THE ROLE OF JAK2 MUTATION IN THROMBOTIC COMPLICATIONS OF CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Disclosure: No potential conflict of interest.
Received: 01.04.14 **Accepted:** 01.06.14
Citation: EMJ Hema. 2014;1:106-113.

ABSTRACT

Patients diagnosed with myeloproliferative neoplasms (MPNs) often develop thrombotic events as an onset of symptoms or in evolution. The pathogenesis of thrombosis in patients with MPN is multifactorial. There are multiple prognostic score systems, but the presence of JAK2V617F (JAK2) mutation is an independent and strong thrombosis risk factor. Patients with MPN and JAK mutational status usually associate thrombocytosis, increased immature circulating platelets, and leukocytosis, with increased expression of CD62P and CD14, increased levels of circulating microparticles and leuko-platelet microaggregates, and altered endothelial function. This review aims to discuss different factors contributing to the increased thrombotic risk in association with JAK2 mutational status. Also, recent reports incriminate this mutation to have a possible role in spontaneous loss of pregnancy.

Keywords: Chronic myeloproliferative neoplasm, JAK2V617F mutation, thrombosis, leuko-platelet microaggregates.

INTRODUCTION

Patients diagnosed with chronic myeloproliferative neoplasms (MPNs) frequently develop thrombotic complications; patients with polycythaemia vera (PV) and essential thrombocythaemia (ET) in particular are exposed to an increased thrombotic risk.[1] These complications may often be the onset manifestation of MPNs. The annual incidence of thrombotic complications in MPNs is around 1-10%.[2] Age, history of thrombosis, leukocytosis, and JAK2V617F (JAK2) mutation are all considered risk factors for thrombotic complications.[2,3] JAK2 mutation represents a G>T transversion at nucleotide 2,343, resulting in a substitution of phenylalanine for valine (V617F) in the JAK2 protein with tyrosine kinase activity (loss of autoinhibitory control).[4] Clinical studies report a correlation between disease phenotype and JAK2 (V617F) mutant alleles introducing the concept of allele burden, considered to be the ratio between mutant and wild type JAK2 in hematopoietic cells.[5]

A predictive risk score for thrombosis in ET patients was identified. This model provides a better prognostic risk stratification of patients compared with the classic one; the thrombotic risk was estimated to be from 0.95% patients/year (low risk), to 2.86% patients/year (high risk). Risk factors included in this model were: age over 60, history of thrombosis, cardiovascular risk factors, and the presence of JAK2 mutation.[6] If for the first 5 years after diagnosis, leukocytosis, age >60 years, and history of thrombosis were considered to be the most important predictive risk factors for thrombosis, after 5 years of evolution the single most powerful risk factor was the presence of JAK2 mutation. These patients are considered to have a 2-fold increased thrombotic

risk compared with JAK2 negative patients.[7-9] JAK2 allele burden is not a predictive risk factor for thrombosis or acute transformation, but JAK2 allele burden >50% is associated with increased risk of progression to primary myelofibrosis (PMF).[8,9]

Approximately 90% of patients with PV present JAKV617F mutation, whereas in ET and PMF patients, the prevalence of the mutation is around 60%. >50% of refractory anaemia with ringed sideroblasts and thrombocytosis patients are also JAK2 positive, as well as 20% of MPN unclassifiable cases.[10] Other somatic mutations that may appear in MPN are exon 12 JAK mutation (<5% of PV cases) and MPL W515F. The latter has been identified in 10% PMF and 1% ET patients, but not in PV.

Recently the presence of insertions or deletions in exon 9 of the CALRE (endoplasmic reticulum chaperone) has been described. CALRE and JAK2 mutations were not observed concomitantly in the analysed patients, and, among those JAK2 negative, 67% and 88% of ET and PMF patients, respectively, presented CALRE mutation. CALRE positive patients associated an increased number of platelets but with a lower risk of thrombosis and decreased overall survival compared to JAK2 positive patients.[11]

CLINICAL FEATURES

Thrombotic complications in MPN patients are represented by microcirculatory events and venous or arterial thrombosis. Microcirculatory symptoms are erythromelalgia, transient ischaemic attacks, transient hearing or visual impairment, recurrent headache, and peripheral paresthaesia. Less common occurrences are: dysarthria, temporary loss of monocular vision, and mono/hemiparesis. Venous commonly affected sites are abdominal veins (portal, hepatic, and mesenteric) and cerebral venous sinuses; less frequent are deep vein thrombosis (DVT) of lower limbs - associated with high risk of pulmonary embolism - and superficial vein thrombosis of the lower limbs.

50% of Budd-Chiari syndromes and 25% of patients with portal vein thrombosis (PVT) are diagnosed with MPNs.[1] For many of these, intra-abdominal thrombosis may be the early sign of an undiagnosed MPN. At this point, the presence of the JAK2 mutation may be the only indication of undiagnosed MPN.[12] Screening for JAK2 in patients diagnosed with splanchnic vein thrombosis (SVT) and no other hematological changes revealed the presence of this mutation in 17.1% of Budd-Chiari syndrome patients and in 15.4% of PVT patients.[13] Thrombotic events in MPN patients may precede the onset of any hematologic abnormalities within 1-2 years.[14]

JAK2 positive MPN patients may develop not only thrombotic complications of abdominal veins, but also thrombosis of cerebral or retinal veins, DVT of lower limbs, and pulmonary thromboembolism. The incidence of such complications in MPN is similar to the percentage of thrombotic events in the general population.[15] MPN cases diagnosed with Budd-Chiari syndrome present a higher incidence of this mutation compared to MPN patients with DVT.[13] Arterial thrombosis is more common than venous thrombosis.[3] Arterial thrombosis accounts for approximately 60-70% of thrombotic events occurring in MPN, with clinical features of stroke, myocardial infarction, or peripheral arterial occlusions. Strokes are by far the most frequently encountered thrombotic events (30-40%).[1] MPL515 mutation was identified in a small percentage of MPN patients with SVT (<1%). The clinical relevance of the presence of this mutation has not been fully understood.[13]

PATHOGENESIS OF THROMBOSIS

The pathogenesis of thrombosis in MPN patients is multifactorial (Figure 1). The hypercoagulant status is the result of blood cell abnormalities (erythrocytes, platelets, and leukocytes) arising from the clonal hematopoietic progenitor cells, which express a prothrombotic phenotype.[1]

Role of Red Blood Cells (RBCs)

Patients with MPN may present increased blood viscosity due to elevated numbers of RBCs and/or platelets. Elevated hematocrit represents a thrombotic risk factor especially for cerebral circulation.[1] This factor is well documented in PV patients. Along with elevated hematocrit, blood flow speed is also important. In veins with turbulent circulation, such as portal vein, the risk of thrombosis is lower. Hepatic veins present a greater risk of thrombotic complications, explaining the increased incidence of Budd-Chiari syndrome in PV patients.[13] Hepatic vein thrombosis is more frequent in PMF, because of enlarged spleen with compressive effect on portal vein system.[13] JAK mutations may affect cell prothrombotic phenotype, resulting in an increased RBC adhesion by modifying surface adhesion molecules.[1]

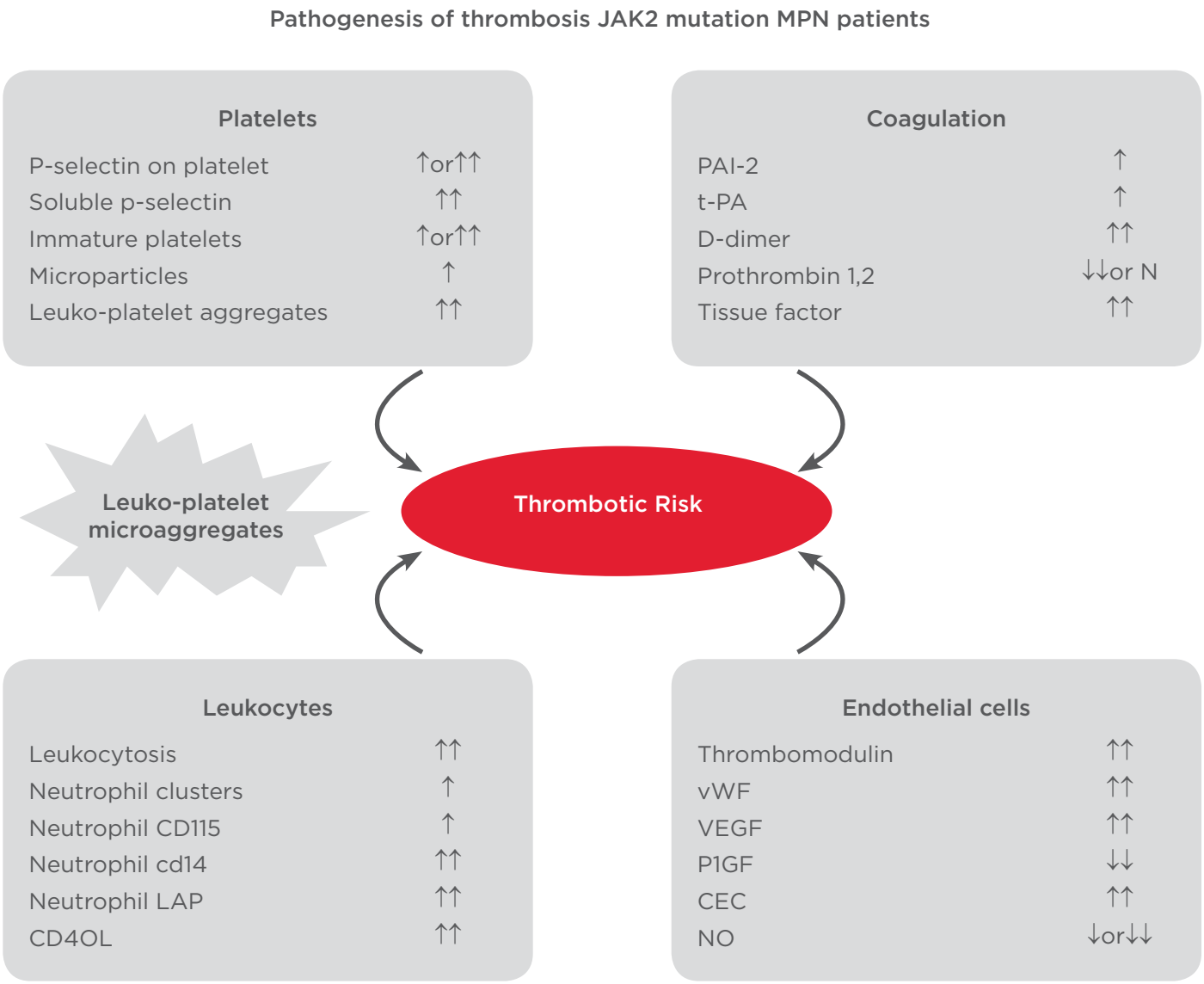

Figure 1: Multifactorial pathogenesis of thrombosis of MPN patients.
JAK: Janus kinase; MPN: myeloproliferative neoplasms; PAI-2: plasminogen activator inhibitor-2; t-PA: tissue plasminogen activator; LAP: leukocyte alkaline phosphatase; vWF: von Willebrand factor; VEGF: vascular endothelial growth factor; P1GF: placental growth factor; CEC: circulating endothelial cells; NO: nitric oxide.

Role of Platelets

Thrombocytosis is a paradoxal contributing factor in thrombosis and the platelet count *per se* has not been significantly correlated with thrombosis. The control of thrombocytosis is correlated with a decrease in the frequency of thrombotic complications. High platelet count (over one million/mmc) is generally protective against thrombosis because of acquired von Willebrand disease. In the ECLAP analysis, antiplatelet therapy, but not cytoreductive treatment, was significantly associated with a lower risk of thrombotic events.[1,3]

In a healthy population, 2% of circulating platelets are immature. Their number reflects the rate of thrombopoiesis. The percentage of immature platelets is increased in MPN patients, associated with an increased response to thrombin and P selectin expression on platelet surface. These platelets are more active in hemostasis. In fact, increased number of such immature platelets is considered a risk factor for thrombotic events. Studies report statistically significant correlations between immature platelets and JAK2 mutational status.[1] Platelets of MPN patients present high levels of CD63 and CD62P associated with acquired dense storage pool disease and ATP release deficiency.[16]

Acquired storage pool disease of dense granules involving ATP, ADP, calcium, serotonin, and pyrophosphate has been reported not only in

chronic MPNs, but also in acute MPNs as well. Defects are the result of granule formation, or the existence of empty vesicles developed after platelet activation. All results suggested that this association is due to chromosomal abnormalities on the megakaryocytic line, leading to a decrease in dense granule formation. The gene coding granule formation is closely located near one gene that in abnormal conditions might be involved in acute leukaemia or myelodysplastic syndrome (MDS) evolution.[17] Acquired storage pool disease of dense granules is associated with an increased secretion from alpha granules in MPN.[18] Increased expression of CD63 on inactive platelet surface and after platelet stimulation in PV patients, the level of P selectin expression on inactive platelet surface and after stimulation with arachidonic acid in ET patients, increased soluble P selectin, soluble CD40 ligand, and tissue factor expression on inactive platelet surface and after stimulation, all represent predictive factors for thrombosis. The level of platelet P selectin (CD62P) is higher in JAK2 positive MPN patients.[19]

JAK2 mutation modulates leukocyte and platelet activation with a great impact on platelet dysfunction. JAK2 mutation leads to an increase in JAK-STAT signalling pathway, activating signalling pathways such as PI3K, RAS, and SAT5 with hypersensitivity to cytokines, increased cell proliferation, and resistance to apoptosis.[20] Exon 12 mutation involves one amino acid located in the pseudokinase domain, suggesting a similar pathogenic mechanism with JAK2 mutation. Platelets of MPN patients present a preactivated conformation as a result of abnormalities of phospho-Tyr527 dephosphorylation with inhibitory effect. Src protein has an important role in this process. Activation of thrombin Src protein-dependent appears before JAK2 activation in normal and pathological platelets.[19,21,22] A strong correlation between the presence of JAK2 mutation and increased activity of platelet-neutrophil with platelet aggregates formation was established. Platelet activated status allows exposure of phosphatidylserine on surface membrane, normally present on the inside of the membrane, thereby providing a catalytic surface for thrombin generation, thus amplifying platelet activation.[23] In MPN patients a high level of plasma microparticles seems to be associated with an increased level of neutrophil - platelet aggregates and CD 62P, especially in those patients with a history of arterial or venous thrombosis.[24]

Platelet and megakaryocyte c-MPL expression is decreased in patients with PV and PMF, but not in patients with ET. Patients with JAK2 V617F mutation have lower levels. The mutation influences both the location and also the stability of c-MPL, contributing to platelet activation.[25-27]

Role of Leukocytes

Leukocytosis was also identified as a potential risk factor for arterial and venous thrombosis.[1] The pathogenic mechanisms are: the active status, the interactions between leukocytes and platelets, endothelial cells, and coagulation factors. The leukocytes contribute to the inflammatory process in atherosclerosis and therefore increase the probability of vascular events. The status of activated leukocytes is proved by CD11b overexpression and increased plasmatic levels of catepsin G, elastase, and myeloperoxidase. The increased expression of integrins and selectins promotes leukocyte adhesion to endothelium and to platelet membrane, thus contributing to the appearance of the aggregates of reactive oxygen species (ROS), and of inflammatory cytokines.[28]

Leuko-Platelet Microaggregates

Leuko-platelet microaggregates are often present in patients with MPN who associate thromboembolic accidents.[29] In JAK2 positive patients, leuko-platelet microaggregates are seen more frequently, as these patients present a higher level of tissue factor, soluble P-selectin, sCD40L, von Willebrand factor (vWF):Ag, and a lower level of free S protein, CD41, and CD42b receptors. Tissue factor expression is highly increased in JAK2 positive patients versus wild type.

Granulocyte-thrombocyte CD11b-CD42b aggregates and monocyte-thrombocyte CD11b-CD14-CD61 aggregates are more common in patients with MPN and MDS.[24,30] The presence of these aggregates does not affect the platelet surface antigen expression.[31] The level of aggregates is correlated with platelet count, P-selectin percentage, thrombospondin (TSP), and GP IV. The incidence is higher in patients with thrombosis and microcirculation abnormalities.[29,32] Alvarez Larran[33] considers that CD11b expression may be used as a marker for PV in patients presenting with Budd-Chiari syndrome. Patients with PMF have an increased risk of spontaneous aggregates. Treatment with aspirin plus hydroxycarbamidum is more effective versus aspirin for the prevention

of leuko-platelet aggregates CD11bCD42b and CD11bCD62P. The important role of hydroxycarbamidum is explained also by the inhibition of endothelin-1 and ICAM-1 expression, and the increased levels of NO, and therefore hydroxycarbamidum, has a strong antithrombotic effect.[25] Trelinski et al.[30] underlined the positive effect of aspirin in preventing leuko-platelet aggregates and the ineffectiveness of hydroxycarbamidum. Microparticles are formed due to alterations of the platelet membrane cytoskeleton and changes in phospholipid asymmetry; the latter ones express phosphatidyl-serine and in some cases active tissue factor, the main coagulation activation.[23]

Aspirin decreases CD11b and neutrophil-platelet aggregates (induced by *in vitro* stimulation), suggesting a lower leukocyte-platelet interaction. G-CSF receptor is connected to JAK2 pathway, and therefore, a constitutive activation of intracellular signalling in the presence of JAK2 mutation might be possible, contributing to the activated leukocyte status. Some markers such as CD14 expression and leukocyte alkaline phosphatase are significantly altered in JAK2 positive patients, while expressions of CD11b and plasma elastase level are not significantly different.[1]

The possible correlation between high levels of C reactive protein and low levels of pentraxin^3 as inflammation markers and thrombotic risk in MPN was studied by Barbui et al.,[34] proving a possible association with high statistical relevance. Both proteins were significantly correlated with increased prevalence of JAK2 mutation in patients with MPN. Still, when pentraxin^3 was >4.5 ng/ml, the thrombotic risk was lower.[28,34] Pentraxin^3 is produced at the inflammation site by endothelial cells, neutrophils, monocytes, and macrophages, and its level increases in sepsis, vasculitis, and autoimmune disorders. Its expression is induced by interleukin-1, tumour necrosis factor-alpha, and low density lipoproteins, but it is not influenced by the level of C reactive proteins.[34]

In patients with MPN, thrombopoietin may induce an increased platelet aggregation and release of dense granule content after exposure to standard stimuli (collagen, epinephrine, ADP). Platelet P-selectin expression and neutrophil CD14 expression are increased in patients with JAK2 positive ET, while CD11b is highly expressed of neutrophils and monocytes of PMF JAK2 positive patients. A higher level of platelet tissue factor and leuko-platelet aggregates was also observed in JAK2 positive ET patients. Also, JAK2 positive patients have increased levels of thrombomodulin and P-selectin.[1] Aside from higher P-selectin, patients with MPN also express a higher TSP level, especially the ones with antecedent thrombotic accidents. These are associated with lower glycoprotein (GP) IIb/IIIa and GPIIb expression.[29] GP IIb/IIIa receptor, an early megakaryocytic marker is higher in MPN, with up to a 33% increase (especially for GP IIIa).[35] While dormant platelets have low GP IIb/IIIa expression, patients with MPN present high levels when the platelets are stimulated with platelet-activating factor (PAF) or PFMA.[36] Fibrinogen induces conformational changes of GP IIb/IIIa receptor, and the altered expression corresponds to the status of activated platelet. Kaplan et al.[37] indicated the presence of a GP IIb/IIIa with normal fibrinogen receptor, but with altered (low) expression in MPN patients. During megakaryocytic maturation, GP IIIa expression decreases.[38] Platelet expression in MPN is correlated with higher thrombotic risk. In addition, patients with MPN present platelet aggregation anomalies due to lower adhesion molecule levels (GP-IIb, GP IIb/IIIa, GPIV, and GPVI) and deficient platelet metabolism (abnormal arachidonic acid metabolism).[1] In ET patients, higher levels of thromboxane A2, B2, PAC-1, PGF1, and PGI2 were observed. These levels are decreased under treatment with Ozagrel, a thromboxane A2 (TxA2) inhibitor, thus, decreasing the thrombotic risk.[39] Recently, an anomaly in the P2Y12 signalling pathway was observed in patients with MPN, which might be one of the contributing pathogenic mechanisms for the bleeding tendency in patients with very high platelet counts. The function of platelet P2Y12 is inversely correlated with white blood cell (WBC) count, platelet count, and JAK2 allele burden.[40]

Role of Endothelial Cells

Besides from the pathogenic mechanisms described above, recent studies have identified the endothelial cell dysfunction as a risk factor for thrombosis.[41] The normal endothelium acts as an antithrombotic surface which inhibits platelet adhesion and coagulation cascade activation. In MPN, it becomes a pro-adhesive and pro-coagulant surface, by exposure to higher levels of ROS, to intracellular proteases released by activated neutrophils, and to cytokines from inflammation site.[1,26] These may induce endothelial cell destruction and release of thrombomodulin, selectin, and vWF.

Selectins are also present on endothelial cells (P-selectin – CD62P, E-selectin – CD62E), platelets (P-selectin), and leukocytes (L-selectin).[1,42] Also, Type 1 intercellular adhesion molecules (ICAM-1, CD54) and vascular cell adhesion molecules (VCAM-1) are expressed, which contribute to leukocyte adhesion to endothelial cells and promote vascular occlusion.[42] The number of different circulating endothelial cells (CECs), the levels of vascular endothelial growth factor (VEGF), of soluble receptors for VEGF (sVEGFR-1,2), and of placental growth factor (P1GF) are all modified in patients with MPN. The number of CECs was significantly higher in patients with ET and PV versus controls, regardless of JAK2 status. Their number correlated with WBC count, probably as a consequence of the existence of a pathological hematopoietic clone.[42] The medium levels of activated CECs were significantly higher in patients with PV and increased WBC (cut off $8.7x10^9$/l). VEGF and soluble receptor sVEGFR-1 plasmatic levels were significantly increased in patients with ET and PV versus controls, while P1GF was decreased. Also, a higher level of D-dimers was observed in patients with MPN. Angiogenesis, reflected by CEC levels, is increased in patients with ET and PV, again regardless of JAK2 status. The presence of an increased level of CECs contributes to the pathogenesis of MPN, reflecting vascular injury. Angiogenic cytokines interact with other known prothrombotic factors.[1,43]

The endothelial cell also releases nitric oxide (NO), generated by oxidation of L-Arginine to L-Citrulline by NO-synthesis. NO mediates vascular relaxation to vasoactive substances, inhibits platelet adhesion, activates platelet secretion, induces platelet de-aggregation, inhibits platelet P-selectin expression, and increases leukocyte adhesion to the endothelium. As a consequence of inflammation, platelets and endothelial cells release small quantities of NO.[28] Deficiency in release of NO – seen in patients with MPN – favours thrombosis.[1] Hydroxycarbamidum increases NO in patients with ET, promoting thrombotic complications, while in PV patients it did modify the prevalence of thrombosis.[1] Endothelial splanchnic cells were found to express JAK2 mutation, possibly as a part of the malignant process.[13] The increase of vWF antigen and serum thrombomodulin in ET may also act as a predictive factor of thrombosis.[19]

Role of Coagulation Factors in Thrombosis

In the assessment of the thrombotic risk, in addition to the quantitative and qualitative changes of the blood and endothelial cells in patients with MPN, abnormalities of the coagulation factors were also observed. MPNs (PV and ET) are associated with acquired resistance to activated C protein.[1,19] Increased levels of prothrombin fragment 1.2 in PMF and tissue factor in ET represent risk factors for thrombosis.[19] Patients with MPN present higher levels of tissue plasminogen activator (t-PA:Ag), plasminogen activator inhibitor-1 (PAI-1:Ag), and D-dimers, indicating the secondary activation of fibrinolysis (t-PA:Ag and D-dimers) and the inhibition of fibrinolysis (PAI-1:Ag). The increase of PAI-1:Ag in MPN is associated with a higher percentage of activated platelets and with altered vascular endothelium.[44]

JAK2 MUTATION IN PREGNANCY

Besides the correlation to an increased risk of thrombotic complications, JAK2 mutation is interestingly correlated with a spontaneous loss of pregnancy in association with ET45 but also independently from other factors.[46,47] Presence of JAK2 mutation was significantly correlated with the risk of pregnancy loss, with odds ratios of 4.63 and 7.20 for embryonic loss and foetal loss, respectively.[46] Nevertheless, there are studies that do not report a negative implication of JAK2 mutation in pregnancy.[48]

CONCLUSIONS

Patients with JAK2 positive MPN have a high incidence of thrombosis and the presence of JAK2 mutation is an important risk factor. The pathogenesis of thrombosis in MPN patients is complex. Platelet and/or endothelial cell dysfunction, leuko-platelet microaggregates, and abnormalities of the coagulation factors are important factors which contribute to the increased risk of thrombosis in MPN. Occasionally, the presence of JAK2 mutation may be the only indication of undiagnosed MPN. The mutation is also interestingly correlated with spontaneous loss of pregnancy, in association with ET or even independently.

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