Expression of CD markers in JAK2V617F positive myeloproliferative neoplasms: Prognostic significance

Saeid Shahrabi,1 Ali Ehsanpour,2 Somayeh Heidary,2 Mohammad Shahjahani,2 Masumeh Maleki Behzad2

1Department of Biochemistry and Hematology, Faculty of Medicine, Semnan University of Medical Sciences, Semnan; 2Thalassemia and Hemoglobinopathy Research Center, Research Institute of Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Abstract

Myeloproliferative neoplasms (MPNs) are clonal stem cell disorders characterized by the presence of JAK2V617F mutation. Thrombohemorrhagic as well as autoimmune or inflammatory phenomena are common clinical outcomes of these disorders. Recent studies have shown that abnormality in frequency and function of blood cells manifested by an alteration in CD markers’ expression patterns play a key role in these complications. So, there may be a relationship between CD markers’ expressions and prognosis of JAK2V617F positive MPNs. Therefore, in this review, we have focused on these abnormalities from the perspective of changing expressions of CD markers and assessment of the relationship between these changes with prognosis of JAK2V617F positive MPNs. It can be stated that the abnormal expression of a large number of CD markers can be used as a prognostic biomarker for clinical outcomes including thrombohemorrhagic events, as well as autoimmune and leukemic transformation in JAK2V617F positive MPNs. Considering the possible role of CD markers’ expressions in JAK2V617F MPNs prognosis, further studies are needed to confirm the relationship between the expression of CD markers with prognosis to be able to find an appropriate therapeutic approach via targeting CD markers.

Introduction

Janus activating kinase 2 (JAK2) V617F is a common mutation in Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs), which include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF).1 This mutation is observed in over 90% of PV patients and in nearly 50% of ET and PMF patients.2 In addition, JAK2V617F mutation has been reported in a number of other malignancies such as myeloid leukemias and has been rarely observed in lymphoid leukemias.3-5 Since MPNs are hematopoietic stem cell (HSC) derived disorders, JAK2V617F mutation can involve any myeloid progenitor, including granulocyte, erythrocyte, platelet, and monocyte.6,7 In addition, since JAK2 is the signaling pathway of several hematopoietic factors and cytokines, the increased activity of this pathway under the influence of JAK2V617F mutation can increase the sensitivity of multipotent progenitor cells to hematopoietic factors such as erythropoietin (EPO), thrombopoietin (TPO), and many other cytokines.8,9 This is an event associated with unchecked hematopoiesis, increased risk of thrombotic events, and bleeding complications that significantly affect the quality of life and disease prognosis. In addition, JAK2V617F mutation can induce thrombosis, ischemia, and other cardiovascular events by affecting cardiac arteries and veins in ET and PV.10 The pathophysiology of clinical findings of these disorders is complex despite the significant overlap in their clinical findings.11 Several studies have indicated that the annual incidence of thrombotic complications in MPNs is around 1-10% and that there is a correlation between MPN manifestations and JAK2V617F mutant alleles burden.12-14 In fact, pervious studies has shown that the presence of JAK2V617F mutation is the most powerful risk factor for thrombotic events and that these patients are considered to bear a 2-fold increased risk of thrombosis than JAK2V617F negative patients.15,16 Although the JAK2V617F mutation can affect the circulating blood cell counts, this mutation does not seem to be responsible for all clinical findings in these disorders by itself; however, a range of factors such as variation in the number and function of different blood cells, increased levels of circulating microparticles, leuko-platelet microaggregates, and altered function of endothelial cells may contribute to the occurrence of these complications.9,17,18 Moreover, it has been shown that for the first five years after initial diagnosis of JAK2V617F positive MPNs, >60 years of age, history of thrombosis, and leukostasis are the most important predictive risk factors for thrombotic events.19 Furthermore, the inflammatory and immunological phenomena
are less frequent complications in these disorders. Recent studies have introduced abnormal immune cell function and the ectopic production of a number of cytokines as responsible for these complications.20,21 A number of studies have provided evidence that JAK2V617F mutation in T, B, and natural killer (NK) cell lineages can effect clonal proliferation of these cells as well as immunological or inflammatory manifestations of JAK2V617F positive MPNs.22-24 Considering the fact that the majority of these changes can appear as the increased or decreased expressions of some CD markers in the involved cells, we attempt to evaluate the variations in the expressions of CD markers in JAK2V617F positive MPNs and their probable role in the prognosis of these disorders.

### CD markers and hemostatic complications in JAK2V617F positive MPNs

JAK2V617F positive MPNs are a group of disorders that can be associated with the incidence of both thrombotic and bleeding complications.11,25 There is growing evidence indicating that a wide range of risk factors such as abnormal proliferation and function of different blood cells can contribute to these complications in addition to the role of JAK2V617F mutation.17,26 Herewith, we mention some of these abnormalities from the viewpoint of changing expression of CD markers in different blood cells and their impact on the prognosis of these disorders.

### Expression of erythroid lineage related CD markers and erythropoiesis

Due to the presence of JAK2V617F mutation in MPN disorders, erythroid lineage usually becomes sensitive to EPO, and the rate of erythropoiesis is thus increased.27 Unchecked erythropoiesis is associated with increased hyperviscosity, which is a risk factor for increased thrombotic events.28 Erythroid maturation is detectable by the altered flow cytometric pattern of erythroid surface markers. Typically, the expression of CD71 (transferrin receptor) is increased in the early maturation stages of this lineage and is then gradually decreased, while the expression of CD235a (glycoporphin A) is increased in the final stages of erythroid maturation.29 Previous in vitro studies showed that the expression rates of these markers in PVT patients were high compared to healthy subjects, which indicate the enhanced proliferation and maturation in these cells.30 It is inferred that the overexpression of CD71 and CD235a in vivo may indicate increased erythropoiesis. Therefore, thrombotic complications could increase in these conditions due to increased hyperviscosity. On the other hand, JAK2V617F mutation can exacerbate thrombosis by affecting the expression of adhesion molecules on red blood cells (RBCs). For example, the phosphorylation and increased expression of CD239 (Lutheran blood group/blast cells adhesion molecule) is directly associated with increased adhesion of RBCs to endothelial cells under the influence of this mutation.31

Although numerous studies have shown that JAK2V617F mutation plays a critical role in increasing proliferation of erythroid lineage, it has also been reported that the in vitro expansion of erythroid progenitors in PV patients is associated with a reduction in JAK2V617F mutation.32 In other words, the rate of JAK2V617F mutation is decreased in differentiated erythroid progenitors, which are characterized by the expression of CD235a. Furthermore, it has been shown that EPO has a higher impact on precursors lacking this mutation.32 Considering the findings of previous studies that have demonstrated the essential role of JAK2V617F mutation in PV pathogenesis, it can be concluded that if the JAK2V617F mutation decreases following in vivo erythropoiesis, then the presence or absence of this mutation cannot serve as an appropriate prognostic factor for prediction of erythropoiesis. Conversely, the changing immunophenotypic patterns of erythroid CD markers in both conditions (presence or absence of JAK2V617F mutation) reinforces their prognostic value in PV patients. Therefore, the flow cytometry immunophenotyping of erythroid CD markers during the onset and progression of PV is likely to reveal the prognostic value of these markers and can be used as predictive factors for the occurrence of thrombotic complications with higher certainty.

### Dysregulation of CD markers’ expressions in megakaryocytic lineage from late progenitors to platelets

The platelets are derived from megakaryocytes in the process of thrombopoiesis. TPO plays an important role in regulating this process by binding to CD110 (TPO receptor).33 When the rate of platelet production is increased, CD110 expression is decreased, and vice versa.34 Several studies have examined the role of TPO and its receptor in JAK2V617F positive MPNs. The results of a majority of these studies have shown that the expression rate and function of CD110 in megakaryocytes and platelets is reduced in these disorders.35-37 Interestingly, ET patients with a heterogeneous-weak pattern of CD110 expression in megakaryocytes show a greater risk of thrombotic events at the start of their diagnosis.38 Since JAK2V617F mutation affects the expression of CD110,39 the reduced expression of this marker in JAK2V617F positive MPNs may be a poor prognostic factor for increased thrombopoiesis and subsequently enhanced risk of thrombotic complications. In contrast to CD110, the expression of P selectin (CD62P) as an activation marker of immature platelets increases in MPNs patients.40 CD62P is a surface glycoprotein mediating the interaction of activated platelets with endothelial cells.41 On the other hand, JAK2V617F mutation has been shown to be significantly associated with increased frequency of these immature cells in JAK2V617F positive MPNs.42 Given that the overexpression of CD62P indicates the presence of activated platelets with a high affinity to interact with endothelial cells, it is assumed that the expression of this marker could be a poor prognostic factor for predicting thrombotic events caused by platelet-endothelial cell interactions. In addition to the effect of TPO, increased sensitivity of megakaryocyte progenitor cells to IL-3 in JAK2V617F positive MPNs leads to the increased production of platelets.43 JAK2 signaling pathways have a central role in the proliferation, survival, and differentiation of different HSCs progenitor cells via signaling of cytokines receptors.44 In addition, it is clear that overactivation of JAK2 signaling due to JAK2V617F mutation plays a central role in oncogenesis.44 Although JAK2V617F mutation requires the activation of cytokine receptors for oncogenic signaling,45 it has been shown that this mutation can induce the colonal expansion of hematopoietic cells by stimulating cytokine receptors despite the absence of cytokines,46 an event that can lead to hypersensitivity to JAK2 signaling in PV, ET, and PMF.47 However, the clinical phenotype resulting from JAK2V617F mutation depends on the activation of specific downstream signaling in these diseases.48 Since JAK2 is a signaling pathway downstream of IL-3 receptor (CD123) that can regulate the formation of megakaryocytes, overexpression of this marker in megakaryocytes of JAK2V617F positive MPNs suggests a
high megakaryopoiesis rate, as well as platelet production and thrombotic events due to impact of JAK2\textsuperscript{V617F} mutation in these disorders.

Although thrombotic and bleeding complications usually result from abnormal platelet counts, these complications can also be seen in patients with normal platelet counts. It seems that in these conditions, the qualitative defect of platelets can play a role in the incidence of these complications. The platelets normally circulate in an inactive state; however, they maintain the hemostasis of body by aggregation and release of their granular contents following activation.\textsuperscript{49} Platelet activation occurs by changing expression patterns of some functional CD markers.\textsuperscript{50} In this regard, several studies have been conducted on the function of both resting and activated platelets in JAK2\textsuperscript{V617F} positive MPN disorders, which indicate the altered expression of functional platelet markers in these disorders.\textsuperscript{51-53} CD61 (glycoprotein IIIa) is a surface glycoprotein that mediates platelet aggregation by binding to fibrinogen. The defective expression of this marker can be associated with an abnormal accumulation of platelets and bleeding complications.\textsuperscript{54} It has been shown that CD61 expression defects in JAK2\textsuperscript{V617F} positive MPN patients (e.g. PV patients) cause impaired binding of platelets to fibrinogen and platelet aggregation.\textsuperscript{55,56} Similarly, the decreased expression of CD41 and CD42b is another change of functional platelet markers influenced by JAK2\textsuperscript{V617F} mutation in ET patients.\textsuperscript{57} Although the association between decreased expressions of these markers with bleeding complications has been less studied, it could be stated that the reduced expression of these markers is a poor prognostic factor for patients with bleeding complications due to the importance of their function in processes such as platelet aggregation and adhesion to vascular subendothelium. In contrast, an increase in the expression of CD36 (glycoprotein IV), a surface glycoprotein in platelets that plays a role in cell adhesion, is associated with the history of thrombosis in ET patients.\textsuperscript{58} Moreover, the changing expression of other platelet functional markers, including CD63 and CD154 (soluble CD40 ligand), has been reported on platelets of JAK2\textsuperscript{V617F} positive MPNs, both of which can be prognostic for thrombotic complications in these disorders (Table 1).\textsuperscript{40,53,59}

Considering the fact that a large number of patients with JAK2\textsuperscript{V617F} positive MPNs show thrombohemorrhagic complications\textsuperscript{28} and that the platelets play a significant role in these complications as the main components of the hemostasis system, the flow cytometric evaluation of their functional CD markers in JAK2\textsuperscript{V617F} positive MPNs may reveal the association between immunophenotypic alterations of platelets with clinical outcomes.

**Expression of leukocyte CD markers and adhesive interactions**

Leukocytosis is another risk factor for arterial and venous thrombosis in JAK2\textsuperscript{V617F} positive MPNs.\textsuperscript{28} Activated leukocytes (such as neutrophils and monocytes) can be involved in the development of these complications through their interaction with platelets and endothelial cells.\textsuperscript{57,60} which are mediated by the expression of integrins like CD11c and CD11b on leukocytes. Several studies have shown that the expressions of these integrins as well as CD14 increase on monocytes and neutrophils of PV, ET, and PMF patients.\textsuperscript{40,61} The interaction of these integrins with CD62P and CD42b on the surface of platelets results in the formation of leuko-platelet aggregations, which is a risk factor for arterial or venous thrombotic events in MPN patients.\textsuperscript{17,62} Moreover, the results of some clinical studies have shown that the leuko-platelet aggregations directly correlate with platelet and leukocyte counts, as well as the increased expression of functional leukocyte markers.\textsuperscript{17,63} Also, high levels of tissue factor in these malignancies enhance leuko-platelet aggregation in these disorders.\textsuperscript{64}

CD56 is another surface adhesion molecule expressed on NK cells,\textsuperscript{65} the aberrant expression of which has been reported in several leukemias and myelodysplastic syndromes (MDS).\textsuperscript{66-68} CD56 expression has been shown to increase in granulocytes of PMF. Although the pathogenesis of aberrant CD56 expression in this malignancy is not clear, increased expression of this marker is likely related to changes in the adhesion pattern of leukocytes.\textsuperscript{69}

According to the above statements, it is concluded that leuko-platelet aggregations are among the risk factors for thrombotic complications, which are widely observed in JAK2\textsuperscript{V617F} positive MPNs. JAK2\textsuperscript{V617F} mutation seems to be an underlying factor for increased expression of adhesion markers on leukocytes that leads to the consolidation of leuko-platelet aggregations. Given the crucial role of leukocyte adhesion CD markers in the formation of leuko-platelet aggregations, efforts to identify the prognostic value of these markers in JAK2\textsuperscript{V617F} positive MPNs may lead to their introduction as potential targets for effective treatment in these disorders.

**CD markers of circulating endothelial cells and activation of coagulation and angiogenesis**

Venous complications such as deep venous thrombosis (DVT) of peripheral vasculature are common vascular complications in most JAK2\textsuperscript{V617F} positive MPN patients.\textsuperscript{70} In addition to blood cells, it seems that the changes in the number and function of endothelial cells as a component of the hemostasis system also play a crucial role in the incidence of this complication. In normal conditions, endothelial cells exhibit antithrombotic properties by expressing some surface markers and preventing platelets from adhesion and aggregation.\textsuperscript{71} However, in JAK2\textsuperscript{V617F} positive MPNs, due to the exposure to a number of proteins and cytokines derived from activated neutrophils, endothelial cells show pro-adhesive and pro-coagulant properties via expression of functional CD markers.\textsuperscript{72,73} CD142 (tissue factor), CD62P (P-selectin), and CD62E (E-selectin) are among the most common CD markers expressed following the activation of endothelial cells, which mediate the adhesion of platelets to these cells.\textsuperscript{74} Furthermore, the increased expressions of intercellular and vascular adhesion molecules (including CD54 and CD106) on these cells, which play a role in the leuko-endothelial adhesion, might contribute to the prognosis of thrombotic events in JAK2\textsuperscript{V617F} positive disorders (Table 1).\textsuperscript{52,74}

Interestingly, in JAK2\textsuperscript{V617F} positive MPNs, an increase in circulating endothelial cells is observed in addition to changing function of these cells.\textsuperscript{72} Endothelial cells are characterized by the expression of CD34 and CD133 in their progenitors as well as CD309 (vascular endothelial growth factor receptor), CD146 (melanoma cell adhesion molecule), and CD31 (platelet and endothelial cell adhesion molecule 1) expression in their mature form.\textsuperscript{75,76} The increase in circulating endothelial cells has a direct relationship with angiogenesis in PV, ET, and especially PMF.\textsuperscript{73,77} It has been shown that vascular endothelial growth factor (VEGF) binding to its receptor of CD309 in endothelial cells can enhance angiogenesis in PMF patients.\textsuperscript{78} Bone marrow (BM) is the first site of angiogenesis, and the rate of angiogenesis can be easily estimated by microvessel density (MVD) and the expression rate of CD34 and VEGF.\textsuperscript{79} Several studies have been conducted on angiogenesis
### Megakaryocyte and platelet surface CD markers

| Markers | Alternative name | Chro. | Function | Type of diseases | Prognosis | Ref. |
|---------|-----------------|-------|----------|------------------|-----------|-----|
| CD71    | TFR             | 3q29  | Necessary for TF absorption and required for erythropoiesis | -        | PV       | 30  |
| CD235a  | GYPA            | 4q31.21 | A sialoglycoprotein that bears the antigenic determinants of the MN and Ss blood groups | -        | PV       | 31  |
| CD239   | BCAM            | 19q13.32 | A glycoprotein for lutheran blood group and a receptor for extracellular laminin | -        | PV       | 53  |
| CD36    | GPIV            | 7q21.11 | It works as a receptor for thrombospondin in platelets | -        | ET       | 58  |
| CD110   | TPO-R           | 1p34.2 | Thrombopoietin receptor in megakaryocytes and platelets | PV, ET, PMF | -       | 35, 37 |
| CD63    | CD63 molecule   | 12q13.2 | A cell-surface protein that mediates signal transduction events and may function as a platelet activation marker | -        | PV, ET   | 40, 52, 59 |
| CD62P   | P-selectin      | 1q24.2 | This protein mediates the interaction of activated platelets with leukocytes | -        | PV, ET, PMF | 17, 60, 62, 63 |
| CD41    | ITGA2B          | 17q21.31 | This receptor plays a crucial role in the platelet aggregation | ET       | -        | 57, 59 |
| CD42a   | GPIX            | 3q21.3 | A platelet surface membrane glycoprotein complex that functions as a receptor for VWF | ET, PV   | -        | 59  |
| CD42b   | GPib            | 17p13.2 | Functions as a receptor for VWF | ET, PV   | -        | 57, 59, 62 |
| CD61    | ITGB3           | 17q21.32 | Participates in cell adhesion as well as platelet aggregation via binding to fibrinogen | ET, PV   | -        | 51, 55 |
| CD154   | CD40L           | Xq26.3 | CD40 Ligand | -        | ET       | 53  |
| CD11b   | MAC-1           | 16p11.2 | Mediates adherence of neutrophils and monocytes to stimulated endothelium and platelet surface molecules | -        | PV, ET, PMF | 60, 61 |
| CD14    | CD14 molecule   | 5q31.3 | Expressed on monocytes/macrophages and mediates the innate immune response to bacterial lipopolysaccharide | -        | PV, ET, PMF | 60-62 |
| CD56    | NCAM-1          | 11q23.2 | Involved in cell-to-cell interactions as well as cell-matrix interactions during development and differentiation | -        | PMF      | 70  |

Continued on the next page.
in JAK2V617F positive MPNs, especially PMF, indicating that the increase in VEGF and CD34-MVD reflects the high density of active angiogenesis in BM of these disorders.77,78 However, it has been shown that CD105-MVD evaluation of BM could better reflect BM angiogenic activity than CD34-MDV in MPNs.18 In addition, the formation of a fibrous network in BM of PMF is a problem complicating BM aspiration in these patients. The expression of CD9 (mobility related protein-1) significantly increases in advanced stages of PMF, and this change is associated with the formation of fibrous networks and mobilization of CD34+ cells into peripheral blood (PB) in advanced stages of PMF.80 Extramedullary hematopoiesis is a constitutive feature of PMF that is usually characterized by spleen neoangiogenesis in this disease. In vitro studies have reported that the expression of spleen CD34+CD133+ HSCs is associated with capillary vascular density (CVD) in spleen of PMF patients, while CD8-staining sinusoidal vascular density (SVD) is inversely correlated with CVD.81 This finding suggests that the overexpression of CD34 and CD133 in spleen specimens of PMF patients can be a poor prognostic factor for active spleen neoangiogenesis and extramedullary hematopoiesis.

Since the increase in activated circulating endothelial cells is closely related to the increase of leuko-platelets adhesion, the overexpression of markers mediating these adhesions could be a poor prognostic factor in clinical outcomes of JAK2V617F positive MPNs. In addition, the presence of excess circulating endothelial cells, which is detected by the expression of MDVs markers, may be a poor prognostic factor for increasing incidence of angiogenesis and the resulting thrombotic events in these disorders. Therefore, it seems that the flow cytometric immunophenotyping of expression levels of these markers from the viewpoint of clinical outcomes and response to treatment in JAK2V617F positive MPNs can provide useful information on the prognosis of these disorders.

Table 1. Continued from previous page.

| Markers | Alternative name | Chromo. | Function | Type of diseases | Prognosis | Ref. |
|---------|------------------|---------|----------|------------------|-----------|------|
| CD34    | CD34 molecule    | 1q22.2  | Hematopoietic stem cell antigen | Decreased expression | PV, ET, PMF | Associated with increased MVD in BM | 78, 79 |
| CD105   | S-endoglin       | 9q34.11 | It works as transforming growth factor beta receptor | Decreased expression | PMF, PV | Associated with augmentation of angiogenesis and fibrosis in BM | 18 |
| CD106   | VCAM1            | 1p21.3  | A cell surface glycoprotein in activated endothelium cells that mediates leuko-endothelial cells adhesion | Decreased expression | PV, ET | Associated with platelet activation and leuko-endothelial cells adhesion that lead to arterial thrombosis and erythroblastia | 52 |
| CD62E   | E-selectin       | 1q34.2  | Responsible for the accumulation of blood leukocytes and mediating the leuko-endothelial cells adhesion | Decreased expression | ET | May be associated with increased leuko-endothelial cells adhesion | 74, 82 |
| CD141   | Thrombomodulin   | 20p11.21| This protein binds to thrombin that results in the activation of protein C | - | PV, ET | May act as a poor prognostic factor for thrombosis | 83 |
| CD142   | TF               | 1p21.3  | This factor enables cells to initiate the blood coagulation | - | PV, ET | Associated with increases adhesion of platelets to the endothelial cells and subsequent thrombotic events | 74 |
| CD54    | ICAM             | 19p13.2 | This glycoprotein binds to some leukocytes integrins including CD11b and CD11a | - | PV, ET | Can be associated with leuko-endothelial cells adhesion | 74 |
| CD9     | MPR-1            | 12p13.31| Function in many cellular processes including differentiation, adhesion, and signal | - | PV, ET, PMF | Associated with increases BM myelofibrosis and mobilization of CD34+ cells into PB | 80 |
| CD309   | VEGFR            | 4q12    | Functions as the main mediator of endothelial cells proliferation, survival, and migration | - | PV, ET, PMF | Associated with increased circulating endothelial cells and angiogenesis that result in thrombotic events | 77 |
| CD146   | MCAM             | 11q23.3 | Functions as a cell adhesion molecule | - | ET | Associated with endothelial activation, increases thrombin generation and thrombotic events | 82 |

chromosome; RBC, red blood cell; TFR, transferrin receptor; CD74, glycoprotein A; ICAM, basal cell adhesion molecule; GPV, glycoprotein IV; TPO-R, thrombopoietin receptor; ITGAM, integrin subunit alpha 2b; GPX, glycoprotein IX; TF, von willebrand factor; GPB, glycoprotein Ib; ITGB1, integrin subunit beta 1; CD49D, CD40 ligand; TF, tissue factor; MASC, macrophage receptor 1; NCAM-1, neural cell adhesion molecule 1; MVD, microvessel density; BM, bone marrow; VCAM-1, vascular cell adhesion molecule 1; ICAM, intercellular adhesion molecule 1; MPR-1, mobility related protein-1; PB, peripheral blood; VEGFR, vascular endothelial growth factor receptor; MCAM, melanoma cell adhesion molecule; Leuko, leukocyte; PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis.
Abnormal expression of immune cell related CD markers and immune complications in JAK2^{V617F} positive MPNs

JAK2^{V617F} positive MPNs are HSC derived disorders that may also be associated with autoimmune and chronic inflammatory complications, as well as thrombotic and bleeding complications. Several studies have detected the aberrant expressions of some genes involved in inflammatory and defensive reactions of the body, which are responsible for immune complications in MPNs (Table 2). There is also growing evidence that the JAK2^{V617F} mutation involves lymphoid subtypes like B, T, and NK cells in addition to myeloid lineage. This mutation can affect the frequency and function of B, T, NK cells, and monocytes in PV patients. Previous studies have shown that different lymphoid subtypes, including TCD4+ and TCD8+ cells, are significantly reduced in PMF patients compared with healthy subjects. In contrast, a small number of these patients showed an increase in BCD5+ and TCD8+ cytotoxic lymphocytes. TCD4+ and TCD8+ cells play an essential role in cellular immunity; therefore, the reduction of these subgroups might be associated with a defect in body’s defensive function. On the other hand, BCD5+ cells account for nearly 10–25% of lymphocytes in healthy subjects and play a crucial role in antibody production. It has also been shown that these lymphocytes play an important role in the occurrence of autoimmune reactions in autoimmune diseases. Thus, there may be a higher likelihood of autoimmune phenomena in PMF patients who show an increase in BCD5+ lymphocytes.

Another feature of PMF is the expansion and activation of monocyte-macrophage system, which is characterized by a significant increase in mature macrophages in BM and monocytes in PB. In PMF patients, CD68 positive monocytosis has been shown to be associated with the onset of accelerated phase of the disease, and patients showing this feature are considered as the high-risk group. There is evidence that younger PMF patients with monocytosis are at increased risk of progression to chronic myelomonocytic leukemia (CMML) and short-term survival. CD68 expression in accelerated phase of PMF may have a prognostic value for predisposition toward CMML.

Regulatory T-cells (Treg) are a subgroup of CD4+ CD25+ FOXP3+ effector T-cells responsible for maintaining peripheral tolerance. These lymphocytes also reduce the function of active T-cells by stimulating the expression of CD279 (programmed death cell-1), a CD28 family inhibitory receptor on these cells. Treg lymphocytes are significantly increased in patients with PV. Recent studies showed that in addition to PV patients, ET and PMF patients treated with interferon alpha (IFN-α) showed an increased number of Treg lymphocytes. Similarly, a recent study on PV and ET patients receiving IFN-α demonstrated an increase in the frequency of another subgroup of Treg lymphocytes characterized by coexpression of CD39a and human leukocyte antigen (HLA-DR). This Treg subtype is highly suppressive, and the decreased number of such cells has been associated with a significant reduction in tolerance.

Since Treg lymphocytes are increased during the onset and progression of PV, their increased number after IFN-α therapy may indicate the resistance or unfavorable response to treatment in these patients. In addition, the unchecked increase in this subgroup can be coupled with excessive suppression of the functional TCD4+ cells. The significance of this issue is elucidated when the PV and ET patients treated with IFN-α show an increase in TCD4+CD279+ lymphocytes. Although this subject has been less studied, the increase in Tregs after IFN-α therapy may increase the number of TCD4+CD279+ lymphocytes. It seems that both of these changes may be accompanied with immune system suppression and predispose to bacterial and viral infections and even

| Markers | Alternative name | Chro. | Function | Decreased expression | Type of diseases | Increased expression | Prognosis | Ref. |
|---------|------------------|-------|----------|----------------------|------------------|----------------------|-----------|-----|
| CD4C    | D4 molecule      | 12p13.31 | TH       | PMF                  |                  |                      |           | 88  |
| CD8     | CD8 molecule     | 2p11.2  | TC       |                      |                  |                      |           |     |
| CD5     | CD5 molecule     | 11q12.2 | B cells  |                      | PMF              |                      |           | 88  |
| CD68    | LAMP4            | 17p13.1 | Monocyte |                      | PMF              |                      |           |     |
| CD177   | NB1 glycoprotein | 19q13.2 | Neutrophil |                  | PV, ET, PMF      |                      |           | 102, 103, 105 |
| CD4 CD25 | CD4 molecule and IL-2R | 12p13.31 and 10p15.1 | Treg |                      | PV, ET, PMF      | Maybe associated with poor prognosis via adverse effect on TCD4+ function and immune system suppression | 97, 98 |
| CD39    | ENTPD1           | 10     | Treg     |                      | PMF              | Maybe associated with poor prognosis through severe immune system suppression | 99        |
| CD274   | PDL1             | 9p24.1 | TH       |                      | PV, ET           | Maybe associated with poor prognosis via immune response failure | 98        |

cro, chromosome; TH, T helper; TC, T cytotoxic; LAMP4, lysosomal associated membrane glycoprotein; CMML, chronic myelomonocytic leukemia; IL-2R, interleukin 2 receptor; Treg, regulatory t cells; ENTPD1, ectonucleoside triphosphate diphosphohydrolase 1; PDL1, programmed death cell-1; PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis.

[page 114] [Oncology Reviews 2018; 12:373]
autoimmune complications in patients. Therefore, flow cytometric immunophenotyping assay of immune cells’ CD markers during follow-up of JAK2V617F positive MPN patients may be useful for understanding the immune system situation and disease management.

Recently, the association between Helicobacter pylori (H. pylori) infection, which is the main cause of gastrointestinal lesions in PV patients, with immune system suppression in JAK2V617F MPNs has received special attention.101 There has also been evidence in favor of the presence of this infection in ET patients.102 Bacterial infections are directly related to neutrophil proliferation and increased expression of CD177 (NB1 glycoprotein) on these cells.103 In addition, CD177 expression has been shown to increase in PV patients.103 Similarly, the increased expression of CD177 has also been reported to a lower extent in other JAK2V617F MPNs, including ET and PMF.104 Although it has been shown that patients with a higher expression of CD177 are at increased risk of thrombocytopenia and bleeding complications due to increased circulating neutrophils,105 it is inferred that the overexpression of this marker in JAK2V617F positive MPNs could be a poor prognostic factor for the presence of bacterial contamination in these disorders.

In general, the relationship between JAK2V617F mutations with changing expression patterns of immune cells’ CD markers in JAK2V617F positive MPNs is not fully understood. However, it seems that the immune cells, like other blood cells differentiated from the HSCs, can be affected by JAK2V617F mutation and exhibit abnormal function by acquiring this mutation at the beginning of their differentiation and keeping it during their maturation. Our hypothesis here is that flow cytometric immunophenotyping assay of immune cells may be useful for understanding these changes and prognosis of inflammatory and autoimmune complications.

Table 3. Possible prognostic CD markers for leukemic transformation in JAK2V617F positive MPNs.

| Initial diagnosis | Number of patient /Age, year/ Gender | Positive CD markers | Prognostic CD markers | Negative CD markers | Transformed malignancy | Ref. |
|-------------------|-------------------------------------|---------------------|-----------------------|---------------------|------------------------|------|
| PV                | 1/69/M                              | CD10, CD19, CD38, HLA-DR | CD23, CD5, CD7, CD11b, CD13, CD133, CD14 | ALL | 124 |
| PV                | 1/79/F                              | CD10, CD5, CD20, CD22, CD23, HLA-DR, CD25 | CD38 | B-CLL | 127 |
| PV                | 2/69,79/F                            | -                   | CD19 | B-CLL | 109 |
| PV                | 1/75/F                              | CD10, CD19, CD20, CD22, CD34, CD78α, TdT | - | ALL | 125 |
| PV                | 1/62/F                              | CD33, CD56, CD138 | CD13, CD14, CD15, CD33, CD34, CD64, MPO | ALL | 128 |
| PV                | 1/69/M                              | CD19/CD5, CD5, CD23 | CD38, Zap-70 | B-CLL | 126 |
| PV                | 1/70/M                              | CD38, Zap-70 | - | CLL | 111 |
| PV                | 1/60/M                              | CD5, CD23 | CD38 | B-CLL | 129 |
| PV                | 1/65/M                              | CD5, CD23, CD22, CD23 | CD38, FMC | - | 128 |
| ET                | 1/58/F                              | CD19/CD5, CD23 | CD38 | CLL | 130 |
| ET                | 3 / 67,72/78, 2F, 1M                 | CD19/CD5, CD5, CD23 | CD38, Zap-70 | B-CLL | 126 |
| ET                | 2/80,82/F                            | CD38, Zap-70 | - | CLL | 111 |
| ET                | 1/72/M                              | CD19/CD5, Zap-70 | CD38, FMC | B-CLL | 110 |
| ET                | 1/82/M                              | CD19/CD5, Zap-70 | - | B-CLL | 110 |
| PMF               | 1/69/M                              | CD38, Zap-70 | - | CLL | 111 |
| PMF               | 1/80/F                              | CD5, CD19, CD20, CD23, CD10, Zap-70 | CD38 | CLL | 108 |
| PMF               | 1/54/M                              | CD10, TdT, CD19, CD20, HLA-DR | CD13, CD14, CD15, CD33, CD34, CD64, MPO | ALL | 131 |

PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis; M, male; F, female; HLA-DR, human leukocyte antigen-DR; TdT, terminal deoxynucleotidyl transferase; Zap-70, zeta chain of T-cell receptor-associated protein kinase 7; POST-PV MF, post-polycythemia vera myelofibrosis; MPO, myeloperoxidase; ALL, acute lymphoblastic leukemia; CLL, chronic lymphoblastic leukemia; AML, acute myeloid leukemia.
leukemic CD markers raises progression towards a specific leukemia in these disorders (Table 3). For example, coexpression of CD10 and terminal deoxynucleotidyl transferase (TdT) in PV patients represents the disease progression toward ALL.\(^{124,125}\) The expression of CD23 and CD5 in these patients can be indicative of a mature B-cell lymphoid neoplasm.\(^{126}\) Similarly, simultaneous expression of CD19 and CD5 could be a poor prognostic factor for progression to CLL in these disorders.\(^{110,111,126}\)

According to the above statements, we can deduce that since JAK2\(^{V617F}\) positive MPNs are disorders involving HSCs, a wide range of clonal proliferation can be seen in different stages of cell maturation in these disorders. Although the JAK2\(^{V617F}\) mutation can affect all the cellular lineages, it is not yet clear whether this mutation can induce leukemic transformation by itself in JAK2\(^{V617F}\) positive MPNs. It seems that the expression of leukemic blasts’ CD markers may be the first recognizable symptom and a poor prognostic factor for leukemic transformation in these disorders.

Discussion

JAK2\(^{V617F}\) positive MPNs are a group of disorders that are prone to both thrombotic and bleeding complications. The former complications are more common and are associated with a higher risk of mortality.\(^{127-132}\) Pathogenesis of thrombotic complications in these disorders is an intricate matter. It seems that the mere presence of JAK2\(^{V617F}\) mutation is not by itself sufficient for the occurrence of these disorders, and a range of factors such as dysfunction and depletion of platelets, leukocytes, and endothelial cells can also be considered as risk factors for this complication.\(^{53}\) These changes can often be reflected in the changing expression patterns of surface CD markers. Therefore, the initial prognosis of these complications can be achieved by a preliminary flow cytometric evaluation of CD markers. For example, the reduced expression of CD110 in platelets and megakaryocytes is associated with an increase in thrombotic complications in JAK2\(^{V617F}\) positive MPNs.\(^{35}\) Meanwhile, the changing frequency and function of platelets and endothelial cells as the main components of hemostasis system are the most important factors that can trigger and reinforce a thrombotic or bleeding event in these disorders.\(^{14,53,72,82}\) On the other hand, several studies have indicated that the coexpression of CD markers such as CD62P, CD62E, CD42a, CD42b, CD14, and CD11b, which mediate the binding of platelets to leukocytes or endothelial cells, aggravates these complications.\(^{52,53}\) Therefore, flow cytometric evaluation of CD markers on different blood cells can be helpful for prognosis of patients’ clinical situations. Immune complications are relatively common in JAK2\(^{V617F}\) positive MPNs, and the changing frequency and function of immune cells are the main causes of these alterations,\(^{21}\) which can be a function of JAK2\(^{V617F}\) mutation, abnormal expression of some biological agents such as cytokines, as well as therapeutic protocols like IFN-α therapy.\(^{20,87,97}\) An abnormal immunophenotype of immune-related CD markers may be a prognostic factor for predicting the immune complications. Therefore, CD markers’ evaluation based on flow cytometry can help determine the patient’s immunologic status and control these complications by appropriate therapeutic interventions. In contrast, leukemic transformation in JAK2\(^{V617F}\) positive MPNs indicates the onset of acute clinical phase, for which limited therapeutic approaches have been reported.\(^{3,133}\) The precise mechanism of leukemic transformation in these disorders has not been identified and there are potential challenges in this way. Although a number of predisposing genetic alterations occurring at the onset of or development towards leukemic transformation could be the first trigger for these complications,\(^{134,135}\) the expressions of leukemic blasts’ CD markers seem to be the first clinically recognizable symptom for leukemic transformation in these disorders. We suggest flow cytometric evaluation of leukemic blasts’ CD markers for the prediction of leukemic transformation in JAK2\(^{V617F}\) positive MPNs. Subsequent timely therapeutic interventions by an appropriate approach may minimize the unfavorable clinical outcomes of leukemic transformation.

Conclusions

Although extensive studies have shown the substantial role of different blood cells in the initiation and progression of clinical outcomes in JAK2\(^{V617F}\) positive MPNs, further clinical studies based on flow cytometric assay are required to provide immunophenotyping patterns of JAK2\(^{V617F}\) positive MPNs to confirm the prognostic value of CD markers’ expressions for clinical outcomes in these disorders.

Highlights

- JAK2 mutation may participate in changing expression patterns of CD markers in JAK2\(^{V617F}\) positive MPNs.
- Abnormality of CD markers’ expressions in different blood cells can be associated with thrombohemorrhagic and immune complications in JAK2\(^{V617F}\) positive MPNs.
- CD markers may be poor prognostic factors for leukemic transformation of JAK2\(^{V617F}\) positive MPNs.

References

1. Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 2005;365:1054-61.
2. Zhan H, Spivak JL. The diagnosis and management of polycythemia vera, essential thrombocytopenia, and primary myelofibrosis in the JAK2 V617F era. Clin Adv Hematol Oncol 2009;7:334-42.
3. Poopak B, Haghi MF, Saki N, et al. JAK2 V617F mutation in Iranian patients with myeloproliferative neoplasms: clinical and laboratory findings. Turk J Med Sci 2013;43:347-53.
4. Valikhanl A, Poopak B, Ferdowsi S, et al. ASXL1 and JAK2V617F gene mutation screening in Iranian patients with chronic myeloid leukemia. Asia Pac J Clin Oncol 2017;13.
5. Wang YL, Lee JW, Kui JS, et al. Evaluation of JAK2V617F in B and T cell neoplasms: identification of JAK2V617F mutation of undetermined significance (JMUS) in the bone marrow of three individuals. Acta Haematol 2007;118:209-14.
6. Ishii T, Bruno E, Hoffman R, et al. Involvement of various hematopoietic-cell lineages by the JAK2V617F mutation in polycythemia vera. Blood 2006;108:3128-34.
7. Mullally A, Lane SW, Ball B, et al. Physiological Jak2V617F expression causes a lethal myeloproliferative neoplasm with differential effects on hematopoietic stem and progenitor cells. Cancer cell 2010;17:584-96.
8. Thepot S, Itzykson R, Seegers V, et al. Treatment of progression of Philadelphia-negative myeloproliferative neoplasms to myelodysplastic syndrome or acute myeloid leukemia by azacitidine: a report on 54 cases on the behalf of the Groupe
1. Ball S, Thein KZ, Maiti A, et al. Thrombosis in Philadelphia negative classical myeloproliferative neoplasms: a narrative review on epidemiology, risk assessment, and pathophysiological mechanisms. J Thromb Thrombolysis 2018;45:516-28.

2. Bogani C, Guglielmelli P, Antonioli E, et al. B-, T-, and NK-cell lineage involvement in JAK2V617F-positive patients with high platelet counts. Platelets 2000;11:183-4.

3. Casini A, Fontana P, Lecompte TP. Thrombotic complications of myeloproliferative neoplasms: risk assessment and risk-guided management. J Thromb Haemost 2013;11:1215-27.

4. Passamonti F, Rumi E. Clinical relevance of JAK2 (V617F) mutant allele burden: Haematologica 2009;94:7-10.

5. Bagheropur S, Ehsanpour A, Birgani MT, et al. JAK2V617F allele burden: innovative concept in monitoring of myeloproliferative neoplasms. Mem-Mag Europ Med Oncol 2018;11:1-6.

6. Ziakas PD. Effect of JAK2 V617F on thrombotic risk in patients with essential thrombocythemia: measuring the uncertain. Haematologica 2008;93:1412-4.

7. Medinger M, Skoda R, Gratwohl A, et al. Impaired expression of platelet activation in myeloproliferative disorders with high platelet counts. Platelets 2000;11:183-4.

8. Medinger M, Skoda R, Gratwohl A, et al. Angiogenesis and vascular endothelial growth factor-receptor expression in myeloproliferative neoplasms: correlation with clinical parameters and JAK2-V617F mutational status. Br J Haematol 2009;146:150-7.

9. Barbui G. An immune dysregulation in MPN. Curr Hematol Malig Rep 2014;9:331-9.

10. Bogani C, Guglielmelli P, Antonioli E, et al. B-, T-, and NK-cell lineage involvement in JAK2V617F-positive patients with idiopathic myelofibrosis. Haematologica 2007;92:258-9.

11. Larsen TS, Christensen JH, Hasselbalch HC, et al. The JAK2 V617F mutation involves B-and T-lymphocyte lineages in a subgroup of patients with Philadelphia-chromosome negative chronic myeloproliferative disorders. Br J Haematol 2007;136:745-51.

12. Pardanani A, Lasho TL, Finke C, et al. Extending Jak2V617F and MPLW515 mutation analysis to single hematopoietic colonies and B and T lymphocytes. Stem Cells 2007;25:2358-62.

13. Elliott MA, Tefferi A. Thrombosis and haemorrhage in polycythaemia vera and essential thrombocythaemia. Br J Haematol 2005;128:275-90.

14. Raszeja-Specht A, Skibowska A, Bieniaszewska M, et al. Relationships between thrombohemorrhagic complications and platelet function in patients with essential thrombocythaemia. Am J Hematol 2001;68:32-6.

15. James C. The JAK2V617F mutation in polycythemia vera and other myeloproliferative disorders: one mutation for three diseases? ASH Educ Prog Book 2008;2008:69-75.

16. Falanga A, Marchetti M. Thrombotic disease in the myeloproliferative neoplasms. ASH Educ Prog Book 2012;2012:571-581.

17. Bruchova H, Yoon D, Agarwal AM, et al. Erythropoiesis in polycythemia vera is hyper-proliferative and has accelerated maturation. Blood Cells Mol Dis 2009;43:81-7.

18. Wautier M-P, El Nemer W, Gane P, et al. Increased adhesion to endothelial cells of erythrocytes from patients with polycythemia vera is mediated by laminin α5 chain and Lu/BCAM. Blood 2007;110:894-901.

19. Gaikwad A, Nussenzeig R, Liu E, et al. In vitro expansion of erythroid progenitors from polycythemia vera patients leads to decreased in JAK2V617F allele. Exp Hematol 2007;35:587-95.

20. Kaushansky K. The molecular mechanisms that control thrombopoiesis. J Clin Invest 2005;115:3339-47.

21. Ng AP, Kauppi M, Metcalf D, et al. Mpl expression on megakaryocytes and platelets is dispensable for thrombopoiesis but essential to prevent myeloproliferation. Proc Natl Acad Sci U S A 2014;111:5884-9.

22. Li J, Xia Y, Kuter DJ. The platelet thrombopoietin receptor number and function are markedly decreased in patients with essential thrombocythaemia. Br J Haematol 2000;111:943-53.

23. Molieterno AR, Hankins WD, Spivak JL. Impaired expression of the thrombopoietin receptor by platelets from patients with polycythemia vera. N Engl J Med 1998;338:572-80.

24. Yoon SY, Li CY, Tefferi A. Megakaryocyte-c-Mpl expression in chronic myeloproliferative disorders. Blood 2000;106:2413-21.

25. Teofili L, Pierconti F, Di Febo A, et al. The expression pattern of c-mpl in megakaryocytes correlates with thrombotic risk in essential thrombocythaemia. Blood 2002;100:714-7.

26. Vlădăreanu A, Popov V, Bumbea H, et al. Pathogenesis of thrombotic and hemorrhagic complications in myeloproliferative and myelodysplastic syndromes. Rev Medico-Chirurg Soc Med Nat Din Iasi 2011;115:14-9.

27. Bermejo E, Alberto MF, Meschengieser SS, et al. Erythropoiesis in essential and myelodysplastic syndromes. Rev Medico-Chirurg Soc Med Nat Din Iasi 2011;115:14-9.

28. Bermejo E, Alberto MF, Meschengieser SS, et al. Assessment of platelet activation in myeloproliferative disorders with complementary techniques. Blood Coagul Fibrinol 2004;15:235-40.

29. Finetelle PS, Denis CV, Weiss L, et al. P-selectin glycoprotein ligand 1 (PSGL-1) is expressed on platelets and can mediate platelet–endothelial interactions in vivo. J Exp Med 2000;191:1413-22.

30. Dai C, Krantz S, Dessypris E, et al. Thrombosis in Philadelphia negative classical myeloproliferative neoplasms: correlation with clinical correlates. Eur J Haematol 2000;65:170-4.

31. Teofili L, Pierconti F, Di Febo A, et al. The expression pattern of c-mpl in megakaryocytes correlates with thrombotic risk in essential thrombocythaemia. Blood 2002;100:714-7.

32. Vlădăreanu A, Popov V, Bumbea H, et al. Pathogenesis of thrombotic and hemorrhagic complications in myeloproliferative and myelodysplastic syndromes. Rev Medico-Chirurg Soc Med Nat Din Iasi 2011;115:14-9.

33. Bermejo E, Alberto MF, Meschengieser SS, et al. Assessment of platelet activation in myeloproliferative disorders with complementary techniques. Blood Coagul Fibrinol 2004;15:235-40.

34. Finetelle PS, Denis CV, Weiss L, et al. P-selectin glycoprotein ligand 1 (PSGL-1) is expressed on platelets and can mediate platelet–endothelial interactions in vivo. J Exp Med 2000;191:1413-22.

35. Dai C, Krantz S, Dessypris E, et al. Polycythemia vera. II. Hypersensitivity of bone marrow erythroid, granulocyte-macrophage, and megakaryocyte progenitor cells to interleukin-3 and granulocyte-macrophage colony-stimulating factor. Blood 1992;80:891-9.

36. Levy DE, Darnell Jr J. Signalling: Stats: transcriptional con-
trol and biological impact. Nat Rev Molecul Cell Biol 2002;3:651.

44. Wernig G, Gonneville JR, Crowley BJ, et al. The Jak2V617F oncogene associated with myeloproliferative diseases requires a functional FERM domain for transformation and for expression of the Myc and Pin proto-oncogenes. Blood 2008;111:3751-9.

45. Lu X, Huang Li-S, Lodish HF. Dimerization by a cytokine receptor is necessary for constitutive activation of Jak2V617F. J Biologic Chemist 2008;283:5258-66.

46. Chen E, Beer PA, Godfrey AL, et al. Distinct clinical phenotypes associated with Jak2V617F reflect differential STAT1 signaling. Cancer Cell 2010;18:524-35.

47. Levine RL, Wadleigh M, Cools J, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell 2005;7:387-97.

48. James C, Ugo V, Le Couédic J-P, et al. A unique clonal Jak2 mutation leading to constitutive signalling causes polycythemia vera. Nature 2005;434:1144.

49. Jennings LK. Mechanisms of platelet activation: need for new strategies to protect against platelet-mediated atherothrombosis. Thromb Haemost 2009;101:248-57.

50. Heemskerk JW, Bevers EM, Lindhout T. Platelet activation and blood coagulation. Thromb Haemost 2002;88:186-93.

51. Le Blanc K, Berg A, Palmblad J, et al. Defective platelet aggregation in polycythemia vera is not caused by impaired calcium signaling, phospholipase D activation or decreased amounts of focal adhesion proteins. Eur J Haematol 2000;65:322-30.

52. Karakantza M, Giannakoulas NC, Zikos P, et al. Markers of endothelial and in vivo platelet activation in patients with essential thrombocythemia and polycythemia vera. Int J Hematol 2004;79:253-9.

53. Arelanno-Rodrigo E, Alvarez-Larrán A, Reverter JC, et al. Platelet turnover, coagulation factors, and soluble markers of platelet and endothelial activation in essential thrombocythemia: relationship with thrombosis occurrence and JAK2V617F allele burden. Am J Hematol 2009;84:102-8.

54. Bennett JS. Platelet–Fibrinogen Interactions. Ann N Y Acad Sci 2001;936:340-54.

55. Kaplan R, Gabbeta J, Sun L, et al. Combined defect in membrane expression and activation of platelet GPIb–IIIa complex without primary sequence abnormalities in myeloproliferative disease. Br J Haematol 2000;111:954-64.

56. Le Blanc K, Lindahl T, Rosendahl K, et al. Impaired platelet binding of fibrinogen due to a lower number of GPIb/IIIa receptors in polycythemia vera. Thromb Res 1998;91:287-95.

57. Falanga A, Marchetti M, Vignoli A, et al. V617F Jak-2 mutation in patients with essential thrombocythemia: relation to platelet, granulocyte, and plasma hemostatic and inflammatory molecules. Exp Hematol 2007;35:702-11.

58. Moles-Moreau M-P, Ternisien C, Tanguy-Schmidt A, et al. Flow cytometry–evaluated platelet CD36 expression, reticulated platelets and platelet microparticles in essential thrombocythemia and secondary thrombocytosis. Thromb Res 2010;126:e394-6.

59. Vladraveanu A, Popov V, Bumbea H, et al. Splanchnic vein thrombosis, the onset manifestation in JAK positive chronic myeloproliferative disorders neoplasms. J Med Life 2011;4:97.

60. Falanga A, Marchetti M, Vignoli A, et al. Leukocyte–platelet interaction in patients with essential thrombocythemia and polycythemia vera. Exp Hematol 2005;33:523-30.

61. Alvarez-Larrán A, García-Pagán JC, Abraldes JG, et al. Increased CD11b neutrophil expression in Budd-Chiari syndrome or portal vein thrombosis secondary to polycythemia vera. Br J Haematol 2004;124:329-35.

62. Villmow T, Kemkes-Matthes B, Matzdorf AC. Markers of platelet activation and platelet-leukocyte interaction in patients with myeloproliferative syndromes. Thromb Res 2002;108:139-45.

63. Jensen MK, de Nully Brown P, Lund BV, et al. Increased circulating platelet–leukocyte aggregates in myeloproliferative disorders is correlated to previous thrombosis, platelet activation and platelet count. Eur J Haematol 2001;66:143-51.

64. Barnard MR, Linden MD, Frelinger A, et al. Effects of platelet binding on whole blood flow cytometry assays of monocyte and neutrophil procoagulant activity. J Thromb Haemos 2005;3:2563-70.

65. Cooper MA, Fehniger TA, Turner SC, et al. Human natural killer cells: a unique innate immunoregulatory role for the CD56bright subset. Blood 2001;97:3146-51.

66. Lacronique-Gazaille C, Chaury M-P, Le Guyader A, et al. A simple method for detection of major phenotypic abnormalities in myelodysplastic syndromes: expression of CD56 in CML. Haematologica 2007;92:859-60.

67. Cruse JM, Lewis RE, Pierce S, et al. Aberrant expression of CD7, CD56, and CD79a antigens in acute myeloid leukemias. Exp Molec Pathol 2005;79:39-41.

68. Kavianpour M, Ketabchi N, Saki N. Prognostic significance of aberrant expression of CD markers in acute lymphoblastic leukemia. Mem-Mag Eur Med Oncol 2017;10:164-9.

69. Feng B, Verstovsek S, Jorgensen JL, et al. Aberrant myeloid maturation identified by flow cytometry in primary myelofibrosis. Am J Clin Pathol 2010;133:314-20.

70. Sekhar M, McVinnie K, Burroughs AK. Splanchnic vein thrombosis in myeloproliferative neoplasms. Br J Haematol 2013;162:730-47.

71. Sagripanti A, Carpi A. Antithrombotic and prothrombotic activities of the vascular endothelium. Biomed Pharmacother 2000;54:107-11.

72. Alonci A, Allegra A, Bellomo G, et al. Evaluation of circulating endothelial cells, VEGF and VEGFR2 serum levels in patients with chronic myeloproliferative diseases. Hematol Oncol 2008;26:235-9.

73. Treliński J, Wierzbowska A, Krawczyńska A, et al. Circulating endothelial cells in postnatal life. Blood 2002;100:3203-8.

74. Barnard MR, Lindem MD, Frelinger A, et al. Effects of platelet binding on whole blood flow cytometry assays of monocyte and neutrophil procoagulant activity. J Thromb Haemos 2005;3:2563-70.
97. Riley CH, Jensen MK, Brimnes MK, et al. Increase in circulating CD4+ CD25+ Foxp3+ T cells in patients with polycythemia vera during treatment with IFN-α. Blood 2011;118:2170-3.

98. Riley CH, Brimnes MK, Hansen M, et al. Interferon-α induces marked alterations in circulating regulatory T cells, NK cell subsets, and dendritic cells in patients with JAK2 V617F-positive essential thrombocythemia and polycythemia vera. Eur J Haematol 2016;97:83-92.

99. Kovacsiovics-Bankowski M, Kelley TW, Efimova O, et al. Changes in peripheral blood lymphocytes in polycythemia vera and essential thrombocythemia patients treated with pegylated-interferon alpha and correlation with JAK2 V617F allelic burden. Exp Hematol Oncol 2015;5:28.

100. Borsellino G, Kleinevietfeld M, Di Mriti D, et al. Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolisis of extracellular ATP and immune suppression. Blood 2007;110:1225-32.

101. Torgano G, Mandelli C, Massaro P, et al. Gastroduodenal lesions in polycythemia vera: frequency and role of Helicobacter pylori. Br J Haematol 2002;117:198-202.

102. Kawamata T, Tojo A. Helicobacter pylori-induced thrombocytosis clinically indistinguishable from essential thrombocythemia. Leuk Lymph 2012;53:1423-4.

103. Göhring K, Wolff J, Doppl W, et al. Neutrophil CD177 (NB1 gp, HNA-2a) expression is increased in severe bacterial infections and polycythemia vera. Br J Haematol 2004;126:252-4.

104. Bench AJ, Pahl HL. Chromosomal abnormalities and molecular markers in myeloproliferative disorders. Semin Hematol 2005;42:196-205.

105. Griesshammer M, Klippel S, Strunck E, et al. PRV-1 mRNA expression discriminates two types of essential thrombocythemia. Ann Hematol 2004;83:364-70.

106. Lee J, Kim Y, Soung Y, et al. The JAK2 V617F mutation in de novo acute myelogenous leukemias. Oncogene 2006;25:1434.

107. Ohanian M, Bueso-Ramos C, Ok CY, et al. Acute myeloid leukemia with MYC rearrangement and JAK2 V617F mutation. Cancer Gen 2015;208:571-4.

108. Kodali S, Chen C, Rathnasabapathy C, et al. JAK2 mutation in a patient with CLL with coexistent myeloproliferative neoplasm (MPN). Leuk Res 2009;33:e236-9.

109. Swierzczek S, Nausova J, Jelinek J, et al. Concomitant JAK2 V617F-positive polycythemia vera and B-cell chronic lymphocytic leukemia in three patients originating from two separate hematopoietic stem cells. Am J Hematol 2013;88:157-8.

110. Tabaczewski P, Nadesan S, Lim SH. Zap-70 positive chronic lymphocytic leukemia co-existing with Jak2V617F positive essential thrombocythemia: a common defective stem cell? Leuk Res 2009;33:854-5.

111. Laurenti L, Tarnani M, Nichele I, et al. The coexistence of chronic lymphocytic leukemia and myeloproliferative neoplasms: a retrospective multicentric GIMEMA experience. Am J Hematol 2011;86:1007-12.

112. Rampal R, Mascarenhas J. Pathogenesis and management of acute myeloid leukemia that has evolved from a myeloproliferative neoplasm. Curr Opin Hematol 2014;21:65-71.

113. Barbui T. The leukemia controversy in myeloproliferative disorders: is it a natural progression of disease, a secondary sequela of therapy, or a combination of both? Semin Hematol 2004;41:15-7.

114. Björkholm M, Derolf ÅR, Hultcrantz M, et al. Treatment-related risk factors for transformation to acute myeloid leukemia and myelodysplastic syndromes in myeloprolifera-
tive neoplasms. J Clin Oncol 2011;29:2410.

115. Tam CS, Kantarjian H, Cortes J, et al. Dynamic model for predicting death within 12 months in patients with primary or post–polycythemia vera/essential thrombocythemia myelofibrosis. J Clin Oncol 2009;27:5587.

116. Huang J, Li CY, Mesa RA, et al. Risk factors for leukemic transformation in patients with primary myelofibrosis. Cancer 2008;112:2726-32.

117. Mesa RA, Verstovsek S, Cervantes F, et al. Primary myelofibrosis (PMF), post polycythemia vera myelofibrosis (post-PV MF), post essential thrombocythemia myelofibrosis (post-ET MF), blast phase MF (PMF-BP): Consensus on terminology by the international working group for myelofibrosis research and treatment (IWG-MRT). Leuk Res 2007;31:737-40.

118. Cervantes F, Dupriez B, Pereira A, et al. New prognostic scoring system for primary myelofibrosis based on a study of the International Working Group for Myelofibrosis Research and Treatment. Blood 2009;113:2895-901.

119. Okamura T, Kinukawa N, Niho Y, et al. Primary chronic myelofibrosis: clinical and prognostic evaluation in 336 Japanese patients. Int J Hematol 2001;73:194-8.

120. Strasser-Weippl K, Steurer M, Kees M, et al. Age and hemoglobin level emerge as most important clinical prognostic parameters in patients with osteomyelofibrosis: introduction of a simplified prognostic score. Leuk Lymph 2006;47:441-50.

121. Tefferi A, Dingli D, Li CY, et al. Prognostic diversity among cytogenetic abnormalities in myelofibrosis with myeloid metaplasia. Cancer 2005;104:1656-60.

122. Barosi G, Bergamaschi G, Marchetti M, et al. JAK2 V617F mutational status predicts progression to large splenomegaly and leukemic transformation in primary myelofibrosis. Blood 2007;110:4030-6.

123. Craig FE, Foon KA. Flow cytometric immunophenotyping for hematologic neoplasms. Blood 2008;111:3941-67.

124. Neilson J, Patton W, Williams M, et al. Polycythemia rubra vera transforming to acute lymphoblastic leukemia with a common immunophenotype. J Clin Pathol 1994;47:471-2.

125. Wu D, Ye B, Shen J, et al. Acute lymphoblastic leukemia in the course of polycythemia vera: a case report and review of literature. India J Hematol Blood Transfus 2016;32:50-5.

126. Musolino C, Allegra A, Penna G, et al. Absence of the V617F JAK2 Mutation in the Lymphoid Compartment in a Patient with Essential Thrombocythemia and B-Chronic Lymphocytic Leukemia and in Two Relatives with Lymphoproliferative Disorders. Acta Haematol 2009;122:46-9.

127. Hussain K, Brakensiek K, Ballmaier M, et al. B-CLL developing in a patient with PV is not affected by V617F mutation of the Janus kinase 2. Eur J Haematol 2006;77:539-41.

128. Chambers I, Truong P, Kallail KJ, et al. Extensive bone marrow necrosis and osteolytic lesions in a case of acute myeloid leukemia transformed from polycythemia vera. Cureus 2016;8.

129. Stijinis C, Kroses W, Balkassmi S, et al. No Evidence for JAK2/V617F Mutation in Monoclonal B Cells in 2 Patients with Polycythemia Vera and Concurrent Monoclonal B-cell Disorder. Acta Haematol 2012;128:183-6.

130. Henry L, Carillo S, Jourdan E, et al. Association of essential thrombocythemia and chronic lymphocytic leukemia: absence of the V617F JAK2 mutation in the lymphoid compartment. Am J Hematol 2007;82:500-1.

131. Ohanian M, Leventaki V, Verstovsek S, et al. Acute lymphoblastic leukemia arising in post-polycythemic myelofibrosis: a rare entity. Leuk Lymph 2012;53:1839-41.

132. Landolfi R, Cipriani MC, Novarese L. Thorbosis and bleeding in polycythemia vera and essential thrombocythemia: pathogenic mechanisms and prevention. Best Pract Res Clin Haematol 2006;19:617-33.

133. Mascarenhas J, Navada S, Malone A, et al. Therapeutic options for patients with myelofibrosis in blast phase. Leuk Res 2010;34:1246-9.

134. Alvarez-Larrán A, Senín A, Fernández-Rodríguez C, et al. Impact of genotype on leukemic transformation in polycythemia vera and essential thrombocythemia. Br J Haematol 2017;178:764-71.

135. Campbell PJ, Baxter EL, Beer PA, et al. Mutation of JAK2 in the myeloproliferative disorders: timing, clonality studies, cytogenetic associations, and role in leukemic transformation. Blood 2006;108:3548-55.