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REVIEW

Essential thrombocythemia: a hemostatic view of thrombogenic risk factors and prognosis

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Abstract

Essential thrombocythemia (ET) is a classical myeloproliferative neoplasm that is susceptible to hypercoagulable state due to impaired hemostatic system, so that thrombotic complications are the leading cause of mortality in ET patients. The content used in this article has been obtained by the PubMed database and Google Scholar search engine from English-language articles (2000–2019) using the following keywords: "Essential thrombocythemia," "Thrombosis," "Risk factors" and "Hemostasis. In this neoplasm, the count and activity of cells such as platelets, leukocytes, endothelial cells, as well as erythrocytes are increased, which can increase the risk of thrombosis through rising intercellular interactions, expression of surface markers, and stimulation of platelet aggregation. In addition to these factors, genetic polymorphisms in hematopoietic stem cells (HSCs), including mutations in JAK2, CALR, MPL, or genetic abnormalities in other genes associated with the hemostatic system may be associated with increased risk of thrombotic events. Moreover, disruption of coagulant factors can pave the way for thrombogeneration. Therefore, the identification of markers related to cell activation, genetic abnormalities, or alternation in the coagulant system can be used together as diagnostic and prognostic markers for the occurrence of thrombosis among ET patients. Thus, because thrombotic complications are the main factors of mortality in ET patients, a hemostatic viewpoint and risk assessment of cellular, genetic, and coagulation factors can have prognostic value and contribute to the choice of effective treatment and prevention of thrombosis.

Keywords Essential thrombocythemia · Thrombosis · Risk factors · Hemostasis

Abbreviations

WBCs	White blood cells
RBCs	Red blood cells
ROS	Reactive oxygen species
MPV	Mean platelet volume
TXA2	Thromboxane-A2
ADP	Adenosine diphosphate
sCD40L	Soluble- CD40 ligand
MPs	Microparticles
vWF	Von Willebrand factor
ECs	Endothelial cells
TM	Thrombomodulin
PAI-1	Plasminogen- activator inhibitor
TG	Thrombin generation
4G	4-Guanosine sequence
5G	5-Guanosine sequence
TF	Tissue factor
NOS3	Nitric oxide synthase-3
NO	Nitric oxide
MTHF	Methylenetetrahydrofolate reductase
TFPI	Tissue factor pathway inhibitor
CAL	Calreticulin

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Introduction

Essential thrombocythemia (ET) is an acquired myeloproliferative neoplasm (BCR-ABL$^-$) characterized by an increase in peripheral blood platelet counts (> 450 G/L) as well as rising megakaryocytes in bone marrow (BM) [1]. Although the risk of fibrotic/leukemic transformation in ET patients is < 1%, the incidence of thrombohemorrhagic events in them has been estimated at approximately 11–39% [2]. Hemostatic abnormalities in these patients often occur as thrombotic events (as opposed to hemorrhage), which are caused by various factors such as increasing counts and function of different cells (i.e. platelets, leukocytes, endothelial cells and erythrocytes), the presence of genetic risk factors including JAK2 V617F mutation (as the most frequent genetic aberration), CALR and MPL mutation, or the increase in coagulant markers (Fig. 1) [3]. Nevertheless, age above 60 years and a history of thrombotic events are known as standard risk factors for thrombosis in these patients [4]. Although life expectancy may not be reduced in ET patients, thrombotic complications are the foremost cause of mortality in them [4]. Identifying the predictors as well as the mechanism of thrombotic events in low-risk ET patients is of high value because they are prone to severe thrombotic attacks in the future [5]. The mechanism of thrombosis pathogenesis in ET patients is not fully understood. However, clinical symptoms (such as vascular/arterial thrombosis and erythromelalgia), laboratory indicators including increasing levels of coagulant markers such as factor VII, VIII, tissue factor [TF], plasminogen activator inhibitor-1 [PAI-1], and genetic analysis can detect thrombosis risk factors [6, 7]. Cellular analysis in ET patients suggests that cell activation and interaction between cells can play an essential role in stimulating platelet aggregation and enhancing thrombosis. Therefore, the evaluation of cell counts and markers associated with cell activation could be considered as a prognostic marker for the occurrence of thrombotic episodes [8]. Besides, genetic studies suggest that the presence of genetic abnormalities such as JAK2 V617F mutation, which is present in nearly 60–65% of ET patients, can augment the procoagulant state in these patients through increasing cellular activity and stimulation of coagulant factors [9]. Hence, genetic evaluation can have diagnostic and prognostic value in ET patients

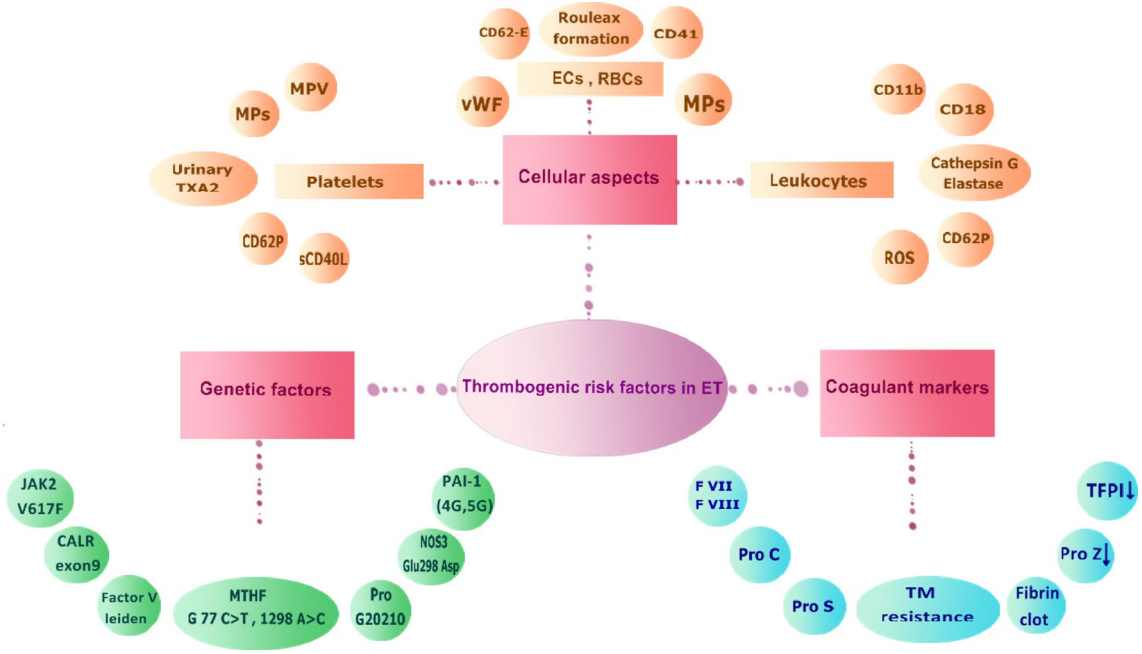

Fig. 1 Cellular, genetic, and coagulation aspects of thrombogenic risk factors in ET. Cellular aspects: Various cells, including leukocytes, platelets, endothelial cells, and erythrocytes, can stimulate thrombotic events by altering the morphology, number, secretion of granules substances, and activating markers. Evaluation of each of these cellular factors may be useful in predicting and detecting early thrombotic attacks in patients. Genetic factors: Genetic abnormalities in patients can lead to excessive proliferation of cells or stimulate the coagulation state. Some of these genetic abnormalities inhibit the fibrinolysis system (such as PAI-1 polymorphisms), and others (such as Pro G20210) enhance the production of thrombin. Genetic evaluation in ET patients can be a prognostic marker for thrombotic attacks and have a high diagnostic value. Coagulant markers: Increased coagulation factors, elevated thrombomodulin resistance, and decreased fibrin sensitivity to plasmin can all provide conditions for stimulating thrombotic events in ET patients. Therefore, the measurement of coagulation factors, along with the risk of cellular and genetic factors, has a high prognostic value

and increase the likelihood of thrombosis. In addition to the mentioned issues, plasma levels of coagulant factors (that are higher in ET patients with thrombosis than in controls) are affected by both cellular activity and genetic polymorphisms [10]. Given that the prevention of thrombotic episodes is the primary goal of treatment for ET patients, in this paper, we investigate a hemostatic viewpoint to investigate cellular, genetic and coagulation risk factors to identify valuable diagnostic and prognostic markers involved in the selection of practical therapeutic and prognostic approach and improvement of patients.

Cellular aspects of thrombosis in ET patients

Neutrophils

Studies have shown that alterations in the number and function of crucial cells like leukocytes, platelets, endothelial cells, as well as erythrocytes may play a role in the pathogenesis of thrombotic complications in patients with ET (Table 1). These patients have high blood viscosity due to leukocytosis, thrombocytosis, or erythrocytosis, which is associated with myeloid hyperplasia of bone marrow [11]. The hyperplasia is partly caused by JAK2 V617F mutation (or an equivalent somatic mutation) that not only increases cell lineage but leads to functional changes in cells [11, 12]. Relative to the increase in other lineages (e.g., erythrocytosis), leukocytosis appears to be more related to vascular complications [13]. Therefore, researchers are paying particular attention to the number and function of polymorphonuclear leukocytes (PMNs) in ET patients. Research has revealed that high WBC counts ($> 11 \times 109$/L) could be considered as a thrombotic risk factor in these patients with poor prognosis [13]. Furthermore, WBCs counts $> 15 \times 109$/L are predictive of arterial/vascular thrombosis in ET patients [11]. Leukocytosis-related thrombotic events can be due to the changing function of activated neutrophils. For example, activated neutrophils can affect the hemostatic system through reactive oxygen species (ROS), the release of intra-granule proteases, or interactions with vascular cells (or platelets, monocytes, and endothelial cells) [14]. ROS, such as nitric oxide (NO), can detect hemostatic components in the blood, including inactive fibrinogen, factor V, factor VII, von willebrand factor (VWF), factor X, plasmin activator inhibitor-1 (PAI-1), and α2-antiplasmin [15]. Moreover, in vitro studies incubating activated neutrophils with endothelial cells in culture medium have revealed that ROS facilitates the release of VWF and thrombomodulin from endothelial cells, which can be considered as a thrombogenic agent [14]. On the other hand, activated neutrophils are capable of secreting intracellular proteases, which can transform the phenotype of platelets and endothelial cells into a procoagulant state and enhance the risk of thrombosis [16]. The most effective neutrophil enzymes are elastase and cathepsin G. These

Table 1 The most Important thrombotic risk factors associated with cell activation

Cells	Activation markers	Thrombotic activity	References
Leucocytes	High WBCs count ($> 11 \times 10^9$/L)	Stimulation of platelet aggregation	[13–15]
	Increases ROS	Facilitate release of vwf from Ecs	[17]
	Increased cathepsin G and elastase	Inhibit coagulant inhibitors, TM and increase PAI-1 secretion	
	Increased expression of CD11b/ CD18	Stimulate super oxide anions synthesis and thrombin formation	[18]
	Increased expression of CD11b/CD62P	Increased platelet degranulation and procoagulant state	[29]
Platelets	Increased MPV and decreased density	Dilation of the dense tubular/open canalicular system	[10]
	Increased urinary TXA2	Increased arachidonic acid metabolism and platelet aggregation	[31]
	Increased microparticles	Increased levels of aminophospholipids and thrombogenesis	[37–39]
	Increased platelet sensitivity to ADP	Stimulation of the platelet aggregation and TG	
	Increased expression of selectin P-selectin	Increased platelet activity and thrombin growth	[42–44]
	Increased levels of sCD40L	Stimulation of agonist-induced platelet and aggregation	
	Decreased pro-apoptotic mediators (BAX)	Increased megakaryocytic and platelet populations	[46–51]
Ecs, RBCs	Increased levels of endothelial-derived MPs	Increased level of coagulability and TG	[52]
	Increased mature vWF	Strengthen the coagulation and aggregation process	
	Increased expression of CD62-E	Increased platelet adhesion and aggregation	[54–57]
	Increased expression of CD62E/CD41	Induction of morphological changes in platelets and aggregation	[59]
	Increased rouleax formation	Stimulation of platelet aggregation	[60]

WBCs white blood cells, *ROS* reactive oxygen species, *MPV* mean platelet volume, *TXA2* thromboxane-A2, *ADP* adenosine diphosphate, *sCD40L* soluble- CD40 ligand, *MPs* microparticles, *vWF* von Willebrand factor, *ECs* endothelial cells, *TM* thrombomodulin, *PAI-1* plasminogen- activator inhibitor, *TG* thrombin generation

enzymes prevent thrombomodulin activation and stimulate PAI-1 release by inhibiting thrombin-induced prostacyclin production, thereby increasing the risk of hypercoagulability thrombosis [14]. Notably, cathepsin G is a degranulating agent and platelet agonist increasing the expression level of glycoproteins (such as platelet receptor PAR4) on platelets for binding to neutrophils, which can contribute to effective aggregation and rising risk of thrombosis [17]. The coagulant activity of neutrophil elastase may be due to the inhibition of several coagulant inhibitors, including protein C, protein S, antithrombin, heparin factor II, or tissue factor pathway inhibitor (TFPI), which is associated with increasing thrombosis [14, 18]. Neutrophils enhance the expression of adhesive molecules such as CD11b to protect proteases by creating a close microenvironment to prevent the effects of proteinase inhibitors [19]. Neutrophil gelatinase-associated lipocalin (NGAL) is a member of the lipocalin family that plays a role in the transport of lipophilic substances and the synthesis of prostaglandins in neutrophils [20]. As an acute-phase protein, NGAL level is elevated in a variety of conditions, including appendicitis, urinary tract infection, or bowel diseases [21, 22]. The prognostic value of NGAL is also significant in malignancies, such as breast cancer and ovarian cancer [23, 24]. Research has shown that NGAL serum levels are significantly increased in ET patients, which may reflect increasing neutrophil counts in these patients because NGAL is produced by neutrophils [25]. Thus, it may be argued that the approaches leading to the inhibition of the production and function of NGAL could be useful in the treatment of ET patients.

The adhesion of neutrophils to other blood cells, which can trigger procoagulant reactions, is an important issue that has been the subject of most studies. For instance, neutrophils bind p-selectin on the surface of platelets via p-selectin glycoprotein ligand-1 (PSGL-1) [26]. Subsequent adhesion is mediated by CD11b/CD18 binding to platelet glycoprotein (or fibrinogen bound to platelet GPIIb/IIIa), resulting in the formation of neutrophil/platelet mix aggregates and superoxide anions [14]. According to investigations, these mix aggregates and superoxide anions are effective in the pathogenesis of thrombotic events in patients with ET. Therefore, measurement of sensitive markers of platelet activation such as neutrophil/platelet mix aggregates by a cytofluorimetric method in whole blood can have prognostic and diagnostic value in these patients [27, 28]. As an example, increasing expression of CD11b (neutrophil marker), CD62p (platelet marker), or CD11b/CD62P + (a marker of neutrophil/platelet mix aggregates) can be used by cytofluorimetric analysis for prognostic and diagnostic purposes [29]. Other studies show that neutrophil activation parameters such as CD11b, leukocyte alkaline phosphatase (LAP), as well as plasma parameters like plasma elastase and myeloperoxidase (MPO) are significantly higher in ET patients than in controls [14, 30, 31], suggesting that the activation and degranulation of neutrophils may be implicated in the pathogenesis of thrombosis in such patients. Interestingly, according to analyses, plasma levels of MPO and elastase were still higher in ET patients than in the control group even after normalizing the WBC count [30]. Consequently, the level of plasma markers (including MPO and elastase) may depend on the activity of the leukocytes, not just their counts [4, 30]. Aspirin can decrease mixed aggregates and thrombotic risks by reducing CD11b expression and neutrophil activity [32]. Accordingly, thrombotic complications can be altered over time through various cell-dependent factors; therefore, the evaluation of blood parameters could have a prognostic value in ET patients as well as in other malignancies (such as leukemia, infection, etc.) [33–35]. While the calculated cutoffs for WBCs are considered equal to 8.48 G/L (above which the risk of thrombosis increases), it is not clear what threshold of WBCs counts is satisfactory as a treatment endpoint for patients [5]. Cytoreductive therapy, including the use of hydroxyurea (HU) to decrease blood cells (especially WBCs), can diminish the risk of vascular thrombotic disease in high-risk ET patients [8]. Thus, leukocytosis, along with functional and morphologic abnormalities, can be a crucial risk factor for thrombotic events in ET patients, and an efficient therapeutic strategy should be considered following possible defects.

Platelets

Along with the previously mentioned changes in neutrophils, some platelet defects like atypical morphology, membrane abnormalities, acquired storage pool diseases, and abnormal arachidonic acid metabolism has been detected in ET patients as well as rising platelet counts [36]. Using automated and ultrastructural analyses, platelet abnormalities such as increased mean platelet volume (MPV), decreased buoyant platelet density, or increased heterogeneity have been observed in ET patients [37]. These findings are due to platelet proliferation or dilation of the dense tubular/open canalicular system. As a result, automated parameters such as MPV and decreasing density reduction may be indicative of increased platelet activity associated with poor prognosis in patients [37–39]. Increased urinary excretion of thromboxane A2 (TX A2), which indicates platelet activation, is also valuable in vivo finding that can be used as a prognostic marker for the occurrence of thrombotic episodes in ET patients [31]. Hemostatically, activation of platelets in ET patients is caused by the exposure of phospholipids present on the surface of platelets to tenase and prothrombinase complexes. Activated platelets can express anionic phospholipids and TF on their surface, thereby providing the conditions for stimulating the coagulation cascade [40, 41]. Concomitant with platelets activation, the tissue factor

(TF) level increases and stimulates the coagulation process. Following platelet activation or cell injury, various cells like platelets, erythrocytes, or leukocytes (mainly platelets) can release microparticles (0.1–1 μm in diameter) into the bloodstream [10]. There are aminophospholipids on the surface of these microparticles that are capable of initiating the thrombotic process. Studies indicate the higher number of these microparticles in ET patients relative to controls [42]. Consequently, microparticles can be considered as biomarkers reflecting the prothrombotic state that increases the chance of thrombosis [10]. On the other hand, surface markers of platelets can enhance platelet aggregation and thrombosis. ADP is a potent platelet agonist affecting three platelet receptors: P2Y1, P2Y12, and P2X1 [43]. Investigations indicate that platelets of ET patients (especially JAK2 V617F positive patients) are more sensitive to ADP and further stimulate platelet aggregation as well as thrombin generation [43]. ADP binding to the P2Y12 receptor increases the secretion of dense granules and α-granules, augmenting TF secretion, and p-selectin translocation to the platelet surface that will eventually be associated with thrombus growth [44]. According to statistics, inhibition of P2Y12 receptor decreases TF-dependent factor Xa activity by 33% in healthy subjects taking clopidogrel (ADP antagonist) [45]. Targeting negatively charged phospholipids on the surface of activated platelets seems to be a promising prospect for antithrombotic therapies among ET patients. Thus, ADP receptor inhibitors (in addition to aspirin) can inhibit platelet function and prevent thrombosis [43]. Alternatively, phagocytes are involved in the clearance of activated circulating platelets, which can prevent thrombotic complications. However, platelet phagocytosis depends on the expression of surface markers on activated platelets, including p-selectin [46]. Since the presence of JAK2 V617F mutation is related with increased leukocyte counts, it may be inferred that the presence of this mutation is effective in reducing the risk of thrombosis in ET patients via increasing clearance and phagocytosis of activated platelets [47]. In addition to the large size and increase in granules of platelets, research has shown that the soluble CD40-L (sCD40-L) level also increases on the surface of platelets [48]. sCD40-L can stimulate the activity, aggregation, and agonist-induced platelet activation through CD40-dependent tumor necrosis receptor and mitogen-activated protein kinase signaling. Accordingly, sCD40-L could be a predictor for platelet activation and thrombosis following rising platelet counts [49]. The impairment of the apoptosis process in megakaryocytes is a crucial mechanism involved in increasing counts of platelets (as well as reticulated platelets) in ET patients. Studies have indicated that decreasing pro-apoptotic mediators (such as BAX activators) can increase the population of megakaryocytes in BM of these patients [50, 51]. Given that the calculated cutoffs for platelet counts are 574.5 G/L, the counts exceeding this threshold can be considered as a thrombotic risk factor [5]. For this reason, apoptosis inducer drugs (such as HU) are used at an appropriate dose to prevent thrombotic events leading to decreased platelet populations. HU may play a role in reducing thrombotic events in these patients by stimulating the apoptotic process and inhibiting platelet dysfunction [51]. Overall, elevated levels of activated platelet markers, platelet dysfunction, and impaired inhibition of the apoptotic process should all be taken into account as they may increase the risk of vascular thrombosis in ET patients.

Endothelial cells and erythrocytes

The stimulatory effect of endothelial cells on the hemostatic system is mainly due to the function of PMNs, granular content, and surface markers associated with endothelial cells. Research has indicated increasing levels of endothelial-derived microparticles under various conditions, including venous thromboembolism [52]. Less than 5% of these microparticles are generated by the activity of endothelial cells, erythrocytes, and monocytes [53]. Endothelial activation plays a role in enhancing the thrombotic state and stimulating thrombin generation. An increase in mature VWF and higher expression of soluble E-selectin (CD62E) are important markers for the identification of endothelial activation [52]. Although endothelial cells do not express CD62E at rest, soluble CD62E levels increase during endothelial activation [54]. Also, some investigations have shown that activated endothelial cells of ET patients express CD41 (a platelet marker) in addition to CD62E-positive microparticles. However, CD41 is not generally detected on endothelial cells [52, 55]. Hence, this double positivity (CD62E/CD41) may have been caused by the interaction between activated platelets and endothelial cells. Elevated CD62E/CD41-positive microparticles increase thrombin generation and raise the risk of thrombosis and coagulation activities, which is an important sign of enhanced platelet and endothelial cell activity [56, 57]. Besides, activated MPNs can invade and disrupt endothelial cells through agonists such as N-formyl-methionyl-leucyl-phenylalanine (fmlP), bacterial endotoxins, cytokines, as well as enzymes generated by their activity [30]. Elastase has the most significant role in cytolysis and damage to endothelial cells compared to other granular proteases [58]. Following this injury, more VWF and thrombomodulin are expressed on the surface of endothelial cells, thereby providing the conditions for the initiation of coagulant processes and thrombosis.

The interaction between activated platelets and erythrocytes has also been observed among ET patients in some investigations, which has been reported to cause changes in platelet morphology (including pseudopodia formation and extreme aggregation) that can affect the hemostatic system [59]. Furthermore, electron light microscopy scans have

shown evidence of platelet-erythrocyte interaction as well as rouleaux formation in ET patients, which may be effective in stimulating platelet aggregation and increasing the risk of thrombosis [59, 60].

Genetic view of thrombogenic risk factors in ET patients

Genetic risk factors can lead to cellular changes and may also be involved in the pathogenesis of thrombosis in ET patients (Table 2). Accordingly, JAK2 V617F mutation is one of the most basic genetic abnormalities in these patients, with a probable incidence of 60–65% [9]. Importantly, JAK2 V617F mutation can also raise the venous thrombosis risk of unusual sites (e.g., cerebral and splanchnic veins) [61]. This mutation has an intrinsic effect on megakaryopoiesis biology and platelet reactivity, increasing the count and function of platelets. For instance, the simultaneous presence of this mutation and thrombophilia causes a fivefold increase in the risk of thrombosis compared to patients lacking this mutation or thrombophilia [11, 62]. Based on investigations, JAK2 V617F mutation spontaneously generates endogenous erythroid colony (EEC) and causes CD177 (PRV-1) overexpression on the surface of granulocytes and leukocytes, leading to higher spontaneous proliferation in megakaryocytes [31]. Moreover, heterozygous/homozygous JAK2 V617F mutations can increase the number and function of leukocytes, which is detectable by the rising score of LAP, CD11b, and CD177 (PRV-1) [31, 63]. On the other hand, overexpression of platelet-leukocyte aggregates and TF is directly associated with the presence of JAK2 V617F mutation, which increases the risk of thrombotic complications in ET patients [64]. The use of JAK2 inhibitors because of failed HU treatment may play an essential role in controlling leukocyte activation as well as lowering the levels of TF and CD11b to minimize the risk of thrombosis in these patients [64].

CALR mutation is the next major genetic risk factor in ET patients. CALR is a chaperone-protected protein involved in differentiation, apoptosis, and cell proliferation [3]. Mutation in exon 9 of CALR has been identified in 20–25% of ET patients, which is associated with a reduced risk of thrombosis compared to JAK2 V617F. Compared to JAK2 V617F mutation, the phenotype of CARL mutation is observed in younger men with lower WBC counts and Hb as well as higher platelet counts and larger BM megakaryocytes [65]. In terms of age and sex distribution, MPL mutation is similar to patients carrying JAK2 V617F mutation and is comparable to $CALR^+$ patients concerning WBC counts [66]. MPL mutation occurs in nearly 5% of ET patients, so it is less prevalent than the two mentioned mutations [9]. It should be noted that the frequency of patients lacking these three mutations (triple-negative) has been estimated to be 10–25%. Triple-negative patients should be considered in ET because they are also at risk of thrombosis [9, 67]. In addition to the mentioned mutations, several genetic abnormalities have been identified concerning the hemostatic system that could be involved in thrombotic events. For example, polymorphisms in coagulation genes like factor V Leiden and prothrombin G20210 mutation have been reported in patients with MPNs (especially ET) [68]. The coexistence of JAK2 V617F and prothrombin G20210 in patients with Budd-Chiari syndrome (BCS) who later progressed to ET is known as a combination of thrombophilic risk factors with an approximate frequency of 28% [69]. In addition to diagnostic value, tracing the coexistence of these two mutations has a prognostic role for progression to ET as well as thrombotic complications [68]. Alternatively, known polymorphisms in the promoter region of PAI-1 (plasminogen activator inhibitor-1) gene, including 4-guanosine (4G) and 5-guanosine sequence (5G), may also contribute to increasing thrombosis [70]. Both of these alleles (4G, 5G) have a binding site for activator of transcription. Studies show that the 5G allele has an additional binding site for repressors relative to the 4G allele, which reduces transcription rates

Table 2 The impact of genetic abnormalities on the process of thrombosis in ET patients

Gene	Gene variant/polymorphism	Outcome	References
JAK2	JAK2 V617F	Increased leukocyte counts, platelets, and factor TF expression	[11, 31, 61–64]
CALR	CALR exon 9	Reduced risk of thrombosis and increased platelet count (compared to JAK2 V617F)	[3, 65]
Factor V	Factor V leiden	Increased factor V resistance and stimulation of thrombogenesis	[68, 69]
Prothrombin	Prothrombin G20210	Increased thrombin levels and procoagulant state	[68, 69]
PAI-1	4G,5G	More plasmin- activator Inhibition and increased thrombin formation	[70, 71]
MTHF	G77 C > T,1298 A > C	Hyperhomocysteinemia and arterial/vascular thrombosis	[11, 72]
NOS_3	Glu298 ASP	Reduced the protective effects of NO and increased platelet aggregation	[73, 74]

4G 4-guanosine sequence, *5G* 5-guanosine sequence, *TF* tissue factor, NOS_3 nitric oxide synthase-3, *NO* nitric oxide, *MTHF* methylenetetrahydrofolate reductase, *PAI-1* plasmin activator-inhibitor

and, consequently, PAI-1 function [71]. Nevertheless, the 4G allele increases the level of PAI-1 (compared to 5G), which is associated with the rising risk of thrombosis in patients [70]. Additionally, common mutations in the methylenetetrahydrofolate reductase gene (MTHF), including G77 C > T and 1298 A > C, are associated with decreasing MTHFR enzymatic activity, which may lead to hyperhomocysteinemia and increased arterial/vascular thrombosis of ET patients [11, 72]. Another important genetic risk factor is the gene polymorphisms of nitric oxide synthase-3 (NOS3). The product of the NOS3 gene is NO, which is capable of inducing antithrombotic effects by reducing leukocyte adhesion and platelet aggregation [73]. Researchers have shown that the Glu298 ASP variant of the NOS3 gene decreases the protective effects, increasing the risk of thrombosis in ET patients [73, 74]. Conversely, haplotypes of factor VII genes, including dekanucleotide polymorphism (-323P0/10), have a protective effect against thrombotic episodes by decreasing factor VII level in plasms [75, 76]. As a result, genetic tests and detection of thrombophilia mutations could be a practical approach for assessing thrombosis risk as well as reducing thrombotic events in ET patients.

Coagulant markers as a trigger of thrombosis in ET patients

ET patients are exposed to hypercoagulant state due to the increase of plasma biomarkers associated with the hemostatic system. According to investigations, increasing levels of several coagulant factors including factor VII, factor VIII, thrombin, prothrombin fragments (F1 and F2), protein S/C, VWF raise the risk of thrombosis in ET patients (Table 3) [1]. With a hemostatic view, impaired TM function and acquired TM resistance can be associated with decreased levels of free protein S, leading to enhanced procoagulant activity in ET patients [10]. Undoubtedly, the evaluation of TF activity is a good indicator to assess coagulation activity. According to studies on ET patients, TF activity is approximately 18-fold higher than the control group [7]. Coagulant activity of TF is inhibited by TFPI, which is profusely secreted by vascular endothelium. TFPI activity has been observed to be lower in JAK2 V617F-negative ET patients relative to JAK2 V617F-positive ones [77]. Therefore, the presence of this mutation is likely to increase the risk of thrombosis by reducing TFPI activity [7]. Along with these factors, protein Z (pro Z), a vitamin K-dependent glycoprotein, may also be involved in the stimulation of thrombogenesis. One of the most critical functions of pro Z is to inhibit factor Xa and XIa [78]. Pro Z and z protein-inhibitor (ZPI) are found in the complex form in the circulation, in such a way that the concentration of one of them affects that of the other [79]. Research shows that pro Z and ZPI levels in JAK2 V617F + patients are much lower than those of JAK2 V617F- patients. Decreased plasma levels of pro-Z are associated with a higher risk of thrombosis and thrombotic state stimulation [78]. Furthermore, the activation of platelets and leukocytes causes damage to endothelial cells (an important source of pro Z), which again results in the decrease of pro Z plasma levels and an increase of thrombotic events [78]. On the other hand, unfavorable alternations to fibrin clot have been shown to decrease the sensitivity of fibrin to the fibrinolytic system, which consequently increases thrombosis [1]. In ET patients, elevated levels of PAI-1, circulating PF4, platelet-specific protein (PF4, sPS), and impaired fibrinolysis lead to the formation of compact fibrin networks that are less sensitive to fibrinolysis and may increase the risk of thrombotic episodes [80]. Since anagrelide increases the risk of arterial thrombosis, hemorrhage, and BM fibrosis, it is preferable to use HU as a safe drug to reduce vascular thrombosis in ET patients [32, 81]. Nonetheless, the use of aspirin acetylates fibrinogen and improves fibrin function by enhancing the influence of the fibrinolysis system, which prevents thrombosis [82, 83]. Therefore, because thrombin formation is dependent upon the balance of the fibrinolytic/procoagulant system, the level of coagulant factors should

Table 3 Thrombogenic coagulant risk factors in ET patients

Coagulant risk factor	Plasma levels	Thrombotic effects	Ref
F VII *F VIII*	Increased	Increased thrombin production and hypercoagulant state	[1, 75, 76]
Pro C *Pro S*	Increased	Increased level of FV, FVIII, and thrombin formation	[1]
vWF	Increased	Stimulation of platelet adhesion and aggregation	[1]
TFPI	Decreased	Stimulation of the extrinsic blood coagulation pathway and thrombogenesis	[7, 77]
Pro Z	Decreased	Increased activity of factors Xa/XI and stimulation of TG	[78]
Unfavorable fibrin clot	Increased	Decreased fibrin sensitivity to fibrinolysis and increased procoagulant state	[1, 80]
Acquired TM resistance	Increased	Decreased free Protein S levels and stimulation of thrombin formation	[10]

vWF von Willebrand factor, *TFPI* tissue factor pathway inhibitor, *TM* thrombomodulin, *TG* thrombin generation

always be evaluated in ET patients to reduce the risk of thrombosis in them.

Discussion

ET is a classical Philadelphia-negative MPN with a prevalence of 0.98–1.7 per 100,000 per year, and the first clinical manifestation of ET is thrombotic complications, usually at arterial, vascular, and microcirculating sites [1, 84]. The incidence of arterial thrombosis is higher than vascular thrombosis among ET patients, and splenic thrombosis is more lethal than other types [85]. Nearly 30–50% of ET patients (especially patients over 60 years of age) suffer from thrombotic complications, and vascular thrombosis accounts for 35–45% mortality of these patients [70]. In terms of thrombosis risk, ET patients are divided into three groups: low-risk (0–1score), intermediate-risk (2 scores) and high-risk (> 3 scores) [86]. The main thrombotic risk factors of ET patients are age over 60 (1 score), cardiovascular risk factors (1 score), previous thrombosis (2 scores), and presence of JAK2 V617F mutation (2 scores) [86]. Conventional thrombotic risk stratification distinguishes ET patients in two risk-groups: A low-risk group includes ET patients younger than 60 years without a history of thrombosis, whereas high-risk group includes ET patients older than 60 years with a history of thrombosis [87]. The International Working Group for MPN Research and Treatment (IWG-MRT) released the prognostic scores to categorizes ET patients in three thrombotic risk groups (low, intermediate, and high-risk groups) based on four risk variables (including age > 60 years, thrombosis history, JAK2 V617F presence, and cardiovascular risk factors), which is known as the International Prognostic Score of thrombosis for ET (IPSET-thrombosis) [88]. Recently, through the re-analysis of the original IPSET-thrombosis data set, a revised IPSET-thrombosis (r-IPSET-t) was accomplished. In r-IPSET-t, three adverse variables (age > 60 years, thrombosis history, and JAK2 V617F presence) are used to designate four risk categories (very low, low, intermediate, and High-risk category) [89]. The most prominent benefit of the r-IPSET-t classification is the prediction of ET patients that require therapeutic intervention. Certainly, this prognostic classification can be useful for risk classification and preventing thrombotic attacks [90]. Other risk factors like hypertension, diabetes, hyperlipidemia, and smoking are also implicated in the induction of thrombosis in ET patients [4]. Although among these prognostic models, only the IPSET-thrombosis system includes cardiovascular risk factors, the role of these risk factors in determining thrombotic events and risk stratification is valuable. Cardiovascular risk factors such as cigarette smoking, diabetes, hypertension, obesity, and dyslipidemia can increase the incidence of thrombotic events and having a significant impact on morbidity and survival of ET patients: therefore, should always be considered in the diagnostic and therapeutic process [87]. In addition to ET, primary myelofibrosis (PMF) and Polycythemia Vera (PV) are two other Philadelphia-negative MPN that propensity for thrombotic events. According to studies, the risk of thrombosis in both ET and PV patients exceeds 20%. The overall prevalence of thrombosis in PMF is almost the same as in ET, while it is significantly lower than in PV [91]. According to the WHO 2016, the most important common risk factors that affect the survival of patients with ET, PV, and PMF include advanced age, leukocytosis, and thrombosis. Other studies indicated that like ET, the presence of cardiovascular risk factors, including hypertension, smoking, diabetes, and dyslipidemia, are independent predictors of thrombosis in PMF [91]. On the other hand, a comparison of the role of thrombogenic mutations in ET, PV, and PMF reveals that the impact of JAK2 V617F is well known as a pro-thrombotic factor in ET and PV. However, its role in the pathogenesis of thrombosis in PMF still needs further research [92]. Although in ET and PMF, CALR-mutated patients are associated with higher platelet count, the presence of CALR mutation leads to decreased thrombotic events and lower incidence of leukocytosis [83, 85, 91].

Changes in the function and count of leukocytes, platelets, endothelial cells, or erythrocytes can increase procoagulant state in ET patients. Interaction among these cells, alteration in their granular content, or increase in phagocytosis play a vital role in boosting platelet aggregation, thrombin generation, or increasing coagulant factors. Assessment of markers related to the activity of each cell type can be considered as a prognostic marker [29, 36, 37]. Several genetic factors have also been identified to exacerbate the mentioned risk factors and enhance the risk of thrombosis in patients. Some mutations (such as JAK2 V617F, CALR, and MPL) can occur at the level of hematopoietic stem cells and lead to clonal myeloproliferation, including overproliferation of megakaryocytes and platelets [93, 94]. In critical situations such as inflammation, the presence of inflammatory biomarkers like pentraxin 3 (PTX3) and high-sensitive C-reactive protein (hs-CRP) may be used as prognostic markers (along with genetic risk factors) for classification and probability determination of thrombosis in ET patients [40, 95]. For this reason, the use of inflammatory status reducers, including statins, along with JAK2 inhibitors, seems to be effective in preventing the progression of inflammation and thrombotic episodes [9]. Other polymorphisms (e.g., factor V Leiden, prothrombin G20210, PAI-1 polymorphisms, MTHF mutations, etc.) can increase the level of coagulant factors and the likelihood of thrombotic events [11, 72]. Similar to cellular analysis, genetic assessment of these mutations has remarkable diagnostic and prognostic value. In contrast, increasing levels of coagulant markers or impaired fibrinolytic system

can cause upregulation in the hemostatic system and raise the chance of thrombosis in ET patients [1]. Some patients with ET have no clinical symptoms upon diagnosis, so evaluating these patients from a cellular, genetic, and coagulant viewpoint may help control and treat the disease (Fig. 1) [7]. Low-dose aspirin (81–100 mg/day) alone or in combination with phlebotomy and HU (depending on the cause and severity of disease) is practically recommended for all ET patients [96]. Therefore, evaluation of cellular, genetic, and hemostatic markers can be of high prognostic value in ET patients and contribute to the selection of effective treatment and improvement of patients considering that hemostatic abnormalities are the most frequent problems in ET patients.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

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