An Update on the Platelet Dysfunction in Chronic Myeloproliferative Syndromes

ANA MARIA VLĂDĂREANU1, CRISTINA CIUFU1, H. BUMBEA1, MINODORA ONISĂI1, S. ARAMĂ2

1Department of Hematology, Emergency University Hospital, “Carol Davila” University of Medicine and Pharmacy
2Department of Physiology, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania

The thrombotic and hemorrhagic diathesis represents a frequent complication in myeloproliferative disorders (CMPD). They are correlated with the number of platelets, but also with their qualitative disorders, such as membrane glycoprotein changes. The latter are revealed by many platelet essays including flow-cytometry and include modified activation, secretion and aggregation patterns. The thrombopoietin platelet receptor (cMPL), affected by the JAK2 V617 mutation encountered in CMPD, may be associated with a prothrombotic status. Its implication reveals the importance of the molecular genetics profile in defining molecular diagnostic hallmarks and makes it a candidate in the early diagnosis of myeloproliferative disorder and a predictor of thrombotic complications in this group of diseases.

Key words: myeloproliferative syndromes, thrombosis, hemorrhage, JAK2 V617 mutation.

The clonal stem cell disorders, including chronic myeloproliferative disorders (CMPD), are associated with qualitative and quantitative disorders of the erythroid, granulocyte, and thrombocyte lineages [1][2].

These dysfunctions are thought to cause bleeding and thrombotic complications. This is the reason why thrombotic and hemorrhagic events occur quite frequently in patients suffering from myeloproliferative syndromes and are also considered important causes of morbidity and mortality [3].

The qualitative defects of the platelets together with their quantitative alterations such as thrombocytosis (which occurs mainly in myeloproliferative syndromes) produce important disorders of hemostasis.

Chronic myeloproliferative disorders are stem-cell disorders, in this group being included essential thrombocythemia, polycythemia vera, myeloid metaplasia with myelofibrosis and chronic myeloid leukemia. In all of these diseases there are described hemorrhagic diathesis, thrombo-embolic complications and qualitative defects of the platelets. Although these diseases have many common features, the spectrum of platelets’ disorders and their clinical picture is different in CMPD. The thrombotic complications are more frequent in essential thrombocythemia and polycythemia vera, and the hemorrhagic complications are more frequent in MMM, especially when there is an associated thrombocytopenia.

32% of the patients with CMPD may develop arterial or microvascular thrombotic complications; venous thrombosis is reported as well [4][5].

The thrombotic risk is increasing with age, a previous thrombosis history and the occurrence of general associated risk factors such as hypercholesterolemia, smoking, diabetes mellitus, arterial hypertension, etc.

THE PLATELET ROLE IN THE ETIOPATHOGENESIS OF THROMBOSIS IN CHRONIC MYELOPROLIFERATIVE DISORDERS (CMPD)

The importance of the platelet’s role in the etiopathogenesis of thrombotic events in CMPD is multifactor through: their number, their qualitative defects, the pattern of platelet activation, the formation of platelet leukocyte aggregates, the appearance of platelet micro-particles, and the impact of JAK mutation [6].

1. In CMPD, especially in polycythemia vera, erythrocytosis leads to increased blood viscosity which may rheologically contribute to the thrombosis tendency. But, this rheologic effect of erythrocytosis is not the only mechanism explaining the thrombotic tendency in MPD [7].

2. Thrombocytosis is a contributing factor in the etiopathogenesis of thrombosis in PV and ET [8]. There is a relative correlation between the
degree of thrombocytosis and the thrombotic complications in chronic myeloproliferative disorders [8] and there is evidence that platelet count control reduces the incidence of thrombosis.

Even if the control of thrombocytosis is decreasing the frequency of thrombotic complications, especially in patients with previous thrombosis, the degree of the thrombocytosis is not correlated with the thrombotic risk all the time [6].

In the ECLAP analysis, antiplatelet therapy, but not cytoreductive treatment, was significantly associated with a lower risk of cardiovascular events [9].

3. Qualitative disorders of the platelets in chronic myeloproliferative disorders

Optic microscopy reveals heterogeneity in size and morphology of platelets, which is expressed by the large mean platelet volume [10] and by anisocytosis and anisochromia on blood smears as shown in Fig. 1.

Electronic microscopy revealed the decrease of alpha granules and mitochondria and also changes of the dense tubular and canalicular system [11]. A decrease of the ADP, ATP and serotonin content of dense granules was also proved [12].

Some patients suffering from chronic myeloproliferative disorder have a prolonged bleeding time, this change being more frequent in MMM than in the other chronic myeloproliferative diseases. The prolonged bleeding time is not correlated with high bleeding risk [13].

Waddell et al. [14] were the first to describe the membrane glycoprotein platelet changes in chronic myeloproliferative disorders, such as: the decrease of the alfaIIb betaIII and Ib receptors and a low fibrinogen surface binding, indicating a decrease of alfaIIb betaIII receptor. Besides the quantitative decrease, the surface receptors alfaIIb betaIII were also proved to have an altered activation pattern [14].

Other qualitative platelet defects in CMPD include: impaired dense granule release, defective Ca mobilization, lipooxygenase accumulation [15].

4. The pattern of platelet activation

The pattern of platelet activation and the changes in the surface receptors are presented in Fig. 2. The platelet secretion and aggregation as a response to epinephrine, ADP, collagen is decreased in chronic myeloproliferative disorders and in myelodysplastic syndromes. This aggregation defect is expressed by the absence of the first aggregation wave [3].

The pathogenesis of platelet activation pattern in chronic myeloproliferative syndromes is not quite defined. It is multifactorial and includes: the lipooxygenase deficiency, the disturbances in bone marrow microenvironment, the hematocrit range, the implication of activated leucocytes, the impaired platelet NO synthetase, elevated thrombopoietin level, the effect of JAK2 mutation [6].

In CMPD, especially in polycythemia vera, the rise of haematocrit increases blood viscosity. The axial migration of the erythrocytes in the bloodstream “pushes” the platelets to the wall of the vessel, increasing the platelet-vessel interaction, especially in high shear conditions – in small arteries and capillaries – contributing to platelet’s activation [7].

As reported by Schafer, a large number of patients have a lipooxygenase deficiency, which could increase the capacity of endoperoxidasis to produce thromboxan A2 [16][17].

Cooper et al. were the first to prove the decrease of adenylate-cyclase activation GPD2 induced, correlated with a decrease of 50% of PGD2 receptors on the platelets, and normal responses to PGE2 and PG12 [18]. This is suggestive for an inhibitor mechanism defect in MPD.

It was recently established that platelets of the PV and MMM patients, but not of ET and CML patients, have a low expression of the thrombopoietin receptor and the tyrosin-phosphorylation thrombopoietin induced is also decreased; tyrosin-phosphorylation thrombin induced is preserved [19].

In normal platelets, PGD2 increases AMP because of adenylate-cyclase stimulation, leading to platelet response inhibition [18].

Fujimoto et al. [20] proved the decrease of Ca mobilization agonist-induced, the signaling by thromboxan-receptor and the proteic phosphorylation because of GMPc protein-kinase deficiency [20]. According to their observations platelet calcium influx was lower than in control patients. Their conclusion was that GP IIb/IIIa anomalies are involved in the decrease of platelet calcium influx in chronic myeloproliferative diseases patients [20].

The increase of P selectin expression, thrombospondin and GP IIb/IIIa was correlated with thrombosis. In chronic myeloproliferative syndrome patients it has been shown an expression and functional decrease of GP IIb/IIIa (CD41/ CD61) receptor, also of fibrinogen receptor, fibronectin receptor, vitronectine receptor, thrombospondin receptor, von Willebrand factor receptor. Also it
has been found a low von Willebrand factor binding on the platelet [21].

Some studies [22] showed an increase of VEGF level that demonstrates increased endothelial activation. The leukocyte activation (especially monocytes and neutrophils) allows the release of the granules content which activate the coagulation pathways, induce platelet aggregation, release inflammatory cytokines and oxygen superoxide, all of the above contributing to the endothelial destruction [22].

The interaction between neutrophils and platelets becomes apparent after the adhesion cascade, when CD62P binds to P-selectin on the neutrophil. The adhesion is stabilised by two other complexes: CD11b of the beta-integrin complexed with CD18 with GP Ib of the platelet and the binding of fibrinogen with the platelet GP IIb/IIIa. Some studies noticed an increase of the CD62P, CD11b, CD42b expression in patients with polycythemia vera and essential thrombocythemia [23]; the level of CD11b/CD42b and CD62P/CD11b complexes was also increased.

Falanga et al. [24] underline that the expression of JAK2 mutation in ET patients confers to neutrophils a different haemostatic property in terms of increased interaction with platelets and increased expression of surface TF and fibrinogen, suggesting a new link of the mutation with the prothrombotic state.

It was also described a deficiency of GP Ia-IIa and an abnormal response to collagen stimulation; GP IV (CD 36) – a membrane glycoprotein implicated in platelet-collagen interaction and platelet-thrombospondin interaction – is known to be decreased in essential thrombocythemia [25].

In chronic myeloproliferative disorders, there are also other alterations of the platelet membrane receptors – GP IV (CD36), thrombospondin receptor which is released by platelet secretion. Its effect is on the strength of the platelet aggregates, making this process irreversible. The expression of this receptor is increased in chronic myeloproliferative disorders – all the patients with essential thrombocythemia presented a proteolysed form of thrombospondin (TSP) and this form was not detected in reactive thrombocytosis [26]. A study showed that the level of normal GP IV and the associated proteins may become normal during the treatment with Interferon, but the altered form of TSP receptor still persists [21].

It was proved over twenty years ago that the Fe-IgG platelet receptors are increased in chronic myeloproliferative diseases [27].

The variability of the platelet receptors is thought to be genetically determined and there are few studies establishing that platelet’s glycoproteins polymorphism could contribute to thrombosis. PLA2 allele of GPIIla was correlated to the increased incidence of arterial thrombosis, but this conclusion must be confirmed by more studies [28].

5. The acquired von Willebrand disease

The acquired von Willebrand disease seems to be an important factor contributing to the bleeding diathesis in patients with thrombocytosis in MPD. The von Willebrand factor multimers analysis on agarosis gel electrophoresis has shown the decrease of ultra-large multimers, similar to von Willebrand disease type 2. It is correlated to the degree of thrombocytosis and could be corrected by cytoreduction therapy [29].

Possible mechanisms of production: high clearance of ultra-large multimers by selective platelet binding, or increased proteolysis of von Willebrand factor, perhaps by the exposure of von Willebrand factor cleavage loci to ADAMT13. Because this acquired defect was also reported in secondary thrombocytosis, it represents more a favoring factor and not the cause of bleeding complications in chronic myeloproliferative diseases, especially in severe thrombocytosis [29][30].

6. The formation of platelet leucocyte aggregates and the appearance of platelet microparticles

The formation of platelet leucocyte aggregates (Fig. 3) is increased in MPD patients [6]. They form after platelet and neutrophil activation and contribute to thrombus formation by the release of granule contents, by inducing tissue factor expression on monocytes, by the release of superoxide and cytokines, by enhancing platelet activation and endothelial activation and damage [6].

Harrison, C.N. (ASH-2005) [6] reported that the increased level of highly thrombogenic platelet microparticles in ET and PV was correlated with the thrombotic tendency.

7. The impact of JAK mutation

An alternative explanation of modified platelet activation includes the effect of JAK2 mutation (Fig. 4). Recent data suggest that JAK2 mutation affects cMPL platelet receptor of thrombopoietin [19].
The JAK2V617F represents a G to T somatic mutation of Jak2 at nucleotide 1849, in exon 14, resulting in the substitution of valine to phenylalanine at codon 617.

In chronic myeloproliferative syndromes, the mutation occurs in multipotent stem-cell [31]. The homozygous progenitors are more frequent in PV patients and very rare in ET patients [32].

The JAK2V617F mutations occur in 90–95% of PV patients, 50–70% of ET patients and 40–50% of MMM patients [33][34]. This mutation has also been demonstrated in other myeloproliferative disorders, including atypical MPD and also in myelodysplastic syndromes [33][36].

The presence of JAK2 mutation has also been revealed in elderly AML patients and it underlines the possibility that these patients have had previously undiagnosed chronic myeloproliferative syndromes. Recent data revealed that JAK2 mutation occurs in a significant number of AML secondary to chronic myeloproliferative syndromes patients. In de novo AML patients the incidence of this mutation is low [41][42].

The intracytoplasmic domain of this receptor is correlated to JAK2, member of kinase family Janus. The correlation between the JAK mutation and the thrombopoietin receptor is shown in Figure 5. The thrombopoietin stimulates the phosphorylation of some proteins, including Janus kinase JAK2 and TYK2. The thrombopoietin and its receptor represent the control-key to regulate the platelet number; the most of PV and MMM patients have a decrease of thrombopoietin receptors and an incomplete glycosylation of these receptors. This defect cannot be found in other platelet membrane glycoproteins (GPIIb), but it is related to the disease duration and extramedullar hematopoiesis [42].

According to Campbell [44] the presence of the JAK2V617 mutation divides essential thrombocytopenia into two distinct subtypes. Patients with the mutation present higher hemoglobin levels, higher white cell counts and bone marrow hypercellularity [44][45]. The risk of thrombosis in mutated ET patient has not been reported by all investigators [46][47].

A group of investigators from Italy – Bergamo [48] have reported that JAK2 mutation in ET brings up a distinct clinical entity with a biological phenotype intermediate between JAK2 wild-type ET and PV. For the first time they present a comparison between the thrombotic risk of ET and PV patients defined on the basis of their JAK2 mutational status [48].

They have reported for JAK2 mutation ET and PV that hemoglobin and hematocrit levels as well as white cells number and activation parameters (PRV1 and LAP) [49] increase whereas platelet number decreases as compared with those with JAK2 wild-type ET.

The association between increased granulocyte PRV-1 and LAP expression and thrombosis is noteworthy. Their recent data showing that leukocytes of JAK2 mutated ET patients present a prothrombotic state indicated by a significantly increased expression of surface tissue factor and fibrinogen and a tendency to form higher numbers of leukocyte-platelet aggregates [24]. These results explain why hydroxyurea is more effective in reducing the thrombotic events particularly in JAK2 mutated ET patients and anagrelide (a megakaryocyte restricted inhibitory agent) is not [44][50], and also hydroxyurea possibly reduces the leukocytes-platelet aggregates by endothelin 1 gene and ICAM-1 overexpression and by increasing the NO level [23][26].

J. Smalberg et al. reported the association between thrombosis of the hepatic veins – the Budd-Chiari syndrome – BCS and the JAK2V617 mutation status. The mutation occurred in 59% of BCS patients [51] and may be used to characterize occult MPD – as a molecular diagnostic hallmark – and could be included in the early diagnosis for MPD in BCS.

Tefferi [52] underlines that it is reasonable to consider the screening of JAK2V617 mutation in the initial evaluation of unexplained thrombocytosis, unusual thrombotic complication including abdominal or cerebral vein thrombosis, arterial events at young age and other MPD characteristic clinical manifestations including erythromelalgia [52]. Despite the rare occurrence of thrombocytopenia, some patients with MPD may sometimes develop life threatening thrombotic complications like PTE (pulmonary thromboembolism), DVT (Deep vein thrombosis) [6][53] highlighting the importance of the functional study of the platelet mainly through the flow-cytometric study of their activation.

**CONCLUSION**

Since the platelet number is not the only factor that controls the thrombotic or hemorrhagic
An update on the platelet dysfunction in MPD, the importance of studying platelet function is increasing. Outlining the genetic profile (hence the molecular changes) may provide early predictors for potentially vital complications in patients diagnosed or susceptible of MPD.

Acknowledgements. The current article was written based on the data collected during a National Research Grant in the Program “Excellence in Research” – MULTRO (A complex, multidiscipline study of platelet in myelodysplastic and myeloproliferative syndromes with 5 partners) sponsored by the Romanian Ministry of Research and Development.

Diateza trombotică şi cea hemoragică reprezintă complicaţii frecvente în sindroamele mieloproliferative cronice (SPMC) şi în sindroamele mielodisplazice (SMD). Aceste modificări sunt corelate atât cu numărul absolut de trombocite, cât şi cu prezenţa anumitor defecte calitative, precum alterări ale glicoproteinelor membranare, care pot fi evidenţiate prin diferite teste plachetare, inclusiv examenul flowcytometric. Modificările glicoproteinelor membranare induc alterarea activării, secreţiei şi agregării plachetare. Receptorul pentru trombopoietina (cMPL) afectat de mutaţia JAK2 V617, întâlnită în sindroamele mieloproliferative, poate fi asociat cu apariţia unui status protrombotic. Implicaţia sa relevă importanţa profilului genetic molecular în definirea unor markeri moleculari de diagnostic, făcându-l astfel candidat pentru diagnosticul precoce al sindroamelor mieloproliferative şi predictor pentru complicaţiile trombotice apărute în acest grup de boli.

Author correspondence: Ana-Maria Vlădăreanu, Assistant Professor
Department of Hematology, Emergency University Hospital
Bucharest, Romania,
169, Splaiul Independenţei, 050098, Bucharest, Romania
Phone no: +4021 318 05 22, Fax: +4021 318 05 70
E-mail: anamariavladareanu@yahoo.com

REFERENCES

31. DELHOMMEAU F., DUPONT S., TONETTI C., MASSE A., GODIN I. et al., Evidence that the JAK2 1849T (V617) mutation occurs in a lymphomyeloid progenitor in polycythemia vera and idiopathic myelofibrosis. Blood. 2007, 109, 1, 71–77
An update on the platelet dysfunction

52. TEFERI A., Classification, Diagnosis and Management of Myeloproliferative Disorders in the JAK2V617F Era. ASH Education Program Book, 2006, 240–245.

Received February 7, 2008