Published in final edited form as:

Leukemia. 2017 September ; 31(9): 1845–1854. doi:10.1038/leu.2017.150.

Assessing the Thrombotic Risk of Patients with Essential Thrombocythemia in the Genomic Era

Lorenzo Falchi¹, Hagop M. Kantarjian², and Srdan Verstovsek²

¹Division of Hematology/Oncology, Columbia University Medical Center, New York, NY

²Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston TX

Abstract

The molecular characterization of myeloproliferative neoplasms, including essential thrombocythemia (ET), has enabled deeper understanding of their pathogenesis. A driver lesion, namely, Janus kinase (*JAK*)2V617F, calreticulin (*CALR*) or myeloproliferative leukemia (*MPL*) gene mutation can be identified in the vast majority of patients. Each of these mutations is associated with distinct clinical features and may modulate the patients' clinical course, risk of complications, including vascular events, and survival. *JAK2V617F* appears to be a risk-modifying mutation and has been shown to increase the likelihood of thrombotic events in patients with ET across studies. As such, it has been included in prognostic models and its presence may influence treatment decisions. The association of *CALR* and *MPL* mutations with the incidence of vascular events has been less clear. Even more limited information is available on the contribution of additional non-driver lesions to the thrombotic risk. In this review we discuss the available evidence on the role of recurrent mutations in the risk of thrombotic complications in patients with ET and how these mutations weigh into modern prognostic scores.

Keywords

Myeloproliferative neoplasms; essential Thrombocythemia; JAK2; calreticulin; thrombosis

Introduction

Essential thrombocythemia (ET) is a Philadelphia-negative myeloproliferative neoplasm (MPN) characterized by clonal proliferation of the megakaryocytic lineage within the bone marrow and elevated platelet count in peripheral blood.¹ It is a rare disease, with an incidence rate of 0.2–2.5/100,000 people per year in western countries,^{2, 3} and it is associated with long overall survival. However, life expectancy of ET patients is shorter than

Corresponding Author: Srdan Verstovsek, Leukemia Department, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 428, Houston TX, 77030. Tel. +1(713)745-3429, Fax +1(713)745-0930, sverstov@mdanderson.org.

Conflict of Interest

The authors declare no conflict of interest.

Authorship Contributions

All authors conceived the research, collected, analyzed and interpreted the literature, wrote and approved the final version of the manuscript.

in the general population,⁴ largely due to the occurrence of thrombotic events. Consequently, the treatment of this disorder is aimed at reducing the risk of vascular complications.⁵

The overall risk of thrombosis associated with ET is 1-3% per patient-year.⁶ The rates of non-fatal arterial and venous events are estimated at 1.2% patient-years and 0.6% patients-years, respectively,^{6, 7} but can fluctuate substantially as a multitude of additional factors variably weigh into each individual's risk.⁸ Although age equal to, or greater than 60 years and prior history of thrombosis are firmly established risk factors for thrombosis in patients with MPN, including ET, several others have been proposed. In particular, the molecular characterization of MPN has enabled the definition of subgroups of patients exhibiting distinct clinical features and potentially, a different risk of thrombosis. In this review, we will discuss the potential role of driver and other recurrent somatic mutations in the modern-day thrombotic risk assessment of ET patients.

Molecular features of ET

Significant advances in understanding the pathobiology of MPN have been made over the last decade. In 2005 independent groups reported the discovery of a gain-of-function mutation of the Janus Kinase (*JAK*)2 gene in nearly all patients with polycythemia vera (PV) and 50–70% of those with ET or primary myelofibrosis (PMF).^{9–12} *JAK*2 is an intracellular non-receptor tyrosine kinase that plays an essential role in normal hematopoiesis. The most frequent mutation of *JAK*2 in patients with MPN is a substitution of valine with phenylalanine at codon 617 (that is, V617F), which leads to constitutive activation of the protein and its signaling pathway.^{9–12} *JAK*2 mutations involving exon 12 have been reported in <5% of patients and are usually not associated with ET.^{13–15} Another driver mutation found in MPN patients affects the myeloproliferative leukemia (*MPL*) gene, which encodes the thrombopoietin (TPO) receptor. Collectively, point mutations at exon 10 in codon 515 of the *MPL* sequence (the most frequent) are found in 3-15% of ET patients. These alterations affect the intracellular domain of the protein and cause ligand-independent signaling through *JAK*2.^{16–18} More recently, somatic mutations of the calreticulin (*CALR*) gene were discovered in 67-71% and 65-88% of patients with *JAK*2- and *MPL*-unmutated ET and PMF, respectively.^{19, 20} *CALR* is a highly conserved chaperone protein that plays an important role in cellular proliferation, differentiation, and apoptosis.²¹ Mutations occur at exon 9 and consist of either a 52-bp deletion (type 1 mutation), or a 5-bp insertion (type 2 mutation).^{19, 20} As a result of these alterations negatively charged amino acids are replaced with positively charged ones at the C-terminus of *CALR*,²⁰ causing loss of the endoplasmic reticulum retention sequence, binding of *CALR* to *MPL* associated with *JAK*2, and consequent overactivation of the *JAK*-*STAT* signaling pathway.^{22–26} Patients who are wild type for the driver mutations *JAK*2, *MPL* and *CALR* are commonly referred to as “triple-negative”. Some of them carry variant gain-of-function *MPL* or *JAK*2 mutations.^{27, 28} For the remaining patients a molecular marker has not been identified.

Other recurring non-driver mutations can be found in patients with MPN, but tend to be less frequent in those with ET (28-46% vs. 96% in PMF).^{29–31} These lesions involve ten-eleven-*(TET)*2, additional sex combs like (*ASXL*)1, enhancer of zeste homolog (*EZH*)2, isocitrate dehydrogenase 1 and 2 (*IDH*1/2), DNA methyltransferase (*DNMT*)3A, SH2B

adaptor protein (*SH2B*),³ Casitas B-cell lymphoma (*CBL*), Ikaros family zinc-finger protein (*IKZF1*), high-mobility group AT-hook (*HMGA*),² *TP53* and *NRAS/KRAS* (reviewed elsewhere³²). These mutations often accumulate in the same patient and can affect prognosis. For example *ASXL1* mutations can co-exist with *CALR* mutations,^{33, 34} and *DNMT3A* alterations can occur in concomitance with those of *JAK2*, *TET2* and *ASXL1*.³⁵ Some mutations (particularly *TET2*, *IDH1* and *2*, *DNMT3A*, *LNK*, *TP53* and *N/KRAS*) are believed to preferentially occur at later stages of the disease course and/or play a role in leukemic transformation. In a recent study 50 patients with either ET (N = 22) or PV (N = 28) were tested through next-generation sequencing using an 18-gene panel. The group of patients, whose disease progressed within 3 years, was enriched in individuals with more than one mutation (including *JAK2V617F*) and an increase in the mutated allele burden. A multivariable model for the risk of progression was not reported, likely due to the small number of patients studied.³⁶

Patient- and disease-related risk factors for thrombosis

An accurate medical history can elicit key information on the vascular risk of a patient presenting with newly diagnosed ET. Age ≥ 60 years and a history of vascular events are well-established risk factors for thrombosis in patients with MPN. The finding of either one assigns an individual to the high-risk category, whereas if none is present patients are considered at low risk.³⁷ Not unexpectedly, general cardiovascular risk factors (for example, tobacco use, hypertension, and diabetes) further increase the likelihood of experiencing a thrombotic episode.^{38–42} For example, younger patients who smoked had nearly 2-fold increased risk of thrombosis³⁸ and a shorter 10-year thrombosis-free survival compared to non-smokers.³⁹ Consequently, cardiovascular risk factors have recently been included as variables in prognostic scores for thrombosis in individuals with ET.³⁷

The presence of palpable splenomegaly has also been proposed as a risk factor for vascular events in patients with ET. In a large retrospective analysis (N = 1297) splenomegaly was independently associated with the occurrence of thrombotic events.⁴³ This finding, however, was not confirmed in a similar study (N = 560), perhaps due to differences in diagnostic criteria used and/or length of follow-up.⁴⁴

Finally, baseline and serial blood counts can provide additional important information on the thrombotic risk of ET patients. Indeed, leukocytosis was found to be correlated with the risk of vascular events and death in several retrospective studies.^{45–47} In a study of 891 patients with ET, arterial, but not venous, thrombosis was associated with a leukocyte count $> 11 \times 10^9/L$.³⁸ Thrombocytosis has also been studied, but found to have a weaker correlation.⁴⁸ Interestingly, some studies revealed that, while the association between blood counts at the time of diagnosis and future vascular events may be weak, leukocytosis or abnormal platelet counts during follow-up were more strongly associated with thrombohemorrhagic events.^{47, 49}

Driver mutations and thrombotic risk in patients with ET

JAK2V617F

Mutated JAK2 predisposes to thrombosis—Both a potentially pro-thrombotic clinical phenotype⁵⁰ and pro-thrombotic alterations in blood cells^{51, 52} have been described in *JAK2-mutated* ET patients. The pathogenesis of such alterations is complex and involves multiple players. Flow cytometry studies revealed abnormalities particularly of platelets (increased expression of CD62P, or P-selectin, CD63, and tissue factor)⁵² and leukocytes (increased expression of CD11b, transferrin and plasminogen activator and increased production of proteases), as well as increased formation platelet–neutrophil aggregates.⁵³ Moreover, an increased production of pro-inflammatory cytokines and various adhesion molecules has been noted in patients with MPN. All these abnormalities were more prominent in patients with mutated allele burden >50%.⁵⁴

Recent studies on heterozygous *JAK2*-positive mouse knock-in models have shed light on the mechanism of *JAK2* mutation-mediated thrombosis. In those animals platelets showed greater tendency to form thrombi, increased reactivity to collagen-related peptide and thrombin, increased aggregation, and increased spreading, regardless of the platelet number (Figure 1). Moreover, gene expression profiles revealed upregulation of genes with a known role in thrombosis.⁵⁵ On the other hand, the presence of *JAK2* mutant liver endothelial cells in patients with Budd-Chiari syndrome suggests that the role of the mutation in promoting thrombosis extends beyond hematopoietic cells (Figure 1).⁵⁶ Despite these important laboratory observations, the relationship between *JAK2* mutation and the increased risk of thrombosis has been more difficult to ascertain in the clinic.

JAK2 mutation is a risk factor for thrombosis—Multiple studies indicated that *JAK2* mutation is associated with a distinct clinical phenotype in MPN patients, that is, older age, higher hemoglobin and hematocrit, lower platelet counts, more frequent need for, and greater sensitivity to cytoreductive treatment, and greater tendency to evolve into PV.^{50, 57} In fact, the *JAK2* mutation may contribute to the disease phenotype independently of the World Health Organisation (WHO)-defined disease classification, as suggested by the fact that *JAK2*-positive ET patients have intermediate clinical features between *JAK2*-wild-type ET and PV (virtually always *JAK2*-positive).^{58, 59} A large number of studies analyzed the effect of *JAK2* mutation on the thrombotic risk of ET patients (Table 1). Most analyses are retrospective in nature and, as such, contain a number of inherent biases. Variables with potential impact on the thrombotic risk that may not have been adequately accounted for include the mutated *JAK2* gene dosage effect, dynamic changes in *JAK2* allele burden, the presence of concomitant non-driver mutations, and the heterogeneous use of cytoreductive and/or antiplatelet therapy, among others.

In a study of very young ET patients, the presence of a *JAK2* mutation did not significantly alter the baseline thrombotic risk.⁶⁰ Other series in adults also showed that the presence of *JAK2* mutation did not have a significant impact on the risk of thrombosis or lost its predictive value when other variables were controlled for in the analysis.^{61–66} A glaring common denominator of all these studies is the small sample size, which weakens the strength of conclusions.

Most studies, on the other hand, suggest a positive correlation between *JAK2* mutation and risk of vascular events. Campbell and colleagues analyzed the clinical and laboratory features of patients enrolled in the prospective randomized primary thrombocythemia-1 trial (which compared hydroxyurea plus aspirin with anagrelide plus aspirin) according to the presence of *JAK2* mutation. Patients carrying the mutation had features that resembled those of PV, including a higher rate of thrombosis, and were more sensitive to hydroxyurea. Interestingly, while both arterial and venous thrombosis, both pre- and post-study enrollment, were more frequent in *JAK2*-positive patients, only the difference in rate of venous thrombosis prior to enrollment was statistically significant, suggesting that disease control may attenuate the thrombogenic potential of *JAK2V617F*.⁵⁰

Subsequent meta-analyses and systematic reviews of the literature showed that, among patients with ET, the risk of thrombosis is about twice as high in those with the *JAK2* mutation compared to those without (odds ratio (OR) range 1.83-1.92). The risk was increased for both arterial (OR range 1.68-2.59) and venous thrombosis (OR range 2.09-2.5).⁶⁷⁻⁶⁹ In the meta-analysis by Dahabreh and colleagues,⁷⁰ that examined 2,436 patients (1,375 of whom *JAK2*-mutated), and included blood counts in the meta-regression, the authors found that leukocytosis might in part mediate the impact of *JAK2* mutation on thrombotic events. In addition, in line with results from the primary Thrombocythemia-1 cohort,⁵⁰ two meta-analyses revealed that the odds of thrombosis may be higher before vs. after ET diagnosis, confirming that control of the disease might reduce the risk of thrombotic events regardless of the *JAK2* mutation status.^{67, 70} Due to an intrinsic lack of homogeneity and detail, these analyses did not clarify the contribution of *JAK2* allelic status (homozygous vs. heterozygous) or burden to the thrombotic risk.

Mutated *JAK2* gene dosage has an effect on thrombotic risk—Although rare in ET patients (2-4%), *JAK2V617F* homozygosity might confer an increased risk of thrombosis compared to a heterozygous or wild-type condition.⁸ Perhaps more importantly, a correlation was noted between the *JAK2* allele burden and a number of parameters, including older age, platelet and leukocyte counts, palpable splenomegaly, and risk of venous thrombosis.⁷¹ Another study showed that an allele burden of 20-25% or higher independently predicted the risk of arterial and venous thrombosis.⁷² Finally, among patients with WHO-defined ET or early PMF a high (>50%) *JAK2* allele burden was found to positively correlate with the thrombotic risk regardless of the WHO diagnosis.⁷³

***JAK2* mutation has an impact on vascular events over time**—In some ET patients, the pro-thrombotic potential of the *JAK2* mutation may manifest itself over time, rather than early in the course of the disease. A study from Carobbio and colleagues⁵⁹ compared and contrasted the impact of the *JAK2* mutation in patients with ET (N = 867) and PV (N = 415). Among patients with wild-type ET, *JAK2*-mutated ET and PV, the rate of arterial and venous thrombosis were similar in the first 5 years, but with additional follow-up the risk of vascular events in patients with *JAK2*-mutated ET and PV became similar, and higher than in patients with wild-type ET. Another analysis of the dynamics of the *JAK2V617F* burden suggested that patients with persistently high or unstable mutation load over time had a trend toward a higher incidence of thrombosis.⁷⁴ Finally, in a retrospective study of 143

individuals with ET and previous major thrombosis 30% experienced recurrence during follow-up. Homozygosity for *JAK2V617F* independently predicted an increased risk of recurrent thrombosis (hazard ratio 6.15), while the risk was similar between heterozygous and wild-type patients.⁷⁵

JAK2 mutation is associated with thrombosis at unusual sites—Venous thrombosis in patients with MPN may occur at unusual sites, such as the splanchnic and cerebral venous systems. However, the prevalence of *JAK2* mutation, with or without “occult” MPN, in patients presenting with various types of venous thrombosis varies substantially in retrospective case series, potentially due to the significant heterogeneity of the populations studied.⁷⁶

For example, in a survey of 24 studies (N = 3,123) *JAK2V617F* was found on average in 32.7% of patients with splanchnic vein thrombosis (SVT) (OR 53.98). The estimated attributable risk of having a SVT for patients with a *JAK2* mutation was 12.74%. In contrast, the mean prevalence of *JAK2* mutation in patients with venous thrombosis at other sites ranged from 0.88% to 2.57%.⁷⁷ Other studies confirmed the low (0.9%) frequency of *JAK2V617F* in patients presenting with non-SVT and without history or signs of MPN, and showed that the median allele burden was low at presentation (around 5%).⁷⁸

Another meta-analysis showed that the prevalence of MPN and *JAK2V617F* in patients presenting with either Budd-Chiari syndrome (N = 1,062) or non-cirrhotic portal vein thrombosis (N = 855) were 40.9% and 41.1%, respectively, in the former and 31.5% and 27.7%, respectively, in the latter. The differences between the two patient groups were statistically significant. MPN was “occult” (that is, absence of symptoms or signs of disease and normal blood counts) in 17.1% and 15.4% of patients with Budd-Chiari syndrome and portal vein thrombosis, respectively.⁷⁹ Although other smaller studies suggested a lower prevalence of *JAK2* mutation (<10%) in patients presenting with isolated SVT,⁸⁰ the current clinical practice contemplates screening for *JAK2V617F* and underlying MPN in patients presenting with isolated SVT of no obvious etiology. The value of such screening in patients with non-SVT remains to be defined. In addition, due to a high recurrence rate, SVT in patients with MPN is generally treated with long-term anticoagulation therapy.

CALR mutations

Studies of platelet activation markers revealed that, compared to *JAK2*-mutated patients, those harboring the *CALR* mutation display decreased platelet-neutrophil complexes and CD14+ expression on neutrophils, and longer platelet function analyzer-100 with collagen and epinephrine cartridge closure time. Leukocyte activation, however, was not different in *CALR*-mutated vs. triple-negative patients, suggesting that the absence of *JAK2* mutation, rather than to the presence of *CALR* mutation, better explains the lesser pro-thrombotic tendency of these patients.⁸¹

Phenotypically, *CALR*-mutated ET patients are more frequently younger males with lower hemoglobin and white blood cell count, higher platelet count, greater marrow megakaryocytic predominance and lower rates of polycythemic transformation, compared to those with *JAK2* mutation.^{19, 20, 82–84}

A synopsis of studies comparing the risk of thrombosis among patients with molecularly annotated ET is presented in Table 2. In an analysis of 576 ET patients, 15.5% of whom had *CALR* mutations, the 10-year cumulative incidence of thrombosis was 14.5% and 5% for *JAK2*-positive and *CALR*-positive patients, respectively. The thrombotic risk of *CALR*-mutated patients was similar to that of triple-negative ones and lower than *JAK2*- and *MPL*-mutated patients.⁸² A second study (N = 745), confirmed these findings, as well as the validity of the International Prognostic Score for Thrombosis in Essential Thrombocythemia (IPSET-thrombosis) model when *CALR* mutations were taken into consideration (see below).⁸¹ In a third series of 217 young patients with WHO-defined ET or early PMF, the lower incidence of thrombosis in patients with *CALR* mutation vs. *JAK2* mutation persisted at 15 years (21.7% vs. 9.1%, respectively $P = 0.04$).⁷³

The type of *CALR* mutation may also influence the risk of thrombosis in individuals with ET. Among 99 patients with *CALR* mutations, 3 of 38 with type 2 vs. 10 of 55 with type 1 mutations had a thrombotic event, although this difference did not reach statistical significance ($P = 0.160$).⁸⁵ Similarly, in large international retrospective data set (N = 1027), the thrombosis-free survival of type 1 and type 2-mutated patients were not different ($P = 0.18$).⁸⁶ A third study showed that patients with type 1-like mutations had an “intermediate” phenotype between those with type 2 mutations and those with *JAK2* mutation. In addition, patients with type 1 mutations tended to have a PMF-like phenotype, whereas those with type 2-like mutations had an “ET-proper” phenotype with higher platelet counts and a more indolent clinical course.⁸⁷ We recommend caution in interpreting the results of these studies given the small number of thrombotic events recorded in each subgroup.

Of note, whereas the prevalence of *JAK2* mutation is high (albeit variable across studies) in patients with SVT, *CALR* mutations are very rarely found in this setting (0-2%). Nonetheless, the population of patients with SVT, who are found to have an MPN and do not harbor *JAK2* mutations may be enriched for these alterations (34.6%) and, thus, be candidate for *CALR* mutation analysis.⁸⁸

***MPL* mutations**

Despite the low frequency, *MPL* mutations have also been associated with distinct clinical features. These individuals resemble *JAK*-mutated patients for age and gender distribution, and *CALR*-mutated ones for blood counts. Marrow erythroid and overall cellularity, however, are uniquely low.⁸⁹

Data on the thrombotic risk associated with *MPL* mutations are scant, primarily due to low overall frequency (Table 2). The 5-year cumulative incidence of thrombosis is estimated to be around 9%.⁹⁰ In the prospective primary thrombocythemia-1 cohort, among 776 patients with ET 32 (4.1%) had *MPL* mutations and these were not predictive of thrombosis, major hemorrhage, myelofibrotic transformation or survival in controlled analyses.⁹¹ In a larger series (N = 994) 3% of patients had the *MPL* mutation, which co-existed with *JAK2* in eight cases. In this study the *MPL* mutation was associated with higher risk of thrombosis only when compared to *JAK2* mutation or wild-type *MPL* (presumably mostly *CALR*-mutated).⁸⁹

“Triple-negative” state

Triple-negative patients constitute a small group, for which available clinical information is limited (Table 2). In an analysis of 300 cases (53% *JAK2*-, 32% *CALR*-, 3% *MPL*-mutated, and 12% triple-negative) 35% experienced arterial (n = 75) or venous (n = 43) thrombosis. In univariate analysis, triple-negative state and *CALR* mutation conferred a lower risk of thrombosis compared to *JAK2* mutation. The triple-negative state remained significant when age and thrombosis history were controlled for.⁹² Another study (N = 217) confirmed the low risk of thrombosis in triple-negative patients, with no events recorded among 28 (13%) patients after a follow-up of 10.2 years.⁷³

Interactions between mutations

The risk of thrombosis may be modulated by the differential combination of driver and non-driver mutations but this hypothesis has been harder to demonstrate, given the exiguous number of such patients. Although the presence of additional mutations may modulate disease course and progression to acute leukemia, limited data exist on how this impacts the thrombotic risk.⁹³ For example, in a cohort of 167 ET patients *JAK2* and *CALR* mutations coexisted in 7 (4.2%) cases. The *JAK2*+/ *CALR*- group appeared to have the highest incidence of thrombosis, but the difference was only statically significant in comparison with the *JAK2*-/ *CALR*- group.⁹⁴ In a study of 190 ET patients, 8 had concomitant *CALR* and *ASXL1* mutation, and seemed to have more pronounced anemia, but no data on the thrombotic risk were reported.⁹⁵ In another experience, *ASXL1* mutations (detected in 8.4% of ET patients, and co-existent with *JAK2* mutations in two-thirds of them) appeared to confer a statistically insignificant increase in the risk of arterial, but not venous thrombosis.⁹⁶

In an elegant study of 24 patients with MPN, hematopoietic stem cells and progenitor cells were isolated and subjected to genotyping or next generation sequencing. Patients who acquired the *JAK2* mutation before the *TET2* mutation were younger, more likely to present with PV (rather than ET), had greater percentage of megakaryocytic and erythroid progenitors, exhibited a higher risk of arterial and venous thrombosis, and their progenitor myeloid cells exhibited greater sensitivity to ruxolitinib *in vitro* compared with those who had acquired a *TET2* mutation first. In fact, in these cells, *JAK2*V617F-mediated up-regulation of genes associated with cell proliferation was prevented.⁹⁷

Current thrombotic risk assessment in patients with ET

Efforts have been made to improve the current dichotomous risk stratification of ET patients based on age and thrombosis history, particularly in light of data suggesting a pro-thrombotic role of *JAK2* mutations. The IPSET-thrombosis incorporated cardiovascular risk factors and *JAK2* mutation in the model and identified patients with low, intermediate or high thrombosis risk (1.03%, 2.35%, and 3.56% patients/year, respectively) (Figure 2).⁹⁸ An external validation study showed that only intermediate- and high-risk patients derived significant clinical benefit from cytoreductive therapy.⁹⁹ Unlike *JAK2*V617F, *CALR* mutations tend to segregate with other low-risk factors in this model and do not appear to modify a patient's risk.¹⁰⁰ We propose that the IPSET-thrombosis score be followed to

inform decisions on the use of antiplatelet and/or cytoreductive therapy in ET patients in routine clinical practice.

The IPSET-thrombosis score was recently revised based on new observations. For conventionally defined high-risk patients, *JAK2* mutations appeared to have an added prognostic value only in patients >60 years without a history of thrombosis. On the other hand, for conventionally defined low-risk patients the thrombotic risk was very low if *JAK2* was unmutated, and even lower in the absence of cardiovascular risk factors (1.05% and 0.44% patient-years, respectively). Patients previously adjudicated low-risk were divided into low risk and very low risk, based on the presence or absence, respectively, of *JAK2* mutations. The revised IPSET-thrombosis, therefore, identified four categories: 1) high risk (thrombosis history or age > 60 years with *JAK2* mutation); 2) intermediate risk (no thrombosis history, age > 60 years and *JAK2*-unmutated); 3) low risk (no thrombosis history, age < 60 years and *JAK2*-mutated); and 4) very low risk (no thrombosis history, age < 60 years and *JAK2*-unmutated) (Figure 2).¹⁰¹ The revised IPSET-thrombosis was also externally validated in a cohort of 585 ET patients, where only *JAK2* mutations and a history of thrombosis were independently predictive of thrombotic events.¹⁰² No data are available yet on the role of other mutations in the setting of the revised IPSET-thrombosis score.

Concluding remarks

The molecular characterization of MPN is among the most comprehensive in hematologic malignancies, as a recognized driver lesion can be identified in nearly all cases and studies have begun to unravel the clonal architecture of these neoplasms. However, establishing clinical-molecular correlations in ET is a difficult exercise given the long life expectancy of patients, the small number of outcome-defining events, and the rarity of some mutations.

Nonetheless, some generalizations relevant for clinical practice can be made. First, among all (driver or non-driver) mutations, only *JAK2V617F* was found to modify the thrombotic risk in ET patients consistently across studies. This is likely because, unlike *CALR* mutations, *JAK2V617F* imparts a disease phenotype closer to that of PV, and thus more prone to thrombosis. In other words, the low-risk profile of *CALR*-positive ET may result from the lack of *JAK2* mutation-associated high-risk features, rather than an effect of the *CALR* mutation *per se*.

Secondly, *CALR* mutations tend to segregate closely with other established low-risk factors, and do not appear to have an independent prognostic significance.^{84, 100} Although longer follow-up and more detailed studies will be necessary, in our estimation this mutation is unlikely to be included in prognostic models in the future.

Third, the contribution of individual mutations to ET patients' thrombotic risk may inform specific clinical decision-making. For instance, given their extremely low thrombotic potential, patients who are classified as very low risk according to the revised IPSET-thrombosis model, are unlikely to benefit from primary prophylaxis with low-dose aspirin.¹⁰¹ Whether management of antiplatelet therapy should be diversified in patients with mutations other than *JAK2V617F*, is less clear. Among 271 patients with *CALR* mutations,

not only low-dose aspirin did not decrease the risk of thrombosis, but it was also associated with higher incidence of bleeding. Notably, the opposite was true for *JAK2*-mutated patients. The results of this study are difficult to interpret because: i) the proportion of patients treated with antiplatelet therapy in *CALR*- vs *JAK2*-mutated patients is unknown, ii) a multivariable model for risk of bleeding was not reported, and iii) cytoreduction was started much earlier in *CALR*- vs. *JAK2*-mutated patients mostly to control the higher platelet count.¹⁰³

In conclusion, testing for somatic mutations has made its way into the thrombotic risk assessment of patients with ET. The presence of *JAK2V617F* independently increases such risk and may inform management at the time of presentation. On the other hand, *CALR* mutations typically cluster in patients who are low risk based on other clinical and laboratory features, and may not affect the decision-making process. Additional data on larger numbers of MPL-mutated or triple-negative patients will be necessary to determine their role, if any, in modulating the vascular risk of ET patients. At the present time, testing for additional non-driver mutations does not appear to have obvious clinical implications based on limited available evidence, and is not warranted in routine clinical practice.

Acknowledgments

This work was supported in part by a Cancer Center Support Grant to MD Anderson Cancer Center (P30 CA016672) from the National Cancer Institute.

References

1. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016 May 19; 127(20):2391–2405. [PubMed: 27069254]
2. Titmarsh GJ, Duncombe AS, McMullin MF, O'Rourke M, Mesa R, De Vocht F, et al. How common are myeloproliferative neoplasms? A systematic review and meta-analysis. *Am J Hematol*. 2014 Jun; 89(6):581–587. [PubMed: 24971434]
3. Moulard O, Mehta J, Fryzek J, Olivares R, Iqbal U, Mesa RA. Epidemiology of myelofibrosis, essential thrombocythemia, and polycythemia vera in the European Union. *Eur J Haematol*. 2014 Apr; 92(4):289–297. [PubMed: 24372927]
4. Wolanskyj AP, Schwager SM, McClure RF, Larson DR, Tefferi A. Essential thrombocythemia beyond the first decade: life expectancy, long-term complication rates, and prognostic factors. *Mayo Clin Proc*. 2006 Feb; 81(2):159–166. [PubMed: 16471068]
5. Michiels JJ, Berneman Z, Schroyens W, Koudstaal PJ, Lindemans J, Neumann HA, et al. Platelet-mediated erythromelalgic, cerebral, ocular and coronary microvascular ischemic and thrombotic manifestations in patients with essential thrombocythemia and polycythemia vera: a distinct aspirin-responsive and coumadin-resistant arterial thrombophilia. *Platelets*. 2006 Dec; 17(8):528–544. [PubMed: 17127481]
6. Casini A, Fontana P, Lecompte TP. Thrombotic complications of myeloproliferative neoplasms: risk assessment and risk-guided management. *J Thromb Haemost*. 2013 Jul; 11(7):1215–1227. [PubMed: 23601811]
7. Passamonti F, Rumi E, Pungolino E, Malabarba L, Bertazzoni P, Valentini M, et al. Life expectancy and prognostic factors for survival in patients with polycythemia vera and essential thrombocythemia. *Am J Med*. 2004 Nov 15; 117(10):755–761. [PubMed: 15541325]
8. Vannucchi AM, Antonioli E, Guglielmelli P, Rambaldi A, Barosi G, Marchioli R, et al. Clinical profile of homozygous *JAK2* 617V>F mutation in patients with polycythemia vera or essential thrombocythemia. *Blood*. 2007 Aug 1; 110(3):840–846. [PubMed: 17379742]

9. Kralovics R, Passamonti F, Buser A, Teo S, Tiedt R, Passweg J, et al. A Gain-of-Function Mutation of JAK2 in Myeloproliferative Disorders. *The New England Journal of Medicine*. 2005; 352:1779–1790. [PubMed: 15858187]
10. Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell*. 2005 Apr; 7(4):387–397. [PubMed: 15837627]
11. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *The Lancet*. 2005; 365(9464): 1054–1061.
12. James C, Ugo V, Couedic J-PL, Staerk Judith, Delhommeau FO, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature*. 2005; 434:1144–1148. [PubMed: 15793561]
13. Scott LM, Tong W, Levine RL, Scott MA, Beer PA, Stratton MR, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. *N Engl J Med*. 2007 Feb 1; 356(5):459–468. [PubMed: 17267906]
14. Pardanani A, Lasho TL, Finke C, Hanson CA, Tefferi A. Prevalence and clinicopathologic correlates of JAK2 exon 12 mutations in JAK2V617F-negative polycythemia vera. *Leukemia*. 2007 Sep; 21(9):1960–1963. [PubMed: 17597810]
15. Passamonti F, Elena C, Schnittger S, Skoda RC, Green AR, Girodon F, et al. Molecular and clinical features of the myeloproliferative neoplasm associated with JAK2 exon 12 mutations. *Blood*. 2011 Mar 10; 117(10):2813–2816. [PubMed: 21224469]
16. Abe M, Suzuki K, Inagaki O, Sassa S, Shikama H. A novel MPL point mutation resulting in thrombopoietin-independent activation. *Leukemia*. 2002 Aug; 16(8):1500–1506. [PubMed: 12145691]
17. Pikman Y, Lee BH, Mercher T, McDowell E, Ebert BL, Gozo M, et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. *PLoS Med*. 2006 Jul. 3(7):e270. [PubMed: 16834459]
18. Pardanani AD, Levine RL, Lasho T, Pikman Y, Mesa RA, Wadleigh M, et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood*. 2006 Nov 15; 108(10):3472–3476. [PubMed: 16868251]
19. Nangalia J, Massie CE, Baxter EJ, Nice FL, Gundem G, Wedge DC, et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. *N Engl J Med*. 2013 Dec 19; 369(25):2391–2405. [PubMed: 24325359]
20. Klampfl T, Gisslinger H, Harutyunyan AS, Nivarthi H, Rumi E, Milosevic JD, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. *N Engl J Med*. 2013 Dec 19; 369(25): 2379–2390. [PubMed: 24325356]
21. Wang WA, Groenendyk J, Michalak M. Calreticulin signaling in health and disease. *Int J Biochem Cell Biol*. 2012 Jun; 44(6):842–846. [PubMed: 22373697]
22. Balligand T, Achouri Y, Pecquet C, Chachoua I, Nivarthi H, Marty C, et al. Pathologic activation of thrombopoietin receptor and JAK2 STAT5 pathway by frameshift mutants of mouse calreticulin. *Leukemia*. 2016 Aug; 30(8):1775–1778. [PubMed: 26987905]
23. Araki M, Yang Y, Masubuchi N, Hironaka Y, Takei H, Morishita S, et al. Activation of the thrombopoietin receptor by mutant calreticulin in CALR-mutant myeloproliferative neoplasms. *Blood*. 2016 Mar 10; 127(10):1307–1316. [PubMed: 26817954]
24. Chachoua I, Pecquet C, El-Khoury M, Nivarthi H, Albu RI, Marty C, et al. Thrombopoietin receptor activation by myeloproliferative neoplasm associated calreticulin mutants. *Blood*. 2016 Mar 10; 127(10):1325–1335. [PubMed: 26668133]
25. Marty C, Pecquet C, Nivarthi H, El-Khoury M, Chachoua I, Tulliez M, et al. Calreticulin mutants in mice induce an MPL-dependent thrombocytosis with frequent progression to myelofibrosis. *Blood*. 2016 Mar 10; 127(10):1317–1324. [PubMed: 26608331]
26. Elf S, Abdelfattah NS, Chen E, Perales-Paton J, Rosen EA, Ko A, et al. Mutant Calreticulin Requires Both Its Mutant C-terminus and the Thrombopoietin Receptor for Oncogenic Transformation. *Cancer Discov*. 2016 Apr; 6(4):368–381. [PubMed: 26951227]

27. Milosevic Feenstra JD, Nivarthi H, Gisslinger H, Leroy E, Rumi E, Chachoua I, et al. Whole-exome sequencing identifies novel MPL and JAK2 mutations in triple-negative myeloproliferative neoplasms. *Blood*. 2016 Jan 21; 127(3):325–332. [PubMed: 26423830]
28. Cabagnols X, Favale F, Pasquier F, Messaoudi K, Defour JP, Ianotto JC, et al. Presence of atypical thrombopoietin receptor (MPL) mutations in triple-negative essential thrombocythemia patients. *Blood*. 2016 Jan 21; 127(3):333–342. [PubMed: 26450985]
29. Li B, Zhang L, Bai J, Xu Z, Qin T, Zhang Y, et al. Non-Driver Mutations Profile Identified By a 206-Gene NGS Panel in Patients with Primary Myelofibrosis and Post-Polycythaemic/Essential Thromocythaemia Myelofibrosis in a Single Center from China. *Blood*. 2016; 128(22):1942.
30. Senín A, Fernández C, Bellosillo B, Camacho L, Longaron R, Angona A, et al. Role of Non-Driver Mutations and JAK2V617F Allele Burden in Myelofibrotic and Acute Myeloid Transformation of Patients with Polycythemia Vera and Essential Thrombocythemia. *Blood*. 2016; 128(22):1952.
31. Tefferi A, Lasho TL, Guglielmelli P, Finke CM, Rotunno G, Elala Y, et al. Targeted deep sequencing in polycythemia vera and essential thrombocythemia. *Blood Advances*. 2016; 1(1):21–30. [PubMed: 29296692]
32. Saeidi K. Myeloproliferative neoplasms: Current molecular biology and genetics. *Crit Rev Oncol Hematol*. 2016 Feb;98:375–389. [PubMed: 26697989]
33. Vannucchi AM, Lasho TL, Guglielmelli P, Biamonte F, Pardanani A, Pereira A, et al. Mutations and prognosis in primary myelofibrosis. *Leukemia*. 2013 Sep; 27(9):1861–1869. [PubMed: 23619563]
34. Tefferi A, Guglielmelli P, Lasho TL, Rotunno G, Finke C, Mannarelli C, et al. CALR and ASXL1 mutations-based molecular prognostication in primary myelofibrosis: an international study of 570 patients. *Leukemia*. 2014 Jul; 28(7):1494–1500. [PubMed: 24496303]
35. Abdel-Wahab O. Genetics of the myeloproliferative neoplasms. *Curr Opin Hematol*. 2011 Mar; 18(2):117–123. [PubMed: 21307773]
36. Luque Paz D, Chauveau A, Boyer F, Buors C, Samaison L, Cottin L, et al. Sequential analysis of 18 genes in polycythemia vera and essential thrombocythemia reveals an association between mutational status and clinical outcome. *Genes Chromosomes Cancer*. 2017 May; 56(5):354–362. [PubMed: 27997717]
37. Barbui T, Barosi G, Birgegard G, Cervantes F, Finazzi G, Griesshammer M, et al. Philadelphia-negative classical myeloproliferative neoplasms: critical concepts and management recommendations from European LeukemiaNet. *J Clin Oncol*. 2011 Feb 20; 29(6):761–770. [PubMed: 21205761]
38. Carobbio A, Thiele J, Passamonti F, Rumi E, Ruggeri M, Rodeghiero F, et al. Risk factors for arterial and venous thrombosis in WHO-defined essential thrombocythemia: an international study of 891 patients. *Blood*. 2011 Jun 2; 117(22):5857–5859. [PubMed: 21490340]
39. Alvarez-Larran A, Cervantes F, Bellosillo B, Giral M, Julia A, Hernandez-Boluda JC, et al. Essential thrombocythemia in young individuals: frequency and risk factors for vascular events and evolution to myelofibrosis in 126 patients. *Leukemia*. 2007 Jun; 21(6):1218–1223. [PubMed: 17519959]
40. Alvarez-Larran A, Cervantes F, Pereira A, Arellano-Rodrigo E, Perez-Andreu V, Hernandez-Boluda JC, et al. Observation versus antiplatelet therapy as primary prophylaxis for thrombosis in low-risk essential thrombocythemia. *Blood*. 2010 Aug 26; 116(8):1205–1210. quiz 1387. [PubMed: 20508163]
41. Jantunen R, Juvonen E, Ikkala E, Oksanen K, Anttila P, Ruutu T. The predictive value of vascular risk factors and gender for the development of thrombotic complications in essential thrombocythemia. *Ann Hematol*. 2001 Feb; 80(2):74–78. [PubMed: 11261328]
42. Besses C, Cervantes F, Pereira A, Florensa L, Sole F, Hernandez-Boluda JC, et al. Major vascular complications in essential thrombocythemia: a study of the predictive factors in a series of 148 patients. *Leukemia*. 1999 Feb; 13(2):150–154. [PubMed: 10025886]
43. Andriani A, Latagliata R, Anaclerio B, Spadea A, Rago A, Di Veroli A, et al. Spleen enlargement is a risk factor for thrombosis in essential thrombocythemia: Evaluation on 1,297 patients. *Am J Hematol*. 2016 Mar; 91(3):318–321. [PubMed: 26748894]

44. Haider M, Gangat N, Hanson C, Tefferi A. Splenomegaly and thrombosis risk in essential thrombocythemia: the mayo clinic experience. *Am J Hematol*. 2016 May; 91(5):E296–297.
45. Carobbio A, Finazzi G, Antonioli E, Guglielmelli P, Vannucchi AM, Delaini F, et al. Thrombocytosis and leukocytosis interaction in vascular complications of essential thrombocythemia. *Blood*. 2008 Oct 15; 112(8):3135–3137. [PubMed: 18587010]
46. Palandri F, Polverelli N, Catani L, Ottaviani E, Baccarani M, Vianelli N. Impact of leukocytosis on thrombotic risk and survival in 532 patients with essential thrombocythemia: a retrospective study. *Ann Hematol*. 2011 Aug; 90(8):933–938. [PubMed: 21287350]
47. Campbell PJ, MacLean C, Beer PA, Buck G, Wheatley K, Kiladjian JJ, et al. Correlation of blood counts with vascular complications in essential thrombocythemia: analysis of the prospective PT1 cohort. *Blood*. 2012 Aug 16; 120(7):1409–1411. [PubMed: 22709688]
48. Falchi L, Bose P, Newberry KJ, Verstovsek S. Approach to patients with essential thrombocythaemia and very high platelet counts: what is the evidence for treatment? *Br J Haematol*. 2017 Feb; 176(3):352–364. [PubMed: 27984634]
49. Lim Y, Lee JO, Kim SH, Kim JW, Kim YJ, Lee KW, et al. Prediction of thrombotic and hemorrhagic events during polycythemia vera or essential thrombocythemia based on leukocyte burden. *Thromb Res*. 2015 May; 135(5):846–851. [PubMed: 25743883]
50. Campbell PJ, Scott LM, Buck G, Wheatley K, East CL, Marsden JT, et al. Definition of subtypes of essential thrombocythaemia and relation to polycythemia vera based on JAK2 V617F mutation status: a prospective study. *Lancet*. 2005 Dec 3; 366(9501):1945–1953. [PubMed: 16325696]
51. Arellano-Rodrigo E, Alvarez-Larran A, Reverter JC, Villamor N, Colomer D, Cervantes F. Increased platelet and leukocyte activation as contributing mechanisms for thrombosis in essential thrombocythemia and correlation with the JAK2 mutational status. *Haematologica*. 2006 Feb; 91(2):169–175. [PubMed: 16461300]
52. Falanga A, Marchetti M, Vignoli A, Balducci D, Russo L, Guerini V, et al. V617F JAK-2 mutation in patients with essential thrombocythemia: relation to platelet, granulocyte, and plasma hemostatic and inflammatory molecules. *Exp Hematol*. 2007 May; 35(5):702–711. [PubMed: 17577920]
53. Falanga A, Marchetti M, Vignoli A, Balducci D, Barbui T. Leukocyte-platelet interaction in patients with essential thrombocythemia and polycythemia vera. *Exp Hematol*. 2005 May; 33(5):523–530. [PubMed: 15850829]
54. Coucelo M, Caetano G, Sevivas T, Almeida Santos S, Fidalgo T, Bento C, et al. JAK2V617F allele burden is associated with thrombotic mechanisms activation in polycythemia vera and essential thrombocythemia patients. *Int J Hematol*. 2014 Jan; 99(1):32–40. [PubMed: 24277659]
55. Hobbs CM, Manning H, Bennett C, Vasquez L, Severin S, Brain L, et al. JAK2V617F leads to intrinsic changes in platelet formation and reactivity in a knock-in mouse model of essential thrombocythemia. *Blood*. 2013 Nov 28; 122(23):3787–3797. [PubMed: 24085768]
56. Sozer S, Fiel MI, Schiano T, Xu M, Mascarenhas J, Hoffman R. The presence of JAK2V617F mutation in the liver endothelial cells of patients with Budd-Chiari syndrome. *Blood*. 2009 May 21; 113(21):5246–5249. [PubMed: 19293426]
57. Tefferi A, Guglielmelli P, Larson DR, Finke C, Wassie EA, Pieri L, et al. Long-term survival and blast transformation in molecularly annotated essential thrombocythemia, polycythemia vera, and myelofibrosis. *Blood*. 2014 Oct 16; 124(16):2507–2513. quiz 2615. [PubMed: 25037629]
58. Finazzi G, Rambaldi A, Guerini V, Carobbio A, Barbui T. Risk of thrombosis in patients with essential thrombocythemia and polycythemia vera according to JAK2 V617F mutation status. *Haematologica*. 2007 Jan; 92(1):135–136. [PubMed: 17229651]
59. Carobbio A, Finazzi G, Antonioli E, Guglielmelli P, Vannucchi AM, Dellacasa CM, et al. JAK2V617F allele burden and thrombosis: a direct comparison in essential thrombocythemia and polycythemia vera. *Exp Hematol*. 2009 Sep; 37(9):1016–1021. [PubMed: 19559071]
60. Giona F, Teofili L, Moleti ML, Martini M, Palumbo G, Amendola A, et al. Thrombocythemia and polycythemia in patients younger than 20 years at diagnosis: clinical and biologic features, treatment, and long-term outcome. *Blood*. 2012 Mar 8; 119(10):2219–2227. [PubMed: 22262773]

61. Cho YU, Chi HS, Lee EH, Jang S, Park CJ, Seo EJ. Comparison of clinicopathologic findings according to JAK2 V617F mutation in patients with essential thrombocythemia. *Int J Hematol.* 2009 Jan; 89(1):39–44. [PubMed: 19093167]
62. Wolanskyj AP, Lasho TL, Schwager SM, McClure RF, Wadleigh M, Lee SJ, et al. JAK2 mutation in essential thrombocythaemia: clinical associations and long-term prognostic relevance. *Br J Haematol.* 2005 Oct; 131(2):208–213. [PubMed: 16197451]
63. Antonioli E, Guglielmelli P, Pancrazzi A, Bogani C, Verrucci M, Ponziani V, et al. Clinical implications of the JAK2 V617F mutation in essential thrombocythemia. *Leukemia.* 2005 Oct; 19(10):1847–1849. [PubMed: 16079890]
64. Palandri F, Catani L, Testoni N, Ottaviani E, Polverelli N, Fiacchini M, et al. Long-term follow-up of 386 consecutive patients with essential thrombocythemia: safety of cytoreductive therapy. *Am J Hematol.* 2009 Apr; 84(4):215–220. [PubMed: 19208420]
65. Cheung B, Radia D, Pantelidis P, Yadegarfar G, Harrison C. The presence of the JAK2 V617F mutation is associated with a higher haemoglobin and increased risk of thrombosis in essential thrombocythaemia. *Br J Haematol.* 2006 Jan; 132(2):244–245. [PubMed: 16398659]
66. Chim CS, Sim JP, Chan CC, Kho BC, Chan JC, Wong LG, et al. Impact of JAK2V617F mutation on thrombosis and myeloid transformation in essential thrombocythemia: a multivariate analysis by Cox regression in 141 patients. *Hematology.* 2010 Aug; 15(4):187–192. [PubMed: 20670476]
67. Qin Y, Wang X, Zhao C, Wang C, Yang Y. The impact of JAK2V617F mutation on different types of thrombosis risk in patients with essential thrombocythemia: a meta-analysis. *Int J Hematol.* 2015 Aug; 102(2):170–180. [PubMed: 25997869]
68. Ziakas PD. Effect of JAK2 V617F on thrombotic risk in patients with essential thrombocythemia: measuring the uncertain. *Haematologica.* 2008 Sep; 93(9):1412–1414. [PubMed: 18641024]
69. Lussana F, Dentali F, Abbate R, d'Aloja E, D'Angelo A, De Stefano V, et al. Screening for thrombophilia and antithrombotic prophylaxis in pregnancy: Guidelines of the Italian Society for Haemostasis and Thrombosis (SISST). *Thromb Res.* 2009 Nov; 124(5):e19–25. [PubMed: 19671474]
70. Dahabreh IJ, Zoi K, Giannouli S, Zoi C, Loukopoulos D, Voulgarelis M. Is JAK2 V617F mutation more than a diagnostic index? A meta-analysis of clinical outcomes in essential thrombocythemia. *Leuk Res.* 2009 Jan; 33(1):67–73. [PubMed: 18632151]
71. Kittur J, Knudson RA, Lasho TL, Finke CM, Gangat N, Wolanskyj AP, et al. Clinical correlates of JAK2V617F allele burden in essential thrombocythemia. *Cancer.* 2007 Jun 1; 109(11):2279–2284. [PubMed: 17440984]
72. Borowczyk M, Wojtaszewska M, Lewandowski K, Gil L, Lewandowska M, Lehmann-Kopydlowska A, et al. The JAK2 V617F mutational status and allele burden may be related with the risk of venous thromboembolic events in patients with Philadelphia-negative myeloproliferative neoplasms. *Thromb Res.* 2015 Feb; 135(2):272–280. [PubMed: 25559461]
73. Palandri F, Latagliata R, Polverelli N, Tieghi A, Crugnola M, Martino B, et al. Mutations and long-term outcome of 217 young patients with essential thrombocythemia or early primary myelofibrosis. *Leukemia.* 2015 Jun; 29(6):1344–1349. [PubMed: 25801912]
74. Alvarez-Larran A, Bellosillo B, Pereira A, Kerguelen A, Hernandez-Boluda JC, Martinez-Aviles L, et al. JAK2V617F monitoring in polycythemia vera and essential thrombocythemia: clinical usefulness for predicting myelofibrotic transformation and thrombotic events. *Am J Hematol.* 2014 May; 89(5):517–523. [PubMed: 24458835]
75. De Stefano V, Za T, Rossi E, Vannucchi AM, Ruggeri M, Elli E, et al. Increased risk of recurrent thrombosis in patients with essential thrombocythemia carrying the homozygous JAK2 V617F mutation. *Ann Hematol.* 2010 Feb; 89(2):141–146. [PubMed: 19582452]
76. Sekhar M, McVinnie K, Burroughs AK. Splanchnic vein thrombosis in myeloproliferative neoplasms. *British journal of haematology.* 2013 Sep; 162(6):730–747. [PubMed: 23855810]
77. Dentali F, Squizzato A, Brivio L, Appio L, Campiotti L, Crowther M, et al. JAK2V617F mutation for the early diagnosis of Ph- myeloproliferative neoplasms in patients with venous thromboembolism: a meta-analysis. *Blood.* 2009 May 28; 113(22):5617–5623. [PubMed: 19273837]

78. Pardanani A, Lasho TL, Hussein K, Schwager SM, Finke CM, Pruthi RK, et al. JAK2V617F mutation screening as part of the hypercoagulable work-up in the absence of splanchnic venous thrombosis or overt myeloproliferative neoplasm: assessment of value in a series of 664 consecutive patients. *Mayo Clin Proc.* 2008 Apr; 83(4):457–459. [PubMed: 18380991]
79. Smalberg JH, Arends LR, Valla DC, Kiladjian JJ, Janssen HL, Leebeek FW. Myeloproliferative neoplasms in Budd-Chiari syndrome and portal vein thrombosis: a meta-analysis. *Blood.* 2012 Dec 13; 120(25):4921–4928. [PubMed: 23043069]
80. Shetty S, Kulkarni B, Pai N, Mukundan P, Kasatkar P, Ghosh K. JAK2 mutations across a spectrum of venous thrombosis cases. *Am J Clin Pathol.* 2010 Jul; 134(1):82–85. [PubMed: 20551270]
81. Torregrosa JM, Ferrer-Marin F, Lozano ML, Moreno MJ, Martinez C, Anton AI, et al. Impaired leucocyte activation is underlining the lower thrombotic risk of essential thrombocythaemia patients with CALR mutations as compared with those with the JAK2 mutation. *Br J Haematol.* 2016 Mar; 172(5):813–815. [PubMed: 26132594]
82. Rotunno G, Mannarelli C, Guglielmelli P, Pacilli A, Pancrazzi A, Pieri L, et al. Impact of calreticulin mutations on clinical and hematological phenotype and outcome in essential thrombocythemia. *Blood.* 2014 Mar 6; 123(10):1552–1555. [PubMed: 24371211]
83. Tefferi A, Wassie EA, Lasho TL, Finke C, Belachew AA, Ketterling RP, et al. Calreticulin mutations and long-term survival in essential thrombocythemia. *Leukemia.* 2014 Dec; 28(12):2300–2303. [PubMed: 24791854]
84. Rumi E, Pietra D, Ferretti V, Klampfl T, Harutyunyan AS, Milosevic JD, et al. JAK2 or CALR mutation status defines subtypes of essential thrombocythemia with substantially different clinical course and outcomes. *Blood.* 2014 Mar 6; 123(10):1544–1551. [PubMed: 24366362]
85. Fu R, Xuan M, Zhou Y, Sun T, Bai J, Cao Z, et al. Analysis of calreticulin mutations in Chinese patients with essential thrombocythemia: clinical implications in diagnosis, prognosis and treatment. *Leukemia.* 2014 Sep; 28(9):1912–1914. [PubMed: 24732593]
86. Tefferi A, Wassie EA, Guglielmelli P, Gangat N, Belachew AA, Lasho TL, et al. Type 1 versus Type 2 calreticulin mutations in essential thrombocythemia: a collaborative study of 1027 patients. *Am J Hematol.* 2014 Aug; 89(8):E121–124. [PubMed: 24753125]
87. Pietra D, Rumi E, Ferretti VV, Di Buduo CA, Milanesi C, Cavalloni C, et al. Differential clinical effects of different mutation subtypes in CALR-mutant myeloproliferative neoplasms. *Leukemia.* 2016 Feb; 30(2):431–438. [PubMed: 26449662]
88. De Stefano V, Qi X, Betti S, Rossi E. Splanchnic vein thrombosis and myeloproliferative neoplasms: molecular-driven diagnosis and long-term treatment. *Thromb Haemost.* 2016 Jan; 115(2):240–249. [PubMed: 26333846]
89. Vannucchi AM, Antonioli E, Guglielmelli P, Pancrazzi A, Guerini V, Barosi G, et al. Characteristics and clinical correlates of MPL 515W>L/K mutation in essential thrombocythemia. *Blood.* 2008 Aug 1; 112(3):844–847. [PubMed: 18519816]
90. Rumi E, Pietra D, Guglielmelli P, Bordoni R, Casetti I, Milanesi C, et al. Acquired copy-neutral loss of heterozygosity of chromosome 1p as a molecular event associated with marrow fibrosis in MPL-mutated myeloproliferative neoplasms. *Blood.* 2013 May 23; 121(21):4388–4395. [PubMed: 23575445]
91. Beer PA, Campbell PJ, Scott LM, Bench AJ, Erber WN, Bareford D, et al. MPL mutations in myeloproliferative disorders: analysis of the PT-1 cohort. *Blood.* 2008 Jul 1; 112(1):141–149. [PubMed: 18451306]
92. Gangat N, Wassie EA, Lasho TL, Finke C, Ketterling RP, Hanson CA, et al. Mutations and thrombosis in essential thrombocythemia: prognostic interaction with age and thrombosis history. *Eur J Haematol.* 2015 Jan; 94(1):31–36. [PubMed: 24889737]
93. Vainchenker W, Kralovics R. Genetic basis and molecular pathophysiology of classical myeloproliferative neoplasms. *Blood.* 2017 Feb 09; 129(6):667–679. [PubMed: 28028029]
94. Kang MG, Choi HW, Lee JH, Choi YJ, Choi HJ, Shin JH, et al. Coexistence of JAK2 and CALR mutations and their clinical implications in patients with essential thrombocythemia. *Oncotarget.* 2016 Jul.;30.

95. Shen H, Chao H, Ding Z, Feng Y, Cen J, Pan J, et al. CALR and ASXL1 mutation analysis in 190 patients with essential thrombocythemia. *Leuk Lymphoma*. 2015 Mar; 56(3):820–822. [PubMed: 25005031]
96. Yonal-Hindilerden I, Daglar-Aday A, Akadam-Teker B, Yilmaz C, Nalcaci M, Yavuz AS, et al. Prognostic significance of ASXL1, JAK2V617F mutations and JAK2V617F allele burden in Philadelphia-negative myeloproliferative neoplasms. *J Blood Med*. 2015; 6:157–175. [PubMed: 26082670]
97. Ortmann CA, Kent DG, Nangalia J, Silber Y, Wedge DC, Grinfeld J, et al. Effect of mutation order on myeloproliferative neoplasms. *N Engl J Med*. 2015 Feb 12; 372(7):601–612. [PubMed: 25671252]
98. Barbui T, Finazzi G, Carobbio A, Thiele J, Passamonti F, Rumi E, et al. Development and validation of an International Prognostic Score of thrombosis in World Health Organization-essential thrombocythemia (IPSET-thrombosis). *Blood*. 2012 Dec 20; 120(26):5128–5133. quiz 5252. [PubMed: 23033268]
99. Fu R, Xuan M, Lv C, Zhang L, Li H, Zhang X, et al. External validation and clinical evaluation of the International Prognostic Score of Thrombosis for Essential Thrombocythemia (IPSET-thrombosis) in a large cohort of Chinese patients. *Eur J Haematol*. 2014 Jun; 92(6):502–509. [PubMed: 24460750]
100. Finazzi G, Carobbio A, Guglielmelli P, Cavalloni C, Salmoiraghi S, Vannucchi AM, et al. Calreticulin mutation does not modify the IPSET score for predicting the risk of thrombosis among 1150 patients with essential thrombocythemia. *Blood*. 2014 Oct 16; 124(16):2611–2612. [PubMed: 25323688]
101. Barbui T, Vannucchi AM, Buxhofer-Ausch V, De Stefano V, Betti S, Rambaldi A, et al. Practice-relevant revision of IPSET-thrombosis based on 1019 patients with WHO-defined essential thrombocythemia. *Blood Cancer J*. 2015; 5:e369. [PubMed: 26617062]
102. Haider M, Gangat N, Lasho T, Abou Hussein AK, Elala YC, Hanson C, et al. Validation of the revised international prognostic score of thrombosis for essential thrombocythemia (IPSET-thrombosis) in 585 Mayo clinic patients. *Am J Hematol*. 2016 Jun; 91(4):390–394. [PubMed: 26799697]
103. Alvarez-Larran A, Pereira A, Guglielmelli P, Hernandez-Boluda JC, Arellano-Rodrigo E, Ferrer-Marin F, et al. Antiplatelet therapy versus observation in low-risk essential thrombocythemia with a CALR mutation. *Haematologica*. 2016 Aug; 101(8):926–931. [PubMed: 27175028]
104. Sun C, Zhou X, Zou ZJ, Guo HF, Li JY, Qiao C. Clinical Manifestation of Calreticulin Gene Mutations in Essential Thrombocythemia without Janus Kinase 2 and MPL Mutations: A Chinese Cohort Clinical Study. *Chin Med J (Engl)*. 2016 Aug 05; 129(15):1778–1783. [PubMed: 27453224]
105. Bertozzi I, Peroni E, Coltro G, Bogoni G, Cosi E, Santarossa C, et al. Thrombotic risk correlates with mutational status in true essential thrombocythemia. *Eur J Clin Invest*. 2016 Aug; 46(8): 683–689. [PubMed: 27271054]
106. Okabe M, Yamaguchi H, Usuki K, Kobayashi Y, Kawata E, Kuroda J, et al. Clinical features of Japanese polycythemia vera and essential thrombocythemia patients harboring CALR, JAK2V617F, JAK2Ex12del, and MPLW515L/K mutations. *Leuk Res*. 2016 Jan; 40:68–76. [PubMed: 26614694]

Figure 1. Suggested role of mutated *JAK2* in the pathogenesis of thrombosis in patients with ET
 The *JAK2* mutation is present clonally in hematopoietic progenitors and affects all blood cell types in the circulation. Thrombosis occurs due to platelet activation and over-production of tissue factor, as well as the formation of platelet-neutrophil aggregates. Moreover, the increased red blood cell mass observed in *JAK2*-mutated individuals may further contribute to the genesis of thrombi (dashed arrow). *JAK2V617F* is also found in other non-blood cell types, such as the liver endothelial cells, where the likelihood of thrombosis may be higher than in the systemic circulation (i.e., Budd-Chiari syndrome, thick arrow). *Abbreviations:* TF, tissue factor; RBC, red blood cell.

Figure 2. Evolution of the thrombotic risk assessment in patients with ET

The figure illustrates how newer prognostic scores for thrombosis in patients with ET were derived from the conventional risk stratification valid for all MPN patients. The incorporation of new risk factors in the models and a different emphasis placed on age and thrombosis history are highlighted. *Abbreviations:* CV, cardiovascular; IPSET, International Prognostic Score for Thrombosis in Essential Thrombocythemia.

Table 1

Relationship between *JAK2* mutation and the occurrence of thrombosis in patients with ET.

First Author, year, reference	N	Number of patients (%)		Median or mean age, years [range] or (±)		Median follow-up		Incidence of thrombosis after study entry, N (%)		P value or OR (95% CI), P-value
		Wild-type	Mutated	Wild-type	Mutated	Wild-type	Mutated	Wild-type	Mutated	
Baxter, 2005 ¹¹	51	22 (43)	29 (57)	54 (17)	55 (16)	NR	NR	4 (18)	8 (27)	NS
Campbell, 2005 ⁵⁰	776	362 (47)	414 (53)	60 (39-77)	52 (32-75)	NR	NR	Ven: 4 (1) Art: 21 (6)	Ven: 12 (3) Art: 25 (6)	Ven: 2.6 (1-6.9), 0.06 Art: 1.1 (0.6-2), 0.8
Ahn, 2007 ^a	24	13 (54)	11 (46)	59 (47-70)	67 (62-72)	27.5 m	37 m	5 (38)	5 (45)	0.744
Alvarez-Larran, 2007 ³⁹	126	59 (47)	44 (53)	29 (7)	30 (7)	10 y		13 (22)	9 (20)	NS
Finazzi, 2007 ³⁸	179	76 (42)	103 (58)	45 (10-78)	50 (16-92)	4.7 y	6.1 y	13 (17)	34 (33)	2.39 (1.16-4.93)
Vannucchi, 2007 ⁸	639	257 (40)	Hetero: 368 (58) Homo: 14 (2)	46 (17)	Hetero: 52 (17) Homo: 56 (21)	5 y		23 (9)	Hetero: 45 (12) Homo: 6 (43)	Homo vs. WT: 3.97 (1.34-11.7), .013
Heller, 2006 ^a	50	26 (52)	24 (48)	30 (11-77)	48 (21-81)	74.5 m	82.5 m	5 (19)	19 (79)	<0.0001
Hsiao, 2007 ^a	53	18 (34)	35 (66)	55 (21)	62 (16)	34.5 m		2 (11)	15 (43)	0.029
Ohyashiki, 2008 ^a	54	21 (39)	33 (61)	57 (18)	63 (14)	NR	NR	0 (0)	6 (18)	P .038
Pemmaraju, 2007 ^a	80	42 (52)	38 (48)	38 (2-80)	59 (27-84)			Art: 13 (31) Ven: 3 (7)	Art: 10 (26) Ven: 0 (0)	NR
Rudzki, 2007 ^a	59	21 (36)	38 (64)	48 (17)	51 (13)	26.8 m	27.6 m	9 (43)	15 (39)	NS
Wong, 2008 ^a	95	35 (37)	60 (63)	54 (21-88)	66 (31-89)	68.4 m		3 (8)	23 (38)	NS
Carobbio, 2009 ⁵⁹	867	491 (57)	376 (43)	49 (8-88)	56 (12-93)	5 y	3.7 y	34 (9)	54 (11)	1.5 (1-2.31), NR
Giona, 2012 ⁶⁰	21	11 (52)	10 (48)	15 (5-19)	18 (6-19)	193 m	150 m	0 (0)	1 (10)	NS
Cho, 2009 ^a	108	47 (43)	61 (57)	61 (27-82)	55 (13-80)	35.7 m		14 (30)	21 (34)	NR
Wolankyj, 2005 ⁶²	150	77 (51)	73 (49)	42 (17-84)	53 (17-98)	11.1 y	11.6 y	22 (28.6)	24 (33)	NS
Antonoli, 2005 ⁶³	130	56 (43)	74 (57)	48 (16-81)	51 (19-84)	NR	NR	13 (23)	17 (23)	NS
Cheung, 2006 ^a	60	31 (52)	29 (48)	41 (8-77)	46 (20-80)	NR	NR	5 (16)	7 (24)	NS
Chim, 2010 ^a	141	61 (43)	80 (57)	50 (14)	62 (14)	70 m		8 (13)	27 (34)	0.006
Kittur, 2007 ^a	176	80 (45)	96 (55)	48 (15-91)	62 (16-88)	59 m		Art: 15 (19) Ven: 2 (3)	Art: 24 (25) Ven: 11 (11)	Art: NS Ven: 0.02

First Author, year, reference	N	Number of patients (%)		Median or mean age, years [range] or (±)		Median follow-up		Incidence of thrombosis after study entry, N (%)		P value or OR (95% CI), P-value
		Wild-type	Mutated	Wild-type	Mutated	Wild-type	Mutated	Wild-type	Mutated	
Patriarca, 2010 ^a	106	46 (43)	60 (57)	68 (25-91)	62 (25-92)	24 m		3 (6)	8 (13)	4.1 (1.7-10.4), <0.003 ^b
Weston, 2011 ^a	61	20 (33)	41 (67)	59 (23-91)	67 (20-90)	33 m		1 (5)	13 (32)	NR
Pich, 2012 ^a	103	44 (43)	59 (57)	63 (16)	56 (16)	43.4 m		Ven: 7/37 (19) Art: 2/37 (5)	Ven: 11/49 (22) Art: 4/48 (8)	NS
Palandri, 2009 ⁶⁴	275	100 (36)	175 (64)	55 (19-80)	63 (16-88)	83 m	73 m	8 (8)	14 (8)	NS
Takata, 2014 ^a	51	20 (39)	31 (61)	51 (12-80)	69 (28-86)	65 m ^c		2 (10)	13 (42)	
Boroczyk, 2015 ^a	186	60 (32)	126 (68)	54 (24-82)	58 (19-83)	34 m	28 m	15 (25)	39 (31)	5.15 (1.16-22.90), 0.024
Yonal, 2016 ^a	107	43 (40)	64 (60)	52 (16)	50 (15)	70 m	70 m	28 (65)	38 (59)	NS

Abbreviations: Art, arterial; CI, confidence interval; ET, essential Thrombocythemia; Hetero, heterozygous; Homo, homozygous; m, months; MPN, myeloproliferative neoplasms; NR, not reported; NS, non-significant; OR, odds ratio; Ven, venous; WT, wild type; y, years.

^aReferenced in Qin *et al.*⁶⁷, Ziakas⁶⁸ and Dahabreh *et al.*⁷⁰

^bFor all thrombosis events.

^cIncludes patients with all MPN.

Only studies that report data specifically for ET, and that report thrombosis after study entry are listed.

Table 2

Relationship between driver mutation and occurrence of thrombosis in patients with molecularly annotated ET.

First Author, year	Number of patients (%)				Median or mean age, years				Median follow-up				Incidence of thrombosis, N (%)				P-value
	JAK2	CALR	MPL	TN	JAK2	CALR	MPL	TN	JAK2	CALR	MPL	TN	JAK2 (A)	CALR (B)	MPL (C)	TN (D)	
Fu, 2014 ⁸⁵	240 (55)	99 (24)	6 (1)	89 (20)	56	50	50	50	37 m	37 m	55 m	38 m	28 (12)	4 (4)	0 (0)	3 (3)	A vs. B 0.029 A vs. D 0.022
Gangat, 2015 ⁹²	160 (53)	95 (32)	8 (3)	37 (12)	59	47	66	42	12.7 y				47 (29)	17 (18)	3 (37)	4 (11)	0.03
Rumi, 2014 ⁸⁴	466 (73)	176 (27)	-	-	50	45	-	-	5.2 y	-	-	-	25/1000 p-y	10/1000 p-y	-	-	0.001
Rotunno, 2014 ⁸²	369 (64)	89 (15)	25 (4)	93 (16)	61	55	54	53	71.9 m				111 (30)	12 (13)	10 (40)	15 (16)	A vs. B 0.011 B vs. C 0.012 B vs. D 0.894
Sun 2016 ¹⁰⁴	149 (49)	92 (30)	65 (21)		62	55	45		102 m				2	1	5		NS
Tefferi, 2015 ⁸⁶	227 (57)	114 (28)	11 (3)	50 (12)	58	47	64	42	NR				55 (24)	20 (18)	3 (27)	4 (8)	0.03
Bertozzi, 2016 ¹⁰⁵	114 (62)	44 (24)	3 (2)	22 (12)	56	49	60	38	9.6 y	11.9 y	13.3 y	9.7 y	26 (23)	6 (14)	1 (33)	0 (0)	0.027
Okabe, 2016 ¹⁰⁶	179 (61)	47 (16)	10 (3)	57 (19)	63	60	68	52	NR				12	6	0	0	NS
Palandri, 2015 ⁷³	132 (71)	54 (29)	-	-	34	34	-	-	10.9 y	9.8 y	-	-	10-y: 11% 15-y: 21.7%	10-y: 3.5% 15-y: 9.1%	-	-	0.042
Torregrosa, 2016 ⁸¹	40 (61)	13 (20)	12 (19)		66	51	72		10.4 y				12 (30)	0	1 (8)		0.023
Alvarez-Larran, 2016 ¹⁰³	162 (28)	271 (62)	-	-	42	42	-	-	2215 p-y	-	-	-	ASA: 9.7/1000 p-y OBS: 6.9/1000 p-y	ASA: 9.7/1000 p-y OBS: 6.9/1000 p-y	-	-	NR
Vannucchi, 2008 ⁸⁹	546 (55)	418 ^a (42)	30 (3)		56	50 ^a	56		59 m				Art: 20 (4) Ven: 12 (2)	Art: 6 (1) ^a Ven: 5 (1) ^a	Art: 4 (13) Ven: 2 (7)		Art: A vs. B: 0.04 A vs. C: 0.05 B vs. C: 0.002 Ven: NS
Beer, 2008 ⁹¹	411 (53)	333 (43) ^a	32 (4)	-	60	52 ^a	67	-	36.5 m				Art: 25 Ven: 11	Art: 19 ^a Ven: 3 ^a	Art: 2 Ven: 2	-	Art: NS Ven: 0.02

Abbreviations: Art, arterial; ASA, low-dose aspirin; CALR, calreticulin; ET, essential Thrombocythemia; JAK2, Janus kinase; m, months; MPL, myeloproliferative leukemia; NR, not reported; NS, non-significant; OBS, observation; p-y, person-years; TN, triple negative; Ven, venous; y, years.

^a Assumed to include TN patients.

Only studies reporting mutation-specific data are listed. Only thrombosis after study entry is indicated.