Chapter 7
Essential Thrombocythemia

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Introduction

Essential thrombocythemia (ET) is classified as a chronic myeloproliferative neoplasm (MPN) involving the megakaryocytic lineage [1]. It was first described by Austrian pathologists, Epstein and Goedel, in 1934 [2], and the definition has evolved over the years. By the most recent diagnostic criteria (Table 7.1), ET is characterized by thrombocytosis $\geq 450 \times 10^9$/L in the peripheral blood; a bone marrow showing increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei; no fibrosis or rare grade 1 reticulin fibrosis; exclusion of other myeloid neoplasms; and the presence of mutations involving Janus kinase 2 (*JAK2*), calreticulin (*CALR*), or thrombopoietin receptor, also known as myeloproliferative leukemia virus oncogene (*MPL*) [3].

Epidemiology

The overall age-adjusted incidence rate for ET is 9.6 per one million persons per year. Differences are seen in incidence rates for Blacks (11.5), Caucasians (9.7), and Hispanics (6.4). The median age at diagnosis is 68 years. ET is rarely seen in children, and incidence rates increase exponentially with increasing age. There is a female predilection, with a M:F ratio of 0.8:1, most prominent in women <60 years of age [4].

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Table 7.1 World Health Organization essential thrombocythemia diagnostic criteria

Major criteria
1. Platelet count ≥450 × 10^9/L
2. Bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers
3. Not meeting WHO criteria for *BCR-ABL1*$^+$ CML, PV, PMF, myelodysplastic syndromes, or other myeloid neoplasms
4. Presence of *JAK2, CALR,* or *MPL* mutation
Minor criterion
Presence of a clonal marker or absence of evidence for reactive thrombocytosis

With permission from Arber et al. [3]
Diagnosis of ET requires meeting all four major criteria or the first three major criteria and the minor criterion

Clinical Features

Up to 50% of patients can be asymptomatic at time of diagnosis, while others may present with vasomotor symptoms, thrombosis, or hemorrhage. Common patient complaints include difficulty sleeping, abdominal discomfort, dizziness/vertigo/lightheadedness, sweats, numbness/tingling in hands/feet and less commonly pruritus, bruising, and fatigue [5].

Morphology

The 2016 World Health Organization (WHO) diagnostic criteria for ET have been updated to include the mutation status of *CALR* and *MPL* (in addition to *JAK2*, included in the 2008 classification), while the morphological criteria have remained essentially the same.

While morphology has always been an indispensable component of the diagnoses of MPNs, the new criteria emphasize the importance of distinguishing prefibrotic/early primary myelofibrosis (pre-PMF) from ET. Absence of fibrosis or only minimal fibrosis (grade 1) is acceptable for a diagnosis of ET. Although this was implied by the 2008 exclusion criteria for PMF and stated in the footnote, the degree of allowable fibrosis is now directly stated under the major criteria heading of the 2016 classification [3]. This distinction is important clinically, as true patients with ET can survive up to 7 years longer than those with pre-PMF [6]. Pre-PMF has a significantly worse prognosis than ET [6–8]. Compared with pre-PMF, patients with true ET have a lower risk of progression to acute leukemia and high-grade fibrosis, superior overall survival, and higher risk of bleeding complications [7].

Peripheral Blood

The major finding in the peripheral blood is marked thrombocytosis. Platelets can vary in size and shape with occasional bizarre forms. The white blood cell count and differential are typically within normal limits, although they may be borderline high. Red blood cells are typically normochromic, normocytic [1].

Bone Marrow

The bone marrow biopsy is normocellular or shows only a slight increase in age-matched cellularity (Fig. 7.1). Only the megakaryocytic lineage shows increased proliferation. Megakaryocytes are enlarged and increased in number, with mature morphology and hyperlobulated nuclei (staghorn appearance) (Fig. 7.2). Neutrophil

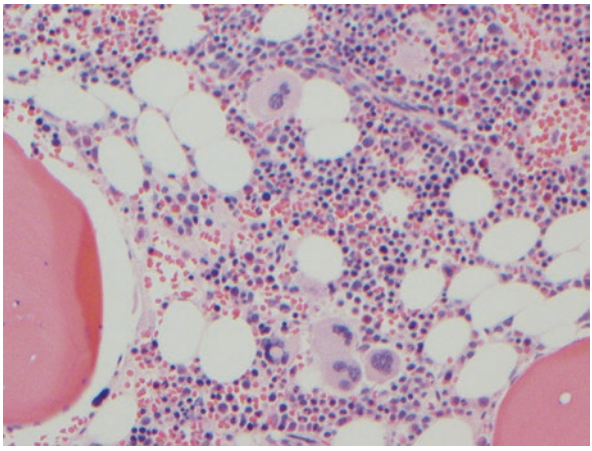

Fig. 7.1 Bone marrow biopsy, normocellular for age with increased megakaryocytes in essential thrombocythemia (Hematoxylin–Eosin, original magnification ×200)

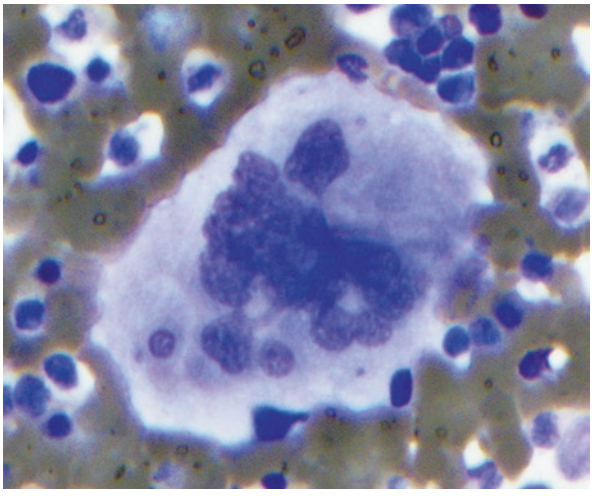

Fig. 7.2 Large, mature megakaryocyte with hyperlobulated (staghorn) nucleus in essential thrombocythemia (Wright–Giemsa, original magnification ×500)

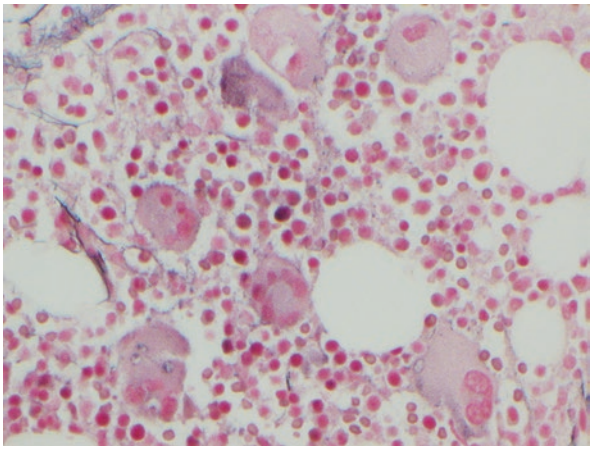

Fig. 7.3 Bone marrow biopsy with no increase in reticulin fibrosis (grade MF-0) in essential thrombocythemia (reticulin, original magnification ×400). Absence of reticulin fibers, bone marrow core biopsy, reticulin stain, 100×

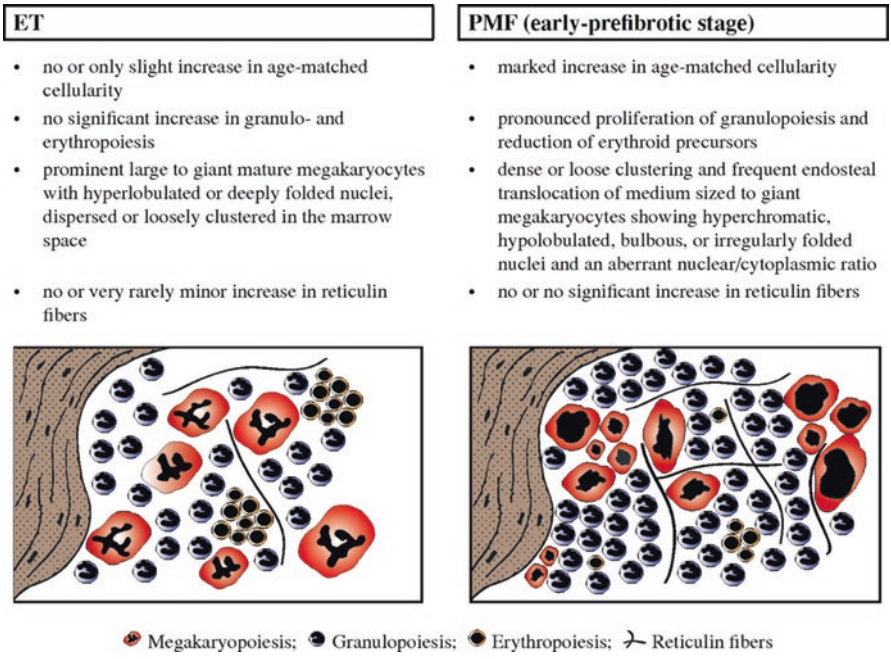

Fig. 7.4 Diagnostic criteria of distinctive value regarding WHO-defined ET (*left*) versus early-prefibrotic stage of PMF (*right*), including standardized morphological features (see Table 7.1 for more details), allowing the generation of characteristic histological bone marrow patterns (Used with permission from Thiele et al. [6])

granulopoiesis and erythropoiesis are not significantly increased or left shifted. Reticulin fibrosis is absent, or very rarely there is minor (grade 1) increase in reticulin fibers [3, 6, 9] (Fig. 7.3). When differentiating ET from pre-PMF, it is important to note that ET lacks marrow hypercellularity, granulocytic proliferation, significant megakaryocyte clustering, and cloud-like or hyperchromatic megakaryocytes (Fig. 7.4).

Molecular Features

Research efforts in the last 10 years have resulted in a much better understanding of the molecular pathogenesis of ET and other *BCR/ABL1*-negative MPNs. Cytogenetic abnormalities occur in less than 5% of ET, and no specific recurrent abnormalities have been documented. In contrast, approximately 90% of ET cases carry driver mutations in one of three genes.

Three main driver mutations have now been well characterized in terms of their incidence and clinical effects. *JAK2* is the most commonly mutated gene in MPNs, followed by *CALR* and *MPL*. These driver mutations ultimately result in overproduction of one or more cell lineages. All three driver mutations activate the Janus kinase 2/signal transducer and activator of transcription (JAK/STAT) signaling pathway. In the case of *JAK2* and *MPL* mutations, the mechanisms of action involve constitutive activation of their mutated counterparts, while mutated CALR has been shown to activate the thrombopoietin receptor, MPL [10]. *JAK2* mutation status was included in the 2008 WHO criteria for a diagnosis. In addition to *JAK2*, the 2016 classification includes mutation status of *MPL* and *CALR*. The clinical features of ET are influenced by which gene is mutated, as discussed further below (Table 7.2).

The first recurrent mutation identified in all three BCR/ABL-negative MPNs was *JAK2* V617F, discovered in 2005 by four groups [11–14]. About 50–60% of ET

Table 7.2 Clinical and laboratory features of 299 patients with essential thrombocytopenia stratified by gene mutation

Variables	*JAK2*-mutated (n = 159)	*CALR*-mutated (n = 95)	*MPL*-mutated (n = 8)	Triple-negative (n = 37)
Age in years; median (range)	59 (16–88)	47 (15–91)	66 (57–85)	42 (16–81)
Age >65 years	42%	27%	50%	22%
Females	65%	48%	38%	73%
Hemoglobin g/dL; median (range)	14.3 (9.8–17.9)	13.3 (6.9–16.4)	12.9 (9.0–15.8)	13 (8.4–15.9)
Leukocytes × 10^9/L; median (range)	10.0 (3.9–53.4)	8.6 (3.3–32.6)	7.0 (4.0–17.7)	7.2 (2.8–12.6)
Platelets × 10^9/L; median (range)	960 (500–3000)	1082 (454–3460)	969 (685–2249)	1000 (557–3300)
Leukocytes ≥ 11 × 10^9/L	39%	32%	25%	16%
Platelets > 1000 × 10^9/L	45%	62%	50%	54%
Microcirculatory symptoms	21%	7%	13%	32%
Postdiagnosis thrombosis	26%	18%	38%	11%
Deaths (maximum follow-up)	52% (44 years)	44% (34 years)	88% (21 years)	27% (36 years)
Leukemic conversions	5%	8%	25%	0%
Fibrotic progression	8%	12%	38%	5%

Adapted from Tefferi et al. [47]; with permission

cases harbor a *JAK2* V617F mutation [15]. JAK2 is a protein tyrosine kinase. In normal megakaryocytes, binding of thrombopoietin to the thrombopoietin receptor results in JAK2 autophosphorylation, recruitment of STAT, and phosphorylation of STAT. The phosphorylated STAT dimerizes and moves to the nucleus, where it activates transcription of genes resulting in proliferation of platelets. The *JAK2* V617F mutation deactivates the repressor pseudokinase domain of JAK2, resulting in activation of JAK2 and downstream signaling pathways in the absence of the appropriate activating ligand.

The percentage of cells with the *JAK2* V617F mutation, or allele burden, affects the clinical features of the disease. Cells homozygous for *JAK2* V617F mutation also contribute to a higher allele burden. Low *JAK2* V617F allele burden is typically seen in ET and pre-PMF, with intermediate levels in polycythemia vera (PV), high levels in fibrotic PMF, and very high levels seen in post-PV MF. The percentage of*JAK2* V617F homozygous granulocytes is also higher in PV and PMF than in ET [16]. Some MPN experts suggest that ET and PV may be different stages of the same disease, with ET representing early, low allelic burden disease, and PV and PMF representing a higher allelic burden later in the disease course (Fig. 7.5) [17]. Recent studies suggest a diagnosis of true ET is unusual in patients with a *JAK2* V617F allele burden over 50% [18]. Increased *JAK2* V617F allele burden in ET is associated with increased splenomegaly, microvessel disease, higher leukocyte count, and history of thrombosis [19, 20]. Although less than 5% of patients with ET are homozygous for *JAK2* V617F, homozygosity also shows correlation with clinical features. Patients with ET homozygous for *JAK2* V617F are more likely than heterozygotes to have splenomegaly (73% vs 28%), cardiovascular events (43% vs 12%), and progression to myelofibrosis (14% vs 5%) [21].

The second most commonly mutated gene in ET is calreticulin (*CALR*). In 2013, two groups discovered *CALR* mutations in the majority of patients with *JAK2*-negative ET [22, 23]. About 20–25% of ET cases have *CALR* mutations [15]. CALR normally acts as a protein chaperone that helps newly synthesized proteins fold properly in the endoplasmic reticulum (ER). CALR is also a calcium ion (Ca^{2+}) transporter that regulates Ca^{2+} levels between the ER and the cytoplasm of cells. The carboxy end of CALR is enriched in negatively charged amino acids that promote binding of Ca^{2+}. The carboxy terminal also has a four amino acid sequence – lysine, aspartic acid, glutamic acid, leucine (KDEL) – that acts as a signal for CALR to be retained in the ER. *CALR* mutations include more than 50 different insertions and deletions (indels). These indels result in altered charge of the carboxy end of the CALR protein, which is the primary calcium-binding domain. The two most common *CALR* mutations are classified as type 1 (a 52 base pair deletion in exon 19) and type 2 (a 5 base pair insertion in exon 19). Type 1 *CALR* mutation is seen in approximately 50% of *CALR*-mutated ET cases, while type 2 is seen in approximately 30%. Type 1-like mutations result in a loss of the majority of the negatively charged amino acids in the calcium-binding region of CALR, and type 2-like mutations result in loss of approximately half the positively charged amino acids in this region. A third group, seen in approximately 10% of cases, includes indels that are typically classified as type 1-like and type 2-like based on the expected change in charge of the mutated CALR carboxy terminus [24].

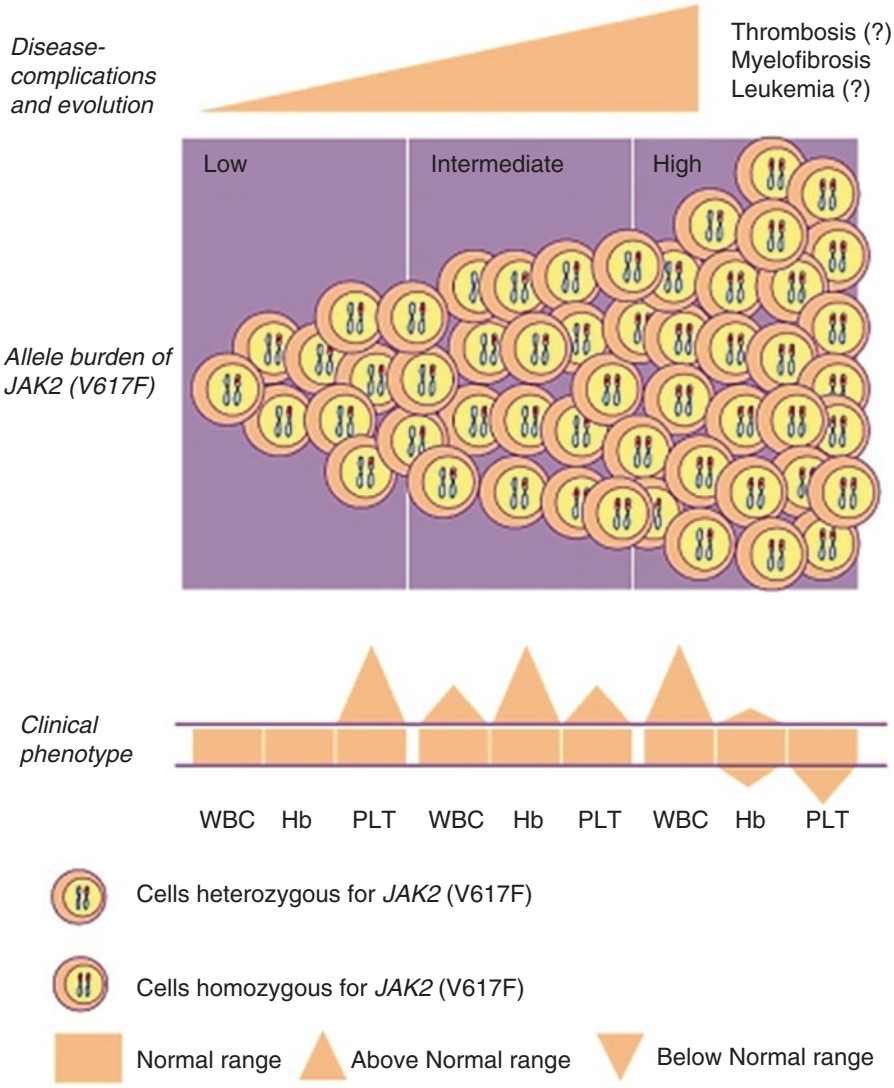

Fig. 7.5 Schematic representation of *JAK2* (V617F) allele burden (*middle panel*) and its relationship with clinical phenotype (*bottom panel*), and disease complications (*top panel*). At low levels of mutant allele, the clinical phenotype is dominated by thrombocytosis, at intermediate levels by erythrocytosis, and at higher levels by leukocytosis. Among complications, current evidence indicates a relationship between allele burden and evolution into myelofibrosis (Used with permission from Passamonti et al. [17])

Compared with *JAK2* V617F-positive ET, *CALR*-mutated patients with ET present at a younger age and have a lower risk of thrombosis and higher platelet counts [25]. Within the group of CALR-mutated cases, type 1 mutations are more frequently associated with features of PMF at presentation than type 2 mutated cases. ET type 1 mutated cases have a higher risk of progression to post-ET MF than type 2 mutated cases. In contrast, patients with type 2 *CALR* mutations more frequently have features of ET at presentation as well as very high platelet counts and lower risk for thrombosis [24]. Increased *CALR* mutant allele burden has been demonstrated in post-ET MF [26].

Approximately 3–5% of ET cases have *MPL* mutations involving exon 515. These include W515L, W515K, W515A, W515S, and W515R [27]. These mutations result in constitutive activation of the JAK-STAT pathway via the thrombopoietin receptor encoded by *MPL*, as well as increased sensitivity of the receptor to thrombopoietin. *MPL*-mutated patients with ET tend to be older and have a higher rate of progression to fibrosis and acute myeloid leukemia (AML).

Approximately 10% of MPNs have none of the three main driver mutations and are referred to as triple-negative MPNs. A small subset of these "triple-negative" cases has been shown to have novel mutations in *JAK2* or *MPL*. The remaining cases have as yet unidentified abnormalities or rare mutations. Whole exome sequencing of triple-negative ET cases in one study uncovered the following mutations: *JAK2* G571S (germline), *ITGAV* R333H, *WBSCR28* A201T, and loss of chromosome 4q [27]. Lymphocyte-specific adapter protein (*LNK*) also known as src homology *2B3SH2B* adapter protein 3 (*SH2B3*) is mutated in rare cases of ET [28].

In addition to driver mutations, many patients with ET harbor mutations in other genes involved in epigenetic modification, RNA splicing, and cell signaling pathways (Table 7.3). Data regarding the frequency of these nondriver mutations in ET specifically are difficult to extract from the literature due to the inclusion of mixed cases of MPNs and small numbers of cases in most series; however, in one study including 69 patients with ET, 62% had only a single driver mutation, 22% had one additional mutation, and 3% had two additional mutations. The most commonly mutated nondriver genes in ET were *DNMT3A*, *TET2*, and *TP53* [29]. *TET2* was reported to occur in approximately 5% of ET in one study [30]. In another study, 15% of patients with ET had *TET2* mutations, 12.5% had *ASXL1* mutations, and less than 1% of patients had mutations in *SRSF2*, *SF3B1*, *IDH1*, *IDH2* or *GATA1* [31]. Single ET cases had mutations involving each of the following genes: *ASXL1*, *EXH2*, *CUX1*, *PIK3R2*, *SH2B3*, and del7q [29].

The order of acquisition of gene mutations also affects the clinical features of disease. Calreticulin mutations generally occur before other mutations. *JAK2*-positive cases may have mutations in *TET2* and/or *DNMT3A* that generally occur before *JAK2* V617F [29].

Cases in which *TET2* or *DNMT3A* mutations occur before *JAK2* mutations are more frequently associated with clinical features of ET, while cases carrying one of these mutations with *JAK2* mutated first are reportedly more frequently associated with features of PV [25].

Table 7.3 Acquired mutations in sporadic essential thrombocythemia

Gene	Mutation location	Genomic location	Protein	Frequency of mutation
JAK2	V617F exon 14	9p24	JAK2	50–60%
JAK2	Various indels, exon 12	9p24	Jak2	Rare
MPL	W515 K/L/A S505 N	1p34	TpoR	3–5%
CBL	Point mutations, exons 8 and 9	11q23	CBL	Rare
TET2	Mutations across gene	4q24	TET2	5%
SH2B3	Various mutations, mainly exon 2	12q24	LNK	3–6%
ASXL1	Mutations across gene	20q11	ASXL1	2–5%
EZH2	Various mutations across gene	7q36	EZH2	1%
DNMT3A	Mutations across gene	2p23	DNMT3A	1–5%
IDH1/IDH2	Mainly IDH1 R132 or IDH2 R140	2q23/15q26	IDH1/IDH2	Rare

Adapted with permission from Jones et al. [48]

Finally, host factors may also predispose patients to ET. Several single nucleotide polymorphisms (SNPs) have been shown to be associated with increased risk of developing MPNs. Compared with other SNPs, the 46/1 haplotype of *JAK2*, found in approximately 50% of healthy Caucasians, carries three to four times the risk of developing a MPN, not only mutated *JAK2* but also wild-type *JAK2* [32, 33]. The risk of ET in first-order relatives of 46/1 *JAK2* patients with ET is increased 12 times [33]. Other less common *JAK2* SNPs have also been associated with increased risk for ET [34]. Additional genes with SNPs associated with increased risk of ET and other Ph-negative MPNs include telomerase reverse transcriptase (*TERT*), *TET2*, and *SH2B* adapter protein 3 (*SH2B3*), also known as *LNK*, *HBS1L/MYB*, and *MECOM* [35, 36].

Prognosis and Therapy

Prognosis in ET is determined by multiple clinical factors, including patient's age, hematologic parameters, mutation status, and type of previous therapy. The international prognostic score for essential thrombocythemia (IPSET-thrombosis) is the current standard for risk stratification in patients with ET and is based on age, history of thrombosis, and *JAK2* V617F mutation status [37–39]. In this model, patients' risk of thrombosis is stratified as follows: very low = age $\leq$ 60, JAK2-negative, no prior thrombosis; low = age $\leq$ 60, *JAK2*-positive, no prior thrombosis; intermediate = age > 60, *JAK2*-negative, no prior thrombosis; and high = prior thrombosis or age > 60 and *JAK2*-positive. The model predicts the risk of vascular events in patients with ET, ranging from very low risk, 0.44% patients per year, to high risk, 4.17% patients per year.

The rates of overall survival and disease progression vary in different studies, most likely due to data collected in studies including a mixed population of patients

diagnosed as ET that include pre-PMF patients. When taken as a pure population, patients with ET have rates of progression to post-ET MF of less than 1% at 5 and 10 years and approximately 9% at 15 years. The rate of progression of ET to AML is less than 1% at 5 and 10 years and approximately 2% at 20 years. Death rates are approximately 3% at 5 years, 5% at 10 years, and 25% at 15 years [11].

Risk factors for progression of ET to MF include older age, anemia, and absence of *JAK2* V6178F. Risk factors for progression of ET to AML include history of thrombosis and extreme thrombocytosis. Risk factors for death include older age, leukocytosis greater than 11×10^9/L, hemoglobin less than 12 g/dL, and history of thrombosis [11].

As described above, there are differences in the clinical features of ET based on the driver mutation causing the disease. Some molecular features have also been found to be correlated with prognosis. Overall survival is similar in *JAK2*-mutated and *CALR*-mutated cases, but is inferior in patients with MPL mutations and better in triple-negative cases [39]. However, patients with *MPL* mutations are older at presentation, accounting for the poor survival in that group [39]. Of the common nondriver mutations, *SRSF2* is associated with inferior survival in post-ET MF, whereas *EZH2*, *ASXL1*, *IDH1*, an *IDH2* mutations had no effect on survival [26]. Mutations in *TP53* are often seen at the time of leukemic transformation [40].

Treatment is primarily directed toward lowering the platelet count and decreasing the risk of thrombosis and bleeding complications. Treatment is tailored to each patient's risk profile. Patients are classified as high risk or low risk based on age and history of thrombosis. High-risk patients are those 60 years of age and older and/or those with a history of thrombosis. Low-risk patients are those under 60 with no history thrombosis. Secondary risk factors that may also be considered when selecting a treatment regimen are *JAK2* V617 status and the presence or absence of cardiovascular risk factors (diabetes, hypertension, and smoking history). The presence of either or both of these risk factors is associated with increased risk of thrombosis and may indicate a need for more aggressive therapy [41].

First-line therapy for all patients with ET is once-daily, low-dose aspirin unless contraindicated. In addition, cytoreductive treatment is recommended for patients 60 years and older and for patients with a history of arterial thrombosis with *JAK2* V617 positivity and/or the presence of cardiovascular risk factors. Patients with a history of venous thrombosis may receive systemic anticoagulation in addition to cytoreductive therapy and aspirin.

Cytoreductive agents frequently used to treat ET include hydroxyurea (HU), anagrelide, and pegylated interferon alpha-2a (PEG-IFNα-2a). HU is the frontline cytoreductive drug most often used. HU treatment has been shown to decrease platelet counts and risk of thrombosis.

PEG-IFNα-2a or anagrelide may be used in patients who do not tolerate HU. Long-term IFNα treatment has been shown to induce complete hematologic remission in 77% of patients with ET and complete molecular remission in 17% of patients with ET. In one study, the efficacy of PEG-IFNα-2a to achieve a complete molecular response was found to be influenced by the presence of somatic mutations in addition to *JAK2* (*CALR* status was unknown at the time of the study).

In particular, patients with *TET2* mutations showed a smaller decrease in *JAK2* allele burden with treatment and a lower rate of complete molecular response [42]. PEG-IFNα-2a may also be effective in treating *CALR*-positive ET. Complete long-term hematologic remission was documented in two patients with *CALR*-positive ET. At the time of the report, these patients had been in remission for 18 months and over 5 years, respectively, after discontinuation IFNα [43].

Anagrelide, a drug that inhibits maturation of megakaryocytes into platelets, has also been used as a cytoreductive treatment in patients with ET who cannot tolerate or are resistant to HU. This drug has been associated with a greater incidence of thrombotic events and a higher incidence of transformation to acute leukemia compared with HU in one study [44], but with no adverse effects relative to HU in another study [45].

Although the Janus kinase inhibitors including ruxolitinib have been used to treat PV and PMF, there are few studies documenting Janus kinase inhibitor treatment in ET. In one study, ruxolitinib decreased platelet and leukocyte counts, reduced spleen size, and improved disease-related symptoms in patients with ET [46].

Conclusion

While morphological criteria for the diagnosis of ET have changed little, our understanding of the molecular underpinnings of this disease have progressed greatly in recent years. The updated 2016 WHO morphological diagnostic criteria remain similar to the 2008 classification, with emphasis on the distinction of ET from pre-PMF based on morphological and laboratory values [3].

In contrast, the molecular diagnostic WHO criteria now incorporate mutation status of *CALR* and *MPL*, in addition to the previously known driver mutations in *JAK2*. Clinical features including age at presentation, cell counts, propensity for thrombosis, and risk of progression to more aggressive disease are influenced by molecular features, including which gene is mutated, the structure of the mutated protein, and in some cases by the mutant allele burden. Furthermore, nondriver mutations and the order in which they are acquired relative to driver mutations also have clinical correlates.

With greater understanding of these molecular features, the future holds great potential for expanding targeted therapy for ET and other MPNs beyond Janus kinase inhibitors.

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