial cohort of myelofibrosis patients with long-term follow-up (11.9 years).
- The presence of the *JAK2-V617F* mutation and a history of prior venous thrombosis are strongly associated with an increased risk of VTE, but not ATE, in patients with myelofibrosis.
- In our study, the *JAK2* allele burden had no effect on the risk of thrombosis.

Authors' Contributions

Study conception and design: O.M., A.H., T.E.; collection of clinical data: O.M., C.C., I.S., A.T.; molecular analyses: J.R., M.M., C.B.; data analyses: O.M.; initial draft of the manuscript: O.M.; correction and approval of the final manuscript: all authors.

Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University Jena (Reg. No.: 2021–2094-Material).

Conflict of Interest

OM: All support for the present manuscript (e.g., funding, provision of study materials, medical writing, article processing charges, etc.): Interdisciplinary Center of Clinical Research (IZKF) of the Medical Faculty Jena (Grant number 413668513).
AT: Grants or contracts from any entity: Interdisciplinary Center of Clinical Research (IZKF) of the Medical Faculty Jena (Advanced clinician scientist-program).
AH: has received honoraria from Novartis, Incyte. He declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
CC, JR, IS, MM, CB and TE have no conflict of interest.

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