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Molecular biomarkers of thrombosis in myeloproliferative neoplasms

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KEYWORDS

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ABSTRACT

JAK2 mutations define polycythemia vera (PV), *CALR* and *MPL* mutations are specific to *JAK2* unmutated essential thrombocythemia (ET) and primary myelofibrosis (PMF). We overviewed the current knowledge on the relationship between these phenotypic driver mutations and thrombotic complications that are major cause of morbidity and mortality in patients with myeloproliferative neoplasms (MPN) particularly PV and ET. The *JAK2* mutation is found in 50-60% of patients with ET and *PMF*. The International Prognostic Score for Thrombosis in ET (IPSET-thrombosis) identified JAK2 mutation as an independent risk factor and a 3-tiered prognostic model was devised. IPSET-thrombosis model outperformed the 2-tiered conventional risk stratification that includes age and thrombotic history. PV is usually associated with a *JAK2* mutation and studies looking at the role of *JAK2V617F* allele burden associated with thrombosis are so far inconclusive. In PMF, the rate of major thrombosis is around 2%pt-yr and JAK2 mutation emerged as an independent risk factor for these events. *Calreticulin/MPL* (CALR) is the second most frequent mutation and occurs in half of JAK2 and MPL wild-type patients with ET and PMF. Despite the fact that these mutations are associated with high platelet counts, the risk of thrombosis compared with JAK2 and MPL mutated cases is significantly lower. The role of MPL in the prediction of thrombosis is of difficult demonstration due to the low frequency in ET and PMF. Therefore, these epidemiologic studies pointed out the role of JAK2V617F mutation as a major contributory factor for the pathogenesis of thrombosis in MPN. Abnormalities of blood cells arising from the clonal proliferation of hematopoietic stem cells may explain the switch to a procoagulant phenotype.

Introduction

Polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) are chronic myeloproliferative neoplasms (MPN) characterized by clonal expansion of an abnormal hematopoietic stem/progenitor cell. Their natural history is marked by thrombo-hemorrhagic complications and a propensity to transform into myelofibrosis (MF) and acute myeloid leukemia (AML). The pathogenesis of thrombosis results from a complex interplay of clinical and disease related factors. Abnormalities of blood cells arising from the clonal proliferation of hematopoietic stem cells involve not only quantitative changes, but also qualitative modifications, which characterize the switch of these cells from a resting to a procoagulant phenotype [1]. By incorporating the body of knowledge in a simple clinically-oriented scheme, conventional classification of patients with either PV or ET in a “high-risk” or “low-risk” for thrombosis according to their age and previous history of thrombosis is currently recommended [2].

This report provides an overview of thrombogenesis pathophysiology in patients with MPN focusing on the role of non-conventional biomarkers of thrombosis such as mutations in phenotypic driver genes possibly leading to a direct link between clonal myeloproliferation and thrombogenesis.

Mutations in phenotypic driver genes: *JAK2, MPL, CALR*

From 2005 onwards, more than 20 somatic mutations have been described. A point mutation at codon 617 in exon 14 of the Janus kinase 2 (JAK2 V617F) gene was documented in the large majority of patients with PV and about 50-60% of those with ET and PMF [3-6]. Mutations in the gene encoding the thrombopoietin receptor (MPL) were discovered soon after in about 5-8% of the patients with PMF and 3-5% with ET [7]. More recently, mutations in the gene encoding the endoplasmic reticulum protein calreticulin (CALR) have been reported in about 20% of ET and PMF patients [8,9], accounting for 70-80% of those lacking the JAK2V617F allele.

Mutations in JAK2, MPL, and CALR are considered phenotypic driver mutations since the expression of the mutated gene in cell lines caused cytokine independent or hypersensitive growth, as known to occur in primary cells from MPN patients. In animal

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models, phenotypes closely resembling a myeloproliferative disease were observed in transgenic or conditional animals [10.]

In addition, other mutations in the epigenetic genes like TET2, DNMT3A, IDH1/2, EZH2, ASXL have been described and found associated with the progression of these neoplasms. They usually occur in hematopoietic cell subclones of variable size, often but not invariably together with one of the phenotypic driver mutations, and may either antedate or follow the acquisition of phenotypic driver mutations [4,11].

At diagnosis, the clinical phenotype varies according to the JAK2, CALR and MPL mutations and this may potentially influence clinical outcomes and the probability of thrombotic incidence. JAK2-V617F patients are older, present higher hemoglobin (Hb) and leukocytes (WBC) levels, lower serum erythropoietin (S-Epo), lower platelet count and increased risk of thrombosis [12]. Patients with CALR insertions/deletions are of younger age, male sex, exhibit lower Hb level and leucocyte count, higher platelet count, and lower incidence of thrombotic events. The hematologic phenotype of MPL mutation is also associated with lower Hb levels and higher platelet counts than wild type (wt) MPL [13].

Thrombosis in MPN

Predominant is arterial thrombosis, particularly cerebrovascular accidents, whereas acute coronary syndromes have been the most common cause of death. Venous thrombosis is less common than arterial thrombosis, but it has been shown to adverse survival. Hepatic vein thrombosis, or Budd-Chiari syndrome, and thromboses of the portal and/or mesenteric veins are also strongly associated with PV and are often a presenting feature. Microvascular symptoms, including headache, visual change, dizziness, and erythromelalgia, are also prominent and affect quality of life.

The pathogenesis of thrombosis in MPN patients is complex. Clinical factors (i.e. age, previous history of thrombotic events, obesity, hypertension, hyperlipemia, etc) as well as the increase in blood cell counts (i.e. leukocytosis, erythrocytosis, thrombocytosis) contribute to different extent to the increased risk of thrombosis in these patients. Abnormalities of blood cells arising from the clonal proliferation of hematopoietic stem cells involve not only quantitative but also qualitative changes that characterize the switch of these cells from a resting to a procoagulant phenotype. Prothrombotic features include the expression by blood cells of procoagulant and proteolytic properties, the secretion of inflammatory cytokines, and the expression of adhesion molecules. In addition to these mechanisms, prothrombotic changes occur in the normal vascular endothelium in response to the insults of inflammatory cytokines, hyperviscosity, and leukocyte derived proteases (i.e. elastase, cathepsin-G, and myeloperoxidase). Specifically, the up-regulation of endothelial adhesion receptors favors the attachment of platelets, erythrocytes and leukocytes to the vascular wall, with subsequent localization of clotting reactions and fibrin deposition.

Therefore, a procoagulant background exists in MPN patients, who present with a hypercoagulable state, a subclinical condition demonstrated by the alterations of plasma thrombotic markers. Among these, the increased levels of circulating procoagulant microparticles and the occurrence of an acquired activated protein C resistance are the most prominent features of hypercoagulability in these subjects [1].

Because survival in strictly WHO-defined ET is near-normal (15-year survival of ~80%) and the 10-year risk of AML or MF less than 1% and 10% respectively, it would be inappropriate to suggest that any current treatment modifies the natural history of the disease. Similarly, in WHO-defined PV, the 10-year projected rates for survival, leukemic transformation and fibrotic progression were >75%, <5% and <10%, respectively, and treatment is tailored to individual patients according to their risk for thrombosis or bleeding.

In the last years, the advanced age (≥60 years) and history of thrombosis have been identified as the most important risk factors for thrombosis in MPN patients, and their absence or presence has been used to stratify patients into high-risk and low-risk disease categories, respectively [13]. Recent studies have also disclosed the additional prognostic value of JAK2 mutations and cardiovascular risk (CV) factors for arterial thrombosis in these patients (Figure 1) [14].

Based on age and previous thrombosis, the potential dangers of cytoreductive therapy, with the intent to prevent thrombotic complications, may be justified in high risk patients [2].

JAK2 V617F mutation and thrombosis

Essential thrombocythemia

Current risk factors for thrombosis in ET are based on relative risk estimates such as odds ratio, risk ratio, or hazard ratio (HR) so that no direct meaning or relevance to prognostication of thrombosis in individual patient can be drawn from a single risk factor assessment. A more reliable risk prediction may be provided by combining multiple variables in prognostic models whose performance needs to be confirmed in other cohorts of patients. Accordingly, an accurate prediction model for thrombosis was recently proposed. In the International Prognostic Score for Thrombosis in essential thrombocythemia (IPSET-thrombosis) [15], age and history of thrombosis were confirmed as independent risk factors for future thrombosis and the study also identified an independent prothrombotic role for CV risk factors and *JAK2*V617F mutation status [15]. Based on multivariate analysis-derived hazard ratio a 3-tiered prognostic model was devised and patients were categorized in (low-, intermediate-, and high-risk using a training cohort of 535 patients. The model was then validated in internal and external cohorts of ET patients. IPSET-thrombosis model outperformed the 2-tiered conventional risk stratification (defined by age and prior events) in predicting future vascular events. Further analysis were performed to quantify the individual contributions of *JAK2* mutations and CV risk factors in conventionally-assigned low and high risk ET, and the results led to revise the IPSET risk stratification scheme [16] that was recently validated in an external cohort of 526 patients from Mayo Clinic [17].

Treatment recommendations for each one of the above-mentioned new risk categories have been proposed to be examined in the context of prospective controlled studies [14].

Polycythemia vera

PV is usually associated with a *JAK2* mutation (98% *JAK2*V617F and 2% other *JAK2* mutations including exon 12 mutations), although rare instances of PV associated with *CALR*, *MPL* or other mutations (e.g. *LNK*) have been reported [18]. In general, *JAK2* exon 12 mutated PV, compared to *JAK2*V617F-mutated PV, is more likely to be associated with younger age and predominantly erythroid myelopoiesis [14].

Several studies have assessed the role of the JAK2 mutant allele burden in the risk of vascular complications [19]. Conflicting results were reported. Allele burden greater than 75% was associated with a 3.56-fold higher relative risk (95% CI 1.47-7.1) of total thrombosis. Thus, patients with PV who harbor the higher V617F allele burden quartile may represent a subgroup at a particularly high risk of thrombosis.

These observations are supported by recently published, retrospective series of patients with PV. Although a significant correlation with thrombosis risk could not be defined, there was a trend towards increased frequency of thrombosis as the *JAK2* V617F allele burden increased. In contrast, in a series of 320 PV patients, no significant correlation between V617F allele burden and thrombosis could be