

Pathogenetic mechanisms of thrombosis in patients with myeloproliferative neoplasm

Abstract

Patients with myeloproliferative neoplasm (MPNs) present at the onset or during evolution thrombotic complications. The microvascular inflammatory and thrombotic complications in myeloproliferative thrombocythemia are caused by aspirin-responsive platelet mediated arteriolar inflammation and thrombosis as the consequence of hypersensitive thrombocytic platelets produced by altered megakaryocytes in the bone marrow of MPN patients with ET and PV. The somatic JAK2V617F mutation induces constitutive activation and proliferation of large hyperlobulated megakaryocytes with the production of hypersensitive platelets. Functionally modified platelet receptor and Src signalling pathway plays an important role in modifications of platelet function. The presence of JAK2V617F mutation results in circulating activated hypersensitive sticky platelets with increased platelet microparticles expression and associated tissue factor expression and platelet-neutrophil aggregates formation.

Keywords: platelet membrane, thrombosis, platelet aggregation, JAK2V617F mutation, microparticles

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Abbreviations: MPNs, myeloproliferative neoplasms; CML, chronic myeloid leukemia; PV, polycythemia vera; MMM, myeloid metaplasia with myelofibrosis; ET, essential thrombocythaemia; CT-1, cardiotrophin-1

Introduction

According to the new WHO criteria published in 2016, chronic myeloproliferative neoplasms (MPNs) include chronic myeloid leukemia (BCR-ABL positive)-CML, chronic leukemia with neutrophilia or eosinophilia, polycythemia vera- PV, myeloid metaplasia with myelofibrosis -MMM, essential thrombocythaemia ET, and the non-classifiable form of chronic myeloproliferative neoplasms.

In the diagnostic criteria of these neoplasms, besides haematological tests and osteomedullary biopsy an important role is played by the JAK2V617F, CALR and Mpl mutational status testing.¹ Numerous researches involved the presence of JAK mutation in increasing the risk of thrombosis; in this way, along with advanced age and history of thrombosis in the classification criteria in "high risk" forms, the addition of JAK2 mutation is added.

Not only the presence of the mutation appeared to be a risk factor but also the value of allele burden expression. Patients with MPN and high allele burden expression (> 75%) have a higher risk of thrombosis.^{2,3} Important in analyzing the risk of thrombosis is also the analysis of additional factors such as hypertension, diabetes, hypercholesterolaemia, hypertriglyceridemia and smoking.⁴ Classification of patients in risk groups is important for the decision to treat anti-aggregation and cytoreduction.⁵

In the international prognosis score for essential thrombocythaemia it has been shown that gender is also a variable risk factor in men, thrombotic complications being more common.⁶ JAK2 mutation plays an important role in the development of cardiovascular thrombotic complications through autocrine mechanisms involving various angiotensin factors such as II (ANG II) and cardiotrophin-1 (CT-1)

factors.⁷ Arterial and venous splanchnic or cerebral thrombosis in the absence of proven myeloproliferation are more commonly associated with the presence of JAK2 mutation, and it is recommended to test the presence of the JAK2 mutation in these patients.⁸⁻¹² Testing for JAK2 mutation is also required in patients with recurrent thrombosis receiving anti-vitamin K.¹³ A significant prevalence of the presence of this mutation in patients with deep vein thrombosis has not been established.¹⁴ CALR mutation is not associated with thrombotic risk; is more common than Mpl in patients with MPN JAK2 negative and is more common in young patients, patients with anaemia, marked thrombocytosis and elevated spleen size.^{15,16} In turn, it has been shown that CALR mutation is more common in patients with chronic myeloproliferative neoplasms that associate haemorrhagic complications. This association is likely due to thrombocytosis seen in patients with chronic myeloproliferative neoplasms, haemorrhagic complications that may worsen by administration of aspirin. The risk of haemorrhagic complications is also increased in patients with a history of bleeding or those with acquired Willebrand disease in primary myelofibrosis.¹⁷ Recent studies indicate a possible association of CALR mutation with low expression JAK2 mutation.¹⁸ In addition to the above mentioned thrombotic risk factors, there are additional factors that contribute to the increased risk of thrombosis: leukocytosis and leuko-trombocyte aggregates, P selectin expression and granulocyte tissue factor, increase mean platelet volume.^{19,20} In the evaluation of the important thrombotic risk to be assessed, there are also the presence of thrombophilia, the qualitative changes in platelets and the degree of expression of the platelet derived micro particles. Non-cirrhotic, non-malignant portal vein thrombosis, not associated with acute abdominal inflammation or abdominal surgery, is often due to thrombophilia. Frequently homozygous PAI1 4G-4G and MTHFR mutations are present, and less frequently the V Leiden 506Q mutation and the G20210A mutation of prothrombin.

Deficiencies of C/S/AT proteins may also coexist.^{21,22} The association between the presence of II, V or PAI1 factor mutations and chronic myeloproliferation frequently leads to intra abdominal thrombosis, the most important role being that of PAI-14G-4G and

MTHFR677TT mutations.²³ In another study, there was a 2.7-fold increase in the incidence of thrombotic complications in patients with chronic myeloproliferative disorders that associated the JAK mutation and factor V Leiden—thrombophilia or resistance to C protein activated. The most common thrombosis is venous thrombosis. However, not all studies have shown the importance of the presence of these mutations in increasing the risk of thrombophilia.²⁰ It seems to be important only the association of the presence of Factor V Leiden that increases the risk of thrombotic complications.²⁴ Platelet has a very important role in the occurrence of thrombotic complications. It have been described quantitative and qualitative changes in platelet receptors that have positively correlated with increased risk of thrombotic complications: GP Ib, GP IIb/IIIa, GP IV, GP VI. Also, activated platelet status including elevated P selectin and thrombospondin and increased GP IIb/IIIa receptor expression were associated with increased risk of thrombosis.²⁵

In addition to the increased level of expression of P selectin, it have been shown to be involved PI3K–Rap1 signaling pathway and activation of α IIB β ₃ integrin but not thrombocyte secretion in the defective platelets response in patients with MPN independently of the JAK mutational status. This pathway is important in regulating α _v β ₃ integrin activity and platelet activation.²⁶ Platelet response to ADP and thrombin has been shown to be more pronounced in patients with MPN, especially those associated with the JAK2 mutation.²⁷ Platelet receptors for adhesion and aggregation are less expressed in chronic myeloproliferative patients compared to the control lot.

Receptors that define the status of activated platelets, P selectin, thrombospondin and CD36 are better expressed in these patients. The degree of expression of these receptors is much greater in patients with thrombotic complications. Platelet stimulation causes a decrease in GP Ib expression and an increase in GP IIb/IIIa and in GP IV expression; in patients with MPN these alterations are attenuated. The presence of a platelet defect in either granule storage or intrinsic cellular defect for receptor mediated granular secretion and GPIIb/IIIa receptor activation has been shown to explain this changes.²⁸ Also, the P2Y₁₂ platelet receptor expression is inversely correlated with the number of leukocytes, platelets and JAK2V617F allele burden in patients with MPN. These changes in this receptor could explain the tendency to haemorrhages of patients with chronic myeloproliferative disorders.²⁹ Patients with MPN may associate acquired Willebrand disease, the presence of JAK2 mutation influencing Willebrand factor endothelial synthesis and a reduced response to ristocetin.³⁰ It has been shown in many studies the altered thrombocyte response to various agonists for patients with chronic myeloproliferative disorders.^{31,32}

In patients with CML treatment with Imatinib they have followed, it has been shown that it has not altered platelet hypo responsiveness, but ristocetin hyper reactivity has been reduced.³¹ Dasatinib and Imatinib influence thrombocyte response, platelet aggregation being diminished for all reagents used.³³ In patients with chronic myeloproliferative neoplasia and thrombosis there is an increased level of lysine oxidase–LOX, the enzyme that contributes to platelet activation and influences platelet receptor function for collagen.^{34,35} Numerous studies have shown a high oxidative status in patients with chronic myeloproliferative neoplasms, a situation that correlates with a higher thrombotic risk.^{32,36,37} Although associations of increases in oxidative stress with MPN have been identified, association with changes in membrane fluidity and thrombocyte function remains to be investigated.^{32,38} The presence of circulating micro particles plays

an important role in the occurrence of thrombosis in patients with chronic myeloproliferative disorders.³⁹

The increase in their expression is associated with the presence of JAK2 mutation and splenomegaly. In these patients have been described in circulation the presence of 4 types of micro particles, derived from platelets, erythrocytes, endothelial cells and tissue factor.²

Microparticles derived from platelets may contain glycoproteins such as GPIIb/IIIa or GPIb/IX. The increase in circulating micro particles was observed not only in thrombosis but also in inflammation, cell activation or dysfunction, angiogenesis or cellular transport.

They may have molecules with cellular adhesion functions, bioactive phospholipids, cytoplasmic components, various antigens corresponding to the status of the cell from which they originated.⁴⁰ Microparticles expressed in chronic myeloproliferative patients have an increased level of phosphatidyl serine expression, phospholipid expressed on the membrane surface of activated cells or in cell apoptosis status^{40,41} (Figure 1).

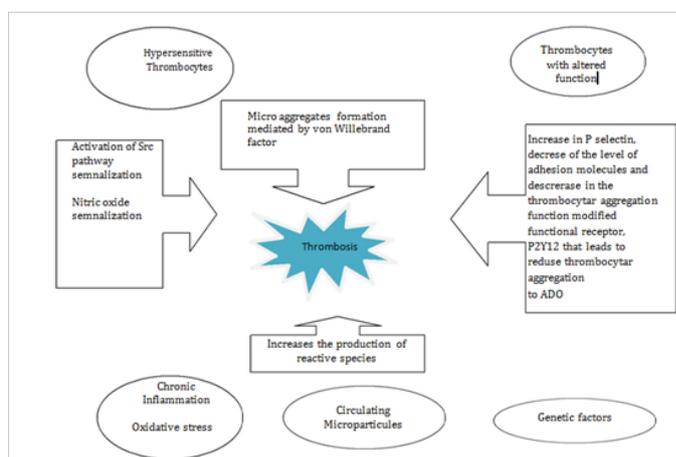


Figure 1 Mechanism of thrombosis in MPN patients.

Besides the increased expression of phosphatidylserine, the increased expression of lactadherine has also been demonstrated. Hydrate treatment lowers the expression of phosphatidylserine and lactadherine on the surface of micro particles released from platelets and erythrocytes.⁴² Also, circulating tissue factor expression has been identified in patients with chronic myeloproliferative disorders that associate thrombotic complications, the level being higher in naive therapeutic patients.⁴³

An imbalance between the number of micro particles expressing the tissue factor and the micro particles expressing the tissue factor inhibitor may lead to the initiation of thrombotic complications. There was identified an in vitro transfer of tissue factor expressed on the surface of derived micro particles containing tissue factor and activated platelet membrane.⁴⁰ However, not all studies confirm the predictive role for thrombosis of circulating micro particles.⁴⁴ The level of expression of the micro particles is a balance between stimulation, cell proliferation and apoptosis. Their production involves the formation of vesicles in the cell membrane as a result of changes in cell cytoskeletal modifications and membrane phospholipid modifications. The degree of their expression is considered a marker for diagnosis and prognosis in some diseases.⁴⁵ It remains to evaluate

the importance of micro particles in the pathogenetic mechanism of thrombosis when MPN patients associate thrombophilia.⁵

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Conflict of interest

Authors declare that there is no conflict of interests.

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