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ORIGINAL ARTICLE

Thromboses and hemorrhages are common in MPN patients with high JAK2V617F allele burden

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Abstract The most common causes of morbidity and mortality in myeloproliferative neoplasms (MPN) are thrombotic and hemorrhagic complications. The JAK2V617F mutation, commonly found in MPN, correlates with several clinical and laboratory characteristics even if the relevance of JAK2V617F allele burden in the natural history of these diseases is unclear. In this study we searched, a relation between thrombotic and hemorrhagic complications and JAK2V617F allele burden level in MPN patients. We evaluated 253 consecutive MPN [121 essential thrombocythemia (ET), 124 polycythemia vera (PV), and 8 primary myelofibrosis (PMF)] patients in whom the JAK2V617F allele burden was available, all studied and followed (median 8.8 years) in our department. Patients were stratified accordingly to their JAK2V617F allele burden, into four quartiles (1st <25%, 2nd 26–50%, 3rd 51–75%, and 4th >75%). Significantly higher incidence of thromboses ($p = 0.001$) and hemorrhages ($p < 0.001$) during follow-up has been observed in higher quartiles when compared to lower ones. Thrombosis- and hemorrhage-free survivals were poorer in patients belonging to the highest quartile. Our data suggest that MPN patients with JAK2V617F allele burden higher than 75% have to be considered as high risk patients, being prone to develop thrombo-hemorrhagic complications during the disease course.

Keywords Myeloproliferative neoplasms · Thrombosis · Hemorrhage · JAK2V617F allele burden

Polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are the so called classical Philadelphia negative myeloproliferative neoplasms (MPN). The most common causes of morbidity and mortality in MPN are hemorrhagic and thrombotic complications [1], the latter mainly represented by myocardial infarction, ischemic stroke, and pulmonary embolism [2].

Bleedings in MPN are less frequent than thromboses, being more common in patients with extreme thrombocytosis. Ecchymosis, epistaxis, menorrhagia, and gingival hemorrhage are the most usual hemorrhagic manifestations, but nowadays, they may also suffer for a major hemorrhagic complication, i.e., gastrointestinal bleeding often associated with aspirin administration [3–6], urogenital, and intracranial [7–9] hemorrhages.

In 2005, the JAK2V617F mutation has been demonstrated in most patients with MPN [10, 11], and its presence is deemed a fundamental diagnostic criterion for MPN by World Health Organization [12].

It has been shown that MPN patients with JAK2V617F mutation carry a higher risk of cardiovascular events and a more frequent evolution into secondary myelofibrosis [13–15] when compared to wild-type patients. At present, JAK2V617F allele burden percentage is considered of significant value only for the evolution into myelofibrosis [16], while no clear correlation has been observed between JAK2-mutation allele burden and cardiovascular complications [17]. The impact of different allele burden on bleeding risk has never been assessed.

In the current retrospective study, we explore whether there is an association between JAK2V617F allele burden and

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thrombotic or hemorrhagic complications in a large cohort of MPN patients with a very long follow-up.

Patients and methods

Study population and definitions

We retrospectively evaluated 253 (121 ET = 47.8%, 124 PV = 49%, and 8 PMF = 3.2%) patients carrying JAK2V617F mutation, detected with real-time PCR, out of 356 MPN patients (71%) in whom a complete molecular analysis was available. The patients were stratified into four groups according to the amount of JAK2 mutant allele, dividing them in four quartiles (1st quartile 1–25%, 2nd quartile 26–50%, 3rd quartile 51–75%, and 4th quartile 76–100%).

All the patients were studied and followed in our department between July 1978 and December 2016, and their median follow-up was 8.8 years (0.1–37.3 years). In the 117 patients diagnosed before 2006, JAK2V617F mutation has been searched after a median time of 7.48 years (2.1–27.5) from diagnosis. Medical history and presence of cardiovascular risk factors or thrombophilia were recorded. The patients were treated with phlebotomies to maintain hematocrit lower than 45%. Most patients received low dose aspirin as primary or secondary prevention of cardiovascular events; patients with a venous thrombosis underwent anticoagulant treatment.

Major thrombotic events were divided in arterial (ischemic stroke, transient ischemic attack, acute myocardial infarction, peripheral arterial thrombosis) and venous (deep vein thrombosis with or without pulmonary embolism, abdominal, or cerebral vein thrombosis). Hemorrhagic complications were classified as "major" or "minor" in agreement with ISTH criteria [18].

The study was approved by local ethic committee and performed according to the principles of the Declaration of Helsinki.

Statistical analysis

Numerical variables were summarized by mean (standard deviation) or median (5–95th percentile). The Fisher's exact test or the X^2 test were used to compare categorical variables among the different patient groups that had been categorized according to the mutated allele burden. Comparison between continuous variables was performed by either the Mann-Whitney *U* test (two groups) or Kruskal-Wallis test (more than two groups). Survivals were calculated with the Kaplan Meier method and compared with the Log Rank test. Cox proportional hazard regression model was used for multivariable analysis. Kaplan Meier and Cox analyses have been evaluated only for groups with different prevalence of events during follow-up. Incidence rate ratio (IRR) has been calculated for groups with different survivals. A two-tailed p value of less than 0.05 was considered significant. All statistical calculations were performed using the SPSS version 23.

Results

The clinical and laboratory data of our patients are summarized in Table 1.

Patients of 1st quartile were younger at diagnosis ($p = 0.031$) and had lower leukocyte count ($p < 0.01$) than those of 4th quartile. Hematocrit was higher in 4th quartile compared both to 1st (<0.001) and 2nd ($p = 0.001$) quartile patients. Platelet count was similar among the quartiles.

Thrombosis

We observed 134 thrombotic events (64 arterials and 70 venous) in 99 patients. In 63 patients (24.9%), cardiovascular event occurred at diagnosis and in 55 (21.7%) during follow-up, even if cardiovascular risk factors and thrombophilia had the same prevalence among different quartiles. Nor the prevalence of all thromboses or the one of the thrombotic events at diagnosis showed differences among quartiles. In contrast, prevalence of thrombosis during follow-up was lower in 1st quartile compared both to 3rd ($p = 0.041$) and to 4th ($p < 0.001$) quartiles. The incidence rate of thrombosis during follow-up was 1.96/100 pats/year for 1st quartile, 3.52/100 pats/year for 2nd quartile, 3.85/100 pats/year for 3rd quartile, and 5.33/100 pats/year for 4th quartile (Fig. 1), with an IRR of 2.7 for 4th quartile vs 1st one. Thrombosis-free survival resulted significantly poorer in 4th quartile comparing to 1st one ($p = 0.003$). A multivariable Cox analysis of thrombotic events during follow-up including allele burden quartile, age at diagnosis, and hematocrit level as covariates confirmed the negative prognostic relevance of higher allele burden quartiles: 3rd vs 1st (HR 3.58; 95% CI 1.42–9.03; $p = 0.007$) and 4rd vs 1st (HR 4.74; 95% CI 2.1–10.69; $p = 0.0002$).

Hemorrhage

Three patients (1.2%) bled at diagnosis (1 major and 2 minor hemorrhages), while 27 (11.8%) suffered for hemorrhages during follow-up (10 major and 17 minor). Prevalence of hemorrhages was higher in 4th quartile compared both to 1st ($p < 0.001$) and to 2nd quartile ($p = 0.003$). Hemorrhage-free survival was poorer in 4th quartile compared to the lower quartiles (4th vs 1st $p < 0.001$; 4th vs 2nd $p = 0.004$). The multivariable analysis of risk factors for bleedings during follow-up (i.e., allele burden quartile, antithrombotic treatment at time of bleeding, platelet count) showed that allele burden in the 4th quartile had an independent negative prognostic role compared both to 1st (HR 1.62; 95% CI 1.15–2.82;

Table 1 Clinical and laboratory data of our patients divided in four quartiles on the basis of allele burden percentage

	JAK2V617F allele burden quartiles			
	1st	2nd	3rd	4th
Patients, *n* (%)	119 (47)	60 (23.7)	33 (13)	41 (16.2)
Females, *n* (%)	83 (69.7)	37 (61.7)	20 (60.6)	19 (46.3)
MPN diagnosis				
ET, *n* (%)	79 (66.4)	30 (50)	8 (24.2)	4 (9.8)
PV, *n* (%)	38 (31.9)	29 (48.3)	23 (69.7)	34 (82.9)
PMF, *n* (%)	2 (1.7)	1 (1.7)	2 (6.1)	3 (7.3)
Median age at diagnosis, years (Percentile range, 5th to 95th)	56.3 (28–79)	53.6 (25–81)	66.1 (25–86)	64.5 (34–82)
Median WBC, $\times 10^9$/L (Percentile range, 5th to 95th)	8.4 (5.2–12.6)	8.9 (5.1–17.7)	9.3 (6.5–18.4)	10.1 (5.6–17.5)
Median Ht, % (Percentile range, 5th to 95th)	45.3 (35.8–58.6)	45.7 (30–58.9)	50.2 (36–67)	53 (37–69)
Median plts count, $\times 10^9$/L (Percentile range, 5th to 95th)	640 (276–1143)	654 (296–1067)	629 (255–1068)	501 (136–1221)
Patients with thrombosis at diagnosis, *n* (%)	31 (26)	18 (30)	9 (27.3)	5 (12.2)
Patients with at least one thrombosis during follow-up, *n* (%)	15 (12.6)	14 (23.3)	9 (27.3)	17 (41.5)
Cardiovascular risk factors and thrombophilia, *n* (%)	65 (54.6)	26 (43.3)	22 (66.7)	26 (63.4)
Patients with at least one hemorrhage during follow-up, *n* (%)	7 (5.9)	4 (6.7)	4 (12.1)	12 (29.3)
Anti-thrombotic treatment at bleeding time				
Low-dose aspirin	4	2	3	8
LMWH/warfarin	2	1	–	3
None	1	1	1	1
Evolution into myelofibrosis post-ET or post-PV, *n* (%)[a]	3 (2.6)	3 (5.1)	3 (9.7)	10 (26.3)
Death, *n* (%)	3 (2.5)	8 (13.3)	6 (18.2)	14 (34.1)

WBC leukocyte, *Ht* hematocrit, *plts* platelets, *LMWH* low molecular weight heparin

[a] PMF patients were excluded in this analysis: for this row 1st $N = 117$, 2nd $N = 59$, 3rd $N = 31$, and 4th $N = 38$

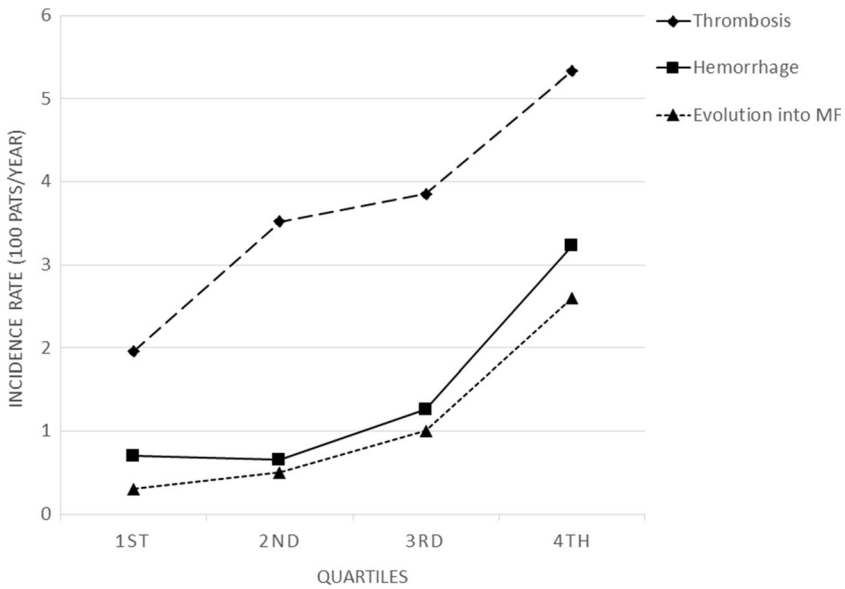

Fig. 1 Changes in incidence rate (100 patients/year) of thrombosis, hemorrhage, and evolution into secondary myelofibrosis (MF) among quartiles. The eight PMF patients were excluded from analysis of incidence rate of evolution into MF

$p = 0.005$) and to 2nd (HR 2.27; 95% CI 1.25–4.14; $p = 0.007$) quartiles. The incidence rate of hemorrhages were respectively 0.7/100 pats/year for 1st quartile, 0.65/100 pats/year for 2nd quartile, 1.26/100 pats/year for 3rd quartile, and 3.23/100 pats/year for 4th quartile (Fig. 1) with a IRR of 4.6 and of 5 for the 4th quartile respectively vs 1st and 2nd one. Among the 30 patients who experienced a bleeding, 26 (86.7%) were under antithrombotic treatment at the time of hemorrhage. No statistically significant difference has been demonstrated in the use of antithrombotic drugs among patients of the different quartiles.

Evolution into myelofibrosis

The eight PMF patients were excluded from this analysis. During follow-up, six ET and 13 PV (7.7% of total cohort) evolved into secondary myelofibrosis after a median time of 11.8 years (1.5–26.1). Prevalence of evolution was higher in 4th quartile in comparison to all the other quartiles (4th vs 1st $p < 0.001$; 4th vs 2nd $p = 0.002$; 4th vs 3rd $p = 0.048$). Myelofibrosis-free survival was significantly lower in 4th compared to 1st and 2nd quartiles ($p < 0.001$). The evolution rate was 0.3/100 pats/year for 1st quartile, 0.5/100 pats/year for 2nd quartile, 1/100 pats/year for 3rd quartile, and 2.6/100 pats/year for 4th quartile (Fig. 1) with a IRR of 8.67 and 5.2 for the 4th quartile respectively vs 1st and 2nd one.

Mortality rates were 0.3/100 pats/year for 1st quartile, 1.2/100 pats/year for 2nd quartile, 1.8/100 pats/year for 3rd quartile, and 3/100 pats/year for 4th quartile: mortality risk rates for the 4th quartile are 10 vs 1st, 2.5 vs 2nd, and 1.7 vs 3rd one.

Discussion

Within the MPN, PV, and ET are relatively indolent disorders, resulting in a modest reduction of lifespan compared with a control population; however, most patients ultimately suffer from one or more severe thrombosis and/or hemorrhage that are potentially fatal complications directly attributable to the disease [19–21]. The cumulative incidence of thrombotic events has been estimated in about 2.5 to 5.0/100 patients/year in PV [2] and 1.9 to 3.0/100 patients/year in ET [22], and nowadays, 5–10% of MPN patients are expected to suffer for a major hemorrhagic complication [4]. Conversely, most PMF patients have a severe course, and their survival is significantly affected [23].

In 2005, the discovery of JAK2V617F mutation in about 95% of patients with PV and in 50–70% of patients with ET or PMF [24] has modified our understanding of the clinical and biologic features of MPN [1]. In numerous studies, the presence of JAK2V617F mutation has been variably associated with greater occurrence of thrombosis, increased BM fibrosis, older age, longer disease duration, or poorer survival in MPN [7, 10, 11, 15, 25].

Several studies have shown that the JAK2V617F allele burden correlates with disease-related symptoms or complications, although it seems quite unlikely that the burden of mutated allele represents the only mechanism underlying MPN pleiotropy [26].

The correlation between allele burden and thrombotic risk is controversial. In a large series of Vannucchi et al. [27], the rate of major thrombosis in PV was not increased in homozygous compared with heterozygous patients, while thrombotic events were more frequent among homozygous ET patients compared to heterozygous ones. Other authors confirmed these results [28], while in the only prospective study addressing the clinical significance of JAK2V617F homozygosity in PV, an allele burden greater than 75% was associated with a 3.56-fold higher relative risk of total thrombosis [29].

In our cohort, we did not find any correlation between JAK2 allele burden and thrombosis at diagnosis. In contrast, the incidence of thrombotic events during follow-up resulted significantly higher in patients belonging to higher quartiles of allele burden. The incidence rate of thrombotic events increases progressively among quartiles, and, as confirmed by multivariable analysis, it achieves a relative risk of thrombosis during follow-up about three times higher in patients belonging to 4th quartile compared to patients of the 1st one.

Risk factors for hemorrhage in MPN are not well-defined, and there is no risk estimation model for this outcome. A platelet count higher than 1500 × 10^9/L due to the consumption of high molecular weight multimers of von Willebrand Factor [3] and use of aspirin have been implicated in bleeding occurrence [3–5]. Previous studies failed to demonstrate a correlation between the presence of JAK2 mutation and bleeding risk [4, 30, 31], and no data are available about the significance of allele burden on this topic. In our cohort, we found a significantly higher incidence of bleeding manifestations during follow-up in patients with higher allele burden. Although most patients who bled were under antithrombotic treatment, there was no difference in the drugs administration among quartiles, suggesting an independent role of JAK2 allele burden in the different occurrence of hemorrhagic events.

Consistently with other studies [27, 38], allele burden influenced also evolution into myelofibrosis and overall survival. Among our patients with ET and PV, those with an allele burden greater of 75% have a risk to evolve into secondary myelofibrosis up to eight times higher than those with a lower burden. Moreover, patients belonging to highest quartile display a risk of mortality up to ten times higher compared to the lower ones. However, the different median age at diagnosis of 1st and 4th quartiles must be considered.

We are conscious that in the patients with a diagnosis obtained before the discovery of JAK2V617F mutation, the molecular study was performed after a long follow-up. Most

published papers [32, 33] have shown, however, that there is not a progressive increase in JAK2 allele burden over years in patients with MPN, and this make us confident of the relevance of our results.

In conclusion, our data show that MPN patients with a JAK2V617F allele burden greater than 75% are more prone to develop thrombotic and hemorrhagic complications during follow-up and not only to progress into myelofibrosis. Therefore, JAK2V617F allele burden can provide prognostic information about the risk of developing thrombo-hemorrhagic complication. Prospective studies are needed to confirm our observations.

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Authors' contributions IB and MLR conceived the study and wrote the paper, GB and GB collected the patients' data, ED and AML performed bio-molecular tests, and FF gave major intellectual contribute.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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