Expression of CD markers in JAK2^{V617F} positive myeloproliferative neoplasms: Prognostic significance

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Abstract

Myeloproliferative neoplasms (MPNs) are clonal stem cell disorders characterized by the presence of JAK2^{V617F} 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 JAK2^{V617F} 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 JAK2^{V617F} 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 thrombohememorrhagic events, as well as autoimmune and leukemic transformation in JAK2^{V617F} positive MPNs. Considering the possible role of CD markers' expressions in JAK2^{V617F} 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.

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Key words: JAK2^{V617F}; CD markers; polycythemia vera; essential thrombocythemia; primary myelofibrosis; prognosis.

Acknowledgments: we wish to thank all our colleagues at Allied Health Sciences School, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Contributions: MMB, conceived the manuscript and revised it; SSh, AE, SH, MSh, wrote the manuscript and prepared the tables.

Conflict of interest: the authors declare no conflict of interest.

Received for publication: 8 May 2018.
Accepted for publication: 20 June 2018

Oncology Reviews 2018; 12:373
doi:10.4081/oncol.2018.373

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, JAK2^{V617F} 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, JAK2^{V617F} 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 JAK2^{V617F} 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, JAK2^{V617F} 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 JAK2^{V617F} mutant alleles burden.[12-14] In fact, pervious studies has been shown that the presence of JAK2^{V617F} 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 JAK2^{V617F} negative patients.[15,16] Although the JAK2^{V617F} 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 JAK2^{V617F} positive MPNs, >60 years of age, history of thrombosis, and leukositosis 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 JAK2^{V617F} mutation in T, B, and natural killer (NK) cells lineages can effect clonal proliferation of these cells as well as immunological or inflammatory manifestations of JAK2^{V617F} 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 JAK2^{V617F} positive MPNs and their probable role in the prognosis of these disorders.

CD markers and hemostatic complications in JAK2^{V617F} positive MPNs

JAK2^{V617F} 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 JAK2^{V617F} 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 JAK2^{V617F} 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 (glycophorin 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, JAK2^{V617F} 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 increasing adhesion of RBCs to endothelial cells under the influence of this mutation.[31]

Although numerous studies have shown that JAK2^{V617F} 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 JAK2^{V617F} 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 JAK2^{V617F} mutation in PV pathogenesis, it can be concluded that if the JAK2^{V617F} mutation decreases following *in vivo* erythroid proliferation, 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 JAK2^{V617F} 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 JAK2^{V617F} 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 JAK2^{V617F} mutation affects the expression of CD110,[39] the reduced expression of this marker in JAK2^{V617F} 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, JAK2^{V617F} mutation has been shown to be significantly associated with increased frequency of these immature cells in JAK2^{V617F} positive MPNs.[28] 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 JAK2^{V617F} positive MPNs leads to the increased production of platelets.[42] JAK2 signaling pathways have a central role in the proliferation, survival, and differentiation of different HSCs progenitor cells via signaling of cytokines receptors.[43] In addition, it is clear that overactivation of JAK2 signaling due to JAK2^{V617F} mutation plays a central role in oncogenesis.[44] Although JAK2^{V617F} mutation requires the activation of cytokine receptors for oncogenic signaling,[45] it has been shown that this mutation can induce the colonal expantion 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 JAK2^{V617F} 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 JAK2^{V617F} positive MPNs suggests a

high megakaryopoiesis rate, as well as platelet production and thrombotic events due to impact of JAK2^{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 hemsotasis of body by aggregation and release of their granular contents following activation.[49] Platelet activation occurs by changing expression patterns of some functional CD markers.[50] In this regard, several studies have been conducted on the function of both resting and activated platelets in JAK2^{V617F} positive MPN disorders, which indicate the altered expression of functional platelet markers in these disorders.[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.[54] It has been shown that CD61 expression defects in JAK2^{V617F} positive MPN patients (*e.g.* PV patients) cause impaired binding of platelets to fibrinogen and platelet aggregation.[55,56] Similarly, the decreased expression of CD41 and CD42b is another change of functional platelet markers influenced by JAK2^{V617F}mutation in ET patients.[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.[58] Moreover, the changing expression of other platelet functional markers, including CD63 and CD154 (soluble CD40 ligand), has been reported on platelets of JAK2^{V617F} positive MPNs, both of which can be prognostic for thrombotic complications in these disorders (Table 1).[40,53,59]

Considering the fact that a large number of patients with JAK2^{V617F} positive MPNs show thrombohemorrhagic complications[25] 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^{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^{V617F} positive MPNs.[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.[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.[60,61] The interaction of these integrins with CD62P and CD42b on the surface of platelets results in the formation of leuko-platelet aggregation, which is a risk factor for arterial or venous thrombotic events in MPN patients.[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.[17,63] Also, high levels of tissue factor in these malignancies enhance leuko-platelet aggregation in these disorders.[64]

CD56 is another surface adhesion molecule expressed on NK cells,[65] the aberrant expression of which has been reported in several leukemias and myelodysplastic syndromes (MDS).[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.[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^{V617F} positive MPNs. JAK2^{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 JAK2V^{617F} 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^{V617F} positive MPN patients.[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.[71] However, in JAK2^{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.[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.[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^{V617F} positive disorders (Table 1).[52,74]

Interestingly, in JAK2^{V617F} positive MPNs, an increase in circulating endothelial cells is observed in addition to changing function of these cells.[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.[75,76] The increase in circulating endothelial cells has a direct relationship with angiogenesis in PV, ET, and especially PMF.[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.[77] 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.[78] Several studies have been conducted on angiogenesis

Table 1. Prognostic value of CD markers' expression of different blood cells in JAK2^{V617F} positive MPNs.

Markers	Alternative name	Chro.	Function	Type of diseases		Prognosis	Ref.
				Decreased expression	Increased expression		
RBC surface CD markers							
CD71	TFR	3q29	Necessary for TF absorption and required for erythropoiesis	-	PV	May be associated with poor prognosis because increases of these markers implicates increases erythroid lineage proliferation	30
CD235a	GYPA	4q31.21	A sialoglycoprotein that bears the antigenic determinants of the MN and Ss blood groups				
CD239	BCAM	19q13.32	A glycoprotein for lutheran blood group and a receptor for extracellular laminin	-	PV	Associated with poor prognosis via adhesion augmentation of RBCs to the endothelial cells	31
Megakaryocyte and platelet surface CD markers							
CD36	GPIV	7q21.11	It works as a receptor for thrombospondin in platelets	-	ET	May be associated with thrombotic complications via increased platelets adhesion	58
CD110	TPO-R	1p34.2	Thrombopoietin receptor in megakaryocytes and platelets	PV, ET, PMF	-	Maybe indicates increased thrombopoiesis and subsequently increased risk of thrombotic complications	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	Associated with poor prognosis via participating in arterial thrombosis and erythromelalgia	40, 52, 59
CD62P	P-selectin	1q24.2	This protein mediates the interaction of activated platelets with leukocytes	-	PV, ET, PMF	Associated with thrombosis and erythromelalgia via mediating leuko-platelets adhesion	17, 60, 62, 63
CD41	ITGA2B	17q21.31	This receptor plays a crucial role in the platelet aggregation	ET	-	May be associated with bleeding complications	57, 59
CD42a	GPIX	3q21.3	A platelet surface membrane glycoprotein complex that functions as a receptor for VWF	ET, PV	-	Associated with poor prognosis with low platelet aggregation response to ristocetin	59
CD42b	GPIb	17p13.2	Functions as a receptor for VWF	ET, PV	-	Associated with poor prognosis via low platelet aggregation response to epinephrine	57, 59, 62
CD61	ITGB3	17q21.32	Participates in cell adhesion as well as platelet aggregation via binding to fibrinogen	ET, PV	-	May be associated with bleeding complications	51, 55
CD154	CD40L	Xq26.3	CD40 Ligand	-	ET	Associated with increased thrombosis via inducing TF expression by endothelial cells	53
Leukocytes surface CD markers							
CD11b	MAC-1	16p11.2	Mediates adherence of neutrophils and monocytes to stimulated endothelium and platelet surface molecules	-	PV, ET, PMF	Poor prognosis via mediate leuko-platelet aggregation and increases of leuko-endothelial cells adhesion	60, 61
CD14	CD14 molecule	5q31.3	Expressed on monocytes/ macrophages and mediates the innate immune response to bacterial lipopolysaccharide	-	PV, ET, PMF	Poor prognosis via interfere leuko-platelet aggregation and increases leukocytes adhesion to the endothelial cells	60-62
CD56	NCAM-1	11q23.2	Involved in cell-to-cell interactions as well as cell-matrix interactions during development and differentiation	-	PMF	Maybe associated with poor prognosis via enhancement of cell adhesion	70

Continued on the next page.

in JAK2^{V617F} positive MPNs, especially PMF, indicating that the increase in VEGF and CD34-MVD reflects the high density of active angiogenesis in BM of these disorder.[77-79] However, it has been shown that CD105-MVD evaluation of BM could better reflect BM angiogenetic 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 vivo* 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 over-expression of markers mediating these adhesions could be a poor prognostic factor in clinical outcomes of JAK2^{V617F} 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 JAK2^{V617F} positive MPNs can provide useful information on the prognosis of these disorders.

Table 1. Continued from previous page.

Markers	Alternative name	Chro.	Function	Type of diseases		Prognosis	Ref.
				Decreased expression	Increased expression		
Endothelial cells surface CD markers							
CD34	CD34 molecule	1q32.2	Hematopoietic stem cell antigen	-	PV, ET, PMF	Associated with increased MVD in BM	78, 79
CD105	S- endoglin	9q34.11	It works as transforming growth factor beta receptor	-	PMF, PV	Associated with augmentation of angiogenesis and fibrosis in BM	18
CD106	VCAM1	1p21.2	A cell surface sialoglycoprotein in activated endothelium cells that mediates leuko-endothelial cells adhesion	-	PV, ET	Associated with platelet activation and leuko-endothelial cells adhesion that leads to arterial thrombosis and erythromelalgia	52
CD62E	E- selectin	1q34.2	Responsible for the accumulation of blood leukocytes and mediating the leuko-endothelial cells adhesion	-	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	-	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

chro, chromosome; RBC, red blood cell; TFR, transferrin receptor; GYPA, glycophorin A; BCAM, basal cell adhesion molecule; GPIV, glycoprotein IV; TPO-R, thrombopoietin receptor; ITGA2B, integrin subunit alpha 2b; GPIX, glycoprotein IX; VWF, von willebrand factor; GPIb, glycoprotein Ib; ITGB3, integrin subunit beta 3; CD40L, CD40 ligand; TF, tissue factor; MAC-1, macrophage receptor 1; NCAM-1, neural cell adhesion molecule 1; MVD, microvessel density; BM, bone marrow; VCAM1, vascular cell adhesion molecule 1; ICAM, intercellular adhesion molecule 1; MPR-1, motility-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.[82-84] 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).[20,85] There is also growing evidence that the JAK2^{V617F} mutation involves lymphoid cells types like B, T, and NK cells in addition to myeloid linage.[22,86] This mutation can affect the frequency and function of B, T, NK cells, and monocytes in PV patients.[87] 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.[88] 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.[89] It has also been shown that these lymphocytes play an important role in the occurrence of autoimmune reactions in autoimmune diseases.[90] Thus, there may be a higher likelihood of autoimmune phenomena in PMF patients who show an increase in BCD5$^+$ lymphocytes.[88]

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.[91] 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.[92] There is evidence that younger PMF patients with monocytosis are at increased risk of progression to chronic myelomonocytic leukemia (CMML) and short-term survival.[93] 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.[94] 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.[95] Treg lymphocytes are significantly increased in patients with PV.[96] 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.[97,98] 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).[99] This Treg subtype is highly suppressive, and the decreased number of such cells has been associated with a significant reduction in tolerance.[100] 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.[97] 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

Table 2. Dysregulated expression of immune cell-related CD markers in JAK2^{V617F} positive MPNs.

Markers	Alternative name	Chro.	Function	Type of diseases		Prognosis	Ref.
				Decreased expression	Increased expression		
CD4C	D4 molecule	12p13.31	TH	PMF	-	Maybe associated with decreased immune function	88
CD8	CD8 molecule	2p11.2	TC				
CD5	CD5molecule	11q12.2	B cells	-	PMF	Maybe associated with autoantibody production and autoimmune complications	88
CD68	LAMP4	17p13.1	Monocyte	-	PMF	Associated with monocytosis, rapid disease progression and increased predisposition toward CMML	92, 93
CD177	NB1 glycoprotein	19q13.2	Neutrophil	-	PV, ET, PMF	Maybe associated with bacterial infections	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

chro, 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.

autoimmune complications in patients. Therefore, flow cytometric immunophenotyping assay of immune cells' CD markers during follow-up of JAK2^{V617F} 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 JAK2^{V617F} 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 JAK2^{V617F} MPNs, including ET and PMF.[104] Although it has been shown that patients with a higher expression of CD177 are at increased risk of thrombotic and bleeding complications due to increased circulating neutrophils,[105] it is inferred that the overexpression of this marker in JAK2^{V617F} positive MPNs could be a poor prognostic factor for the presence of bacterial contamination in these disorders.

In general, the relationship between JAK2^{V617F} mutations with changing expression patterns of immune cells' CD markers in JAK2^{V617F} 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 JAK2^{V617F} 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.

CD markers' expression during leukemic transformation in JAK2^{V617F} positive MPNs

JAK2^{V617F} is a common mutation in MPNs.[1] To a lower extent, this mutation has also been reported in other malignancies, including acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), chronic lymphoblastic leukemia (CLL), and some patients with MDS.[3-5] Recently, there have been cases of JAK2^{V617F} mutation in some leukemic patients without a history of JAK2^{V617F} positive MPNs.[106,107] The results of these studies suggest that this mutation is not specific to JAK2^{V617F} positive MPNs and that it may also be seen in normal individuals and underlie malignancies. Furthermore, there are case reports that indicate the co-existence of JAK2^{V617F} positive MPNs and leukemia in a single patient.[108-111] The progression towards leukemia is a feature of JAK2^{V617F} positive MPNs. Although this tendency is often due to the nature of these disorders, factors such as age, anemia, leukocytosis, thrombocytosis, increased blasts in BM and PB, some mutations, certain treatment protocols, and cytogenetic abnormalities have been identified as the risk factors.[112] Detection of pathogenesis mechanisms is difficult in such a situation; however, several studies have emphasized the role of therapeutic protocol in leukemic transformation of these disorders.[113-116]

Leukemic transformation is often identified by the presence of at least 20% leukemic blasts in BM or PB of JAK2^{V617F} positive MPNs.[117] This complication is observed in all the three JAK2^{V617F} positive disorders, including PV, ET, and PMF, but there is a higher risk of its development in PMF than other disorders.[118] Several studies have been conducted concerning PMF leukemic transformation with large numbers of samples, and their results have shown that factors such as hemoglobin level, leukocyte count, circulatory blasts, platelet counts, monocyte counts, advanced age, male sex, and cytogenetic abnormalities are risk factors for this complication in PMF patients.[119-121] Furthermore, JAK2^{V617F} mutation status is another prognostic factor for leukemic transformation in PMF.[122] Leukemic blasts often express surface CD markers that differentiate them from natural populations.[123] Therefore, the expression of leukemic blasts' CD markers in JAK2^{V617F} positive MPNs can be the first indication that these disorders are progressing towards leukemia. Unlike PMF, most studies on PV and ET regarding leukemic transformation have been case reports, and their results suggest that the expression of certain

Table 3. Possible prognostic CD markers for leukemic transformation in JAK2^{V617F} positive MPNs.

Initial diagnosis	Number of patient /Age, year/ Gender	Prognostic CD markers		Transformed malignancy	Ref.
		Positive CD markers	Negative CD markers		
PV	1/76/M	CD10, CD19, CD38, HLA-DR	CD23, CD5, CD7, CD11b, CD13, CD133, CD14	ALL	124
	1/79/M	CD19, CD5, CD20, CD22, CD23, HLA-DR, CD25	CD38	B-CLL	127
	2/67,79/F	CD19	-	B-CLL	109
	1/75/M	CD10, CD19, CD20, CD22, CD34, CD79α, TdT	-	ALL	125
	1/62/F	CD33, CD56, CD138	-	AML	128
	1/ 69/ M	CD19/CD5, CD5, CD23	CD38, Zap-70	B-CLL	126
	1/ 76/M	CD38, Zap-70	-	CLL	111
	1/60/M	CD5, CD23	CD38	B-CLL	129
	1/65/M	CD5, CD23, CD22	CD38, FMC		
ET	1/58/F	CD19/CD5, CD23	CD38	CLL	130
	3 / 67,72,78/ 2F, 1M	CD19/CD5, CD5, CD23	CD38, Zap-70	B-CLL	126
	2/80,82/F	CD38, Zap-70	-	CLL	111
	1/72/M	CD19/CD5, Zap-70	CD38, FMC	B-CLL	110
	1/82/ M	CD19/CD5, Zap-70	-	B-CLL	110
PMF	1/ 69/ M	CD38, Zap-70	-	CLL	111
	1/80/F	CD5, CD19, CD20, CD23, CD10, Zap-70	CD38	CLL	108
post-PV MF	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 70; 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.[18,53,72,82] 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.[52,57] 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 JAK^{2V617F} 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.[8,133] 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.

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