Literature Review

Essential Thrombocytocemia

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Essential Thrombocytocemia (ET), a clonal hematopoietic stem cell disorder, is one of the classic Philadelphia negative myeloproliferative neoplasms and is characterized by thrombocytosis with bone marrow megakaryocytic hyperplasia. Mutations in Janus kinase 2 (JAK2), calreticulin (CALR), or myeloproliferative leukemia (MPL) are found in approximately 80-90% of patients. Over the past decade, new molecular and clinical knowledge in ET has led to a significant improvement in the diagnostic, prognostic, and therapeutic processes. Despite these advancements, many uncertainties remain regarding clinical decision-making. In the 2016 revised World Health Organization (WHO) classification true ET requires meeting all 4 major criteria described below or 3 major criteria and one minor criterion. Because of the revised WHO classification, study data has been re-evaluated and the revised International Prognostic Score of Thrombosis for Essential Thrombocytocemia risk stratification was devised allowing clinicians to assign patients to the appropriate risk group in a 4-tiered system. In ET patient’s transformation to acute myeloid leukemia and/or post-ET myelofibrosis are rare events. Current treatment in ET is primarily indicated for the purposes of preventing vascular complications which have been reported to be the leading cause of death. Thrombotic complications can occur in up to 24% of patients with 13% developing a vascular event before diagnosis. Mutational status has an impact on thrombotic risk with a lower rate of thrombosis seen in CALR-mutants as compared to JAK2 V617F/MPL mutants and triple-negative cases. Mutations other than JAK2, CALR, or MPL have been found in approximately 53% of patients with ET with the most frequent being TET2, ASXL1, DNMT3A, and SF3B1.

Key words: Essential Thrombocytocemia, JAK2 mutation, CALR mutation, MPL mutation, Platelets.

Introduction

Essential thrombocytocemia (ET) is a rare but serious myeloproliferative neoplasm (MPN) characterized by thrombocytosis with bone marrow megakaryocytic hyperplasia and a tendency to develop thrombotic and hemorrhagic complications. Essential thrombocytosis (primary thrombocytocemia) is a nonreactive, chronic myeloproliferative neoplasm predominantly occurring in the age group of 50-60 years equally in the male and female population. A sustained megakaryocyte proliferation leads to an increase in the number of circulating platelets. Mutations in Janus kinase 2 (JAK2), calreticulin (CALR), or myeloproliferative leukemia (MPL) are found in approximately 90% of patients with essential thrombocytosis.¹ As in primary myelofibrosis (PMF) and polycythaemia Vera (PV),

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dysregulated signalling in the JAK pathway plays a role in the pathophysiology of ET. Over the past decade, new molecular and clinical knowledge in ET has led to a significant improvement in the diagnostic, prognostic, and therapeutic processes. Despite these advancements, many uncertainties remain regarding clinical decision-making.

**Epidemiology, Etiology and Pathogenesis**

The incidence rate refers to the number of new cases of a disease diagnosed within a specific time. Annual incidence rate for ET has been reported to be 1.03 per 100,000 in Europe and North America with higher rate in males.\(^1\) Prevalence is the number of cases of a disease in the population at a specific point in time. The prevalence rate of ET has been found to be 11.00 to 42.52 per 100,000.\(^1\) Female to male incidence is 2:1, generally with median age of about 60 years.\(^3\) Most frequent recurrent chromosomal defects seen in ET patients include trisomy 1q, trisomy 8, trisomy 9, del(13q) and del(20q). ET generally has a better prognosis compared to other MPN with an expected survival rate of 18 - 19.8 years. Survival is significantly better in patients with a lower risk of thrombosis and the incidence of blast and fibrotic transformation in ET is lower than other MPN.\(^4\)

Three main mutations seen in most MPN including PV, ET and PMF involve JAK2 gene or MPL gene that provides instructions for making the thrombopoietin receptor protein and CALR. However, their absence does not rule out ET diagnosis, as about 20% ET patients are triple negative.\(^5\) JAK2 is a member of the tyrosine kinase family of enzymes and is involved in signal transduction for erythropoietin, thrombopoietin, and granulocyte colony-stimulating factor (G-CSF). JAK2 gene is located on chromosome 9p24. The V617F is the most frequent mutation of JAK2.\(^6\) MPL gene, also known as myeloproliferative leukemia or thrombopoietin receptor gene, is on chromosome 1p34 and has a mutation cluster in exon 10 including MPLW515L/K and MPL550SN which is both a germline and somatic (ET) mutation resulting in hereditary thrombocythemia. CALR gene is on chromosome 19p13.2 and is responsible for a multifunctional Ca\(^{2+}\) binding protein chaperone mostly localized in the endoplasmic reticulum. These mutations are also found in cases with thrombocytosis other than MPN such as myelodysplastic syndrome with ring sideroblasts (MDS-RS), MDS/myeloproliferative neoplasm with RS and thrombocytosis (MDS/MPN-RS-T) and prefibrotic PMF (pre-PMF). Hereditary thrombocytosis has also been reported with germline JAK2 mutation (JAK2V617I) and associated with vascular events but not fibrotic/leukemic progression. JAK2 mutated patients are usually older with either higher hemoglobin or platelet count and abnormal serum erythropoietin levels. These patients are more likely to develop a thrombosis.\(^5\)\(^7\)

The JAK2V617F mutation occurs in about 55% of patients with ET, CALR mutation is seen in 15%-24% of the ET patients and MPL gene mutation occurs in about 4% of affected patients.\(^5\)\(^8\)\(^9\) CALR or MPL mutations may coexist in some patients. Unfortunately, the clinical significance of the coexistence of multiple mutations is still unclear.\(^3\) Mutations other than JAK2, CALR, or MPL have been found in approximately 53% of patients with ET with the most frequent being the tet-methylcytosine dioxygenase 2 (TET2) [16%], ASXL transcription regulator 1 (ASXL1) [11%], DNA-methytransferase 3A (DNMT3A) [6%], and component of the U2 snRNP (SF3B1) [5%].\(^1\)

The most common risk for patients with ET is thrombosis. This increased risk may also be due to the presence of giant platelets that may lead to microvascular occlusion and large vessel thrombosis. Bleeding may also result in patients with extreme thrombocytosis. The reason could be due to acquired deficiency of von Willebrand Factor (VWF).

**Clinical and Diagnostic Findings**

The revised 2016 World Health Organization (WHO) diagnostic criteria for ET include four major and one minor criteria. Diagnosis of ET requires meeting all 4 major criteria or the first 3 major criteria including peripheral thrombocytosis, large megakaryocytes in bone marrow, Philadelphia negative MPN or positive
Table 1. Essential Thrombocytethemia WHO diagnostic criteria

<table>
<thead>
<tr>
<th>Diagnostic Criteria</th>
<th>Major</th>
<th>Minor</th>
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<tr>
<td>1. Platelet count ≥450 × 10^9/L.</td>
<td>X</td>
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<td>2. Bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyper-lobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers.</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>3. Not meeting WHO criteria for BCR-ABL1+ CML, PV, PMF, myelodysplastic syndromes, or other myeloid neoplasms.</td>
<td>X</td>
<td></td>
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<tr>
<td>4. Presence of JAK2, CALR, or MPL mutation.</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>5. Presence of a clonal marker or absence of evidence for reactive thrombocytosis.</td>
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JAK2, CALR, or MPL mutation. The minor criterion demonstrates the presence of a clonal marker or absence of evidence for reactive thrombocytosis (Table 1).3,10,11 Peripheral blood morphology and bone marrow examination are often necessary to make an accurate morphologic diagnosis of ET and distinguish it from other myeloid neoplasms, like pre-PMF. Platelets in peripheral blood show anisocytosis with small to giant platelets which may be hypo granular or agranular (Figure 1).

Megakaryocytes in ET are large and mature-appearing and form loose clusters (Figure 2), whereas those in pre-PMF display abnormal maturation with hyperchromatic and irregularly folded nuclei and form tight clusters.12 Patients may present with microcytic hypochromic blood picture due to impaired platelet function resulting in chronic blood loss from the gastrointestinal tract. Generally, bone marrow is hypercellular showing increase in large mature megakaryocytes with hyper-lobulated nuclei, often forming clusters within the bone marrow. If ET is triple negative, that is all three common mutations are absent (about 12% ET patients fall in this category), in these cases the bone marrow may show an increase in pleomorphic megakaryocytes forming loose clusters (Figure 3). Similar megakaryocytic clusters are also seen in the bone marrow of patients with CALR positive ET (Figure 4). These can also be seen with CD61(cluster of differentiation marker found on thrombocytes) immunohistochemical stain (Figure 5).

Figure 1. Peripheral Blood (magnification x600) JAK2 positive ET. Platelet count 1287x10^9/L with platelet anisocytosis including small and large platelets.
Figure 2. Aspirate (magnification x100) JAK2 positive ET. Increased numbers of megakaryocytes can be seen.

Figure 3. Triple negative ET (Magnification x200) Increase in pleomorphic megakaryocytes forming loose clusters.

Figure 4. CALR positive ET (Magnification x400) Increase in pleomorphic megakaryocytes forming loose clusters.
Risk Stratification
Risk stratification is an early step that follows diagnosis and is critically important to guide clinicians towards appropriate therapeutic interventions. Historically, risk stratification looked at two risk parameters; whether the patient was >60 years of age and if there was a history of thrombosis. Patients who met these criteria were high risk. The absence of both risk factors i.e. <60 years of age and no history of thrombosis were classified as low risk. A new 3-tiered score, which included an intermediate risk group, was proposed in 2012 and was called the “International Prognostic Score for Thrombosis for Essential Thrombocythemia” (IPSET-Thrombosis model). The IPSET-Thrombosis score accounted for the presence of cardiovascular risk factors and JAK2/V617F mutation. Previously, classification had allowed a degree of bone marrow fibrosis and morphologic features more resembling myelofibrosis. With the revised WHO classification true ET was characterized by lower white blood cell (WBC) counts, lower hemoglobin levels, lower lactate dehydrogenase levels in plasma, and more importantly a better prognosis, with close to normal survival rates. The data was re-evaluated, and the revised IPSET risk stratification was devised. The revised IPSET-Thrombosis score changed to a 4-tiered system of very-low-risk, low-risk, intermediate risk, and high-risk categories (Table 2) and included the integration of MPL-mutation status. According to the revised 2016 WHO criteria, in triple-negative patients, testing by next generation sequencing (NGS) for the most frequent additional mutations (e.g., ASXL1, DNMT3A, TET2, EZH2 [histone-lysine-N-methyltransferase enzyme], IDH1/2 [isocitrate dehydrogenase 1 or 2], SRSF2 [serine and arginine rich splicing factor 2]) may be helpful to determine the clonal nature of the disease and complement the morphological criteria. Another advantage of using NGS for mutation detection in triple-negative MPNs is the possibility of simultaneous testing of rare variants in JAK2, CALR, or MPL otherwise not detected by conventional assays.

Prognosis and Disease Progression
In ET patient’s, the transformation to acute myeloid leukemia (AML) and/or post-ET myelofibrosis (post-ET MF) are rare events. Risk of transformation to acute leukemia was
reported at 2-3% at 10 years and 5% at 15 years.18 A history of thrombosis and male sex were independent predictors of death.19 ET is considered to have a favorable prognosis A 2014 study reported the median survival of patients with ET to be 19.8 years, inferior to that of the age- and sex-matched United States population and unaffected by driver mutation status.20 Life expectancy in ET for patients younger than 60 years of age is mildly compromised with median survival approaching 33 years. A more recent study in 2019 concluded ET has a similar or slightly lower survival than the general population.18 The distinction made in 2016 by the WHO in pre-PMF and overt myelofibrosis has improved the prognosis, as several cases of ET have been reclassified to pre-PMF which has a slightly worse prognosis.18

The prognostic relevance of the somatic mutations by NGS has been investigated in large ET cohorts of patients.1 SH2B3/LNK (lymphocyte adapter protein), SF3B1, U2AF1 (U2 small nuclear RNA auxiliary factor 2), TP53 (tumor protein p53), IDH2, and EZH2 have been shown to impact on overall, leukemia-free, and myelofibrosis-free survival based on age-adjusted multivariable analysis. Their presence was associated with poor survival in ET (median 9 years vs. 22 years). A study of 502 molecularly annotated ET patients allowed mutational information to be incorporated into a new prognostic model, the Mutation-Enhanced International Prognostic Scoring System (MIPSS) specific for ET (Table 3).22 Overall survival was adversely affected by spliceosome mutations SF3B1 and SRSF2 while U2AF1 and SF3B1 adversely affected myelofibrosis-free survival; earlier studies showing TP53 to be predictive of leukemic transformation were confirmed.1 The number of somatic mutations has also been reported to impact on overall survival with reported hazard risk values of 6.6 for 3 mutations, and hazard risk of 2.2 for 1 or 2 mutations.21 Data on the thrombotic risk associated with MPL mutations are scant, primarily due to low overall frequency. In a 2013 study, the 5-year cumulative incidence of thrombosis was estimated to be around 9% while in the 2008 prospective primary thrombocythemia-1 cohort MPL mutation (4.1% of ET patients) was not predictive of thrombosis.23,24 However, a larger 2008 study, (N=994) in which 3% of subjects had the MPL mutation, demonstrated that it was associated with higher risk of thrombosis when compared to JAK2 mutation or wild-type MPL.25

In an analysis of 576 ET patients, 15.5% of whom had CALR mutations, the 10-year cumulative incidence of thrombosis was 5%. The thrombotic risk of CALR mutated patients was similar to that of triple-negative ones and lower than JAK2- and MPL-mutated patients.26 Later studies confirmed these findings, with a 2015 study of 217 patients with WHO-defined ET or early PMF demonstrating that the lower incidence of thrombosis in patients with CALR mutation vs. JAK2 mutation persisted at 15 years (9.1% vs. 21.7% respectively P=0.04).27 CALR-mutated ET shows clinical features different from JAK2 V617F-positive ET with CALR-mutated patients presenting with a higher platelet count at diagnosis, a lower hemoglobin and WBC count, coupled with a lower thrombotic risk if compared to JAK2 V617F-positive ET patients.28,29 An extreme thrombocytosis may result in minor bleeding or major hemorrhagic complications as a platelet count >1000 x 10^9/L can induce an acquired von Willebrand syndrome (AVWS), a rare but probably underestimated bleeding disorder. AVWS is usually associated with a range of underlying disorders and according to
the International Society on Thrombosis and Hemostasis registry, 15% of cases are linked to an underlying myeloproliferative neoplasm. AVWS is caused by the proteolytic reduction of VWF multimers due to the passive adsorption to the platelet membrane with an inverse relationship between platelet count and the plasma defect of high molecular weight multimers. ET may develop into PMF or PV in small number of patients probably due to pathophysiological continuum, starting from ET and ending in MF. The fact that made the continuum theory attractive was the detection of the V617F mutation in the tyrosine pseudokinase region of the JAK2 gene, being a gain-of-function mutation, resulting in uncontrolled cellular growth in the hematopoietic compartment. The presence of a JAK2V617F mutation indicates MPNs but does not differentiate between them.

Treatment

Current treatment in ET is primarily indicated for the purposes of preventing vascular complications which have been reported to be the leading cause of death. Thrombotic complications can occur in up to 24% of patients with 13% developing a vascular event before diagnosis. Mutational status has an impact on thrombotic risk with a lower rate of thrombosis seen in CALR-mutants as compared to JAK2 V617F/MPL mutants and triple-negative cases. Thrombosis can occur in unusual sites such as the splanchnic vessels (Budd-Chiari syndrome) or cerebral venous sinus. These thrombotic events in unusual sites are more frequent among JAK2 V617F carriers and may represent the first sign of disease onset. In 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) 1.83-1.92]. The risk is increased for both arterial (OR range 1.68-2.59) and venous thrombosis (OR range 2.09-2.5). The JAK2 V617F mutation burden also influences thrombotic risk. JAK2 V617F homozygosity is rare in ET patients but it is thought to confer an increased risk of thrombosis when compared to a heterozygous or wild-type condition. A 2015 study showed that an allele burden of 20-25% or higher independently predicted the risk of arterial and venous thrombosis. Another study found that among WHO-defined ET or early PMF a high JAK2 allele burden (>50%) positively correlated with the thrombotic risk regardless of the WHO diagnosis.

Treatment for patients with ET varies according to individual risk stratification, and ranges from the use of aspirin (acetylsalicylic acid [ASA]) to that of cytoreductive therapy. Current treatments are not intended to be curative but rather directed at reducing the thrombo-hemorrhagic risk. There is general agreement that high risk patients with ET should be treated with low-dose ASA with most doctors giving ASA to practically all ET patients. However, the European Leukemia Net consensus recommendations advise a more restrictive approach with ASA only being given to patients who have microvasculature symptoms and those with cardiovascular risk factors. Antiplatelet therapies should be used with caution in patients with platelet counts >1000x10³/L due to the possibility of the acquired von Willebrand syndrome. In patients with marked thrombocytosis and previous hemorrhages, the use of antiplatelet therapy should be avoided. Worldwide, hydroxyurea [HU (also known as hydroxyurea)] is the most widely used cytoreductive agent for patients with ET. It works by inhibiting the enzyme ribonucleotide reductase, thereby decreasing the production of deoxyribonucleotides. It has long been considered first-line treatment for ET, given its favorable toxicity profile. The most common side effects, which are generally mild, include hyperpigmentation of the skin, oral ulcers, and rashes. A cessation rate of about 10% has been reported due mostly to the presence of leg ulcers. HU produces macrocytosis, and dysplasia can be seen in the bone marrow due to its action on DNA formation. This is usually disregarded as clinically irrelevant HU has been suspected of increasing the risk for leukemia transformation. The issue is contentious, as various studies over several decades either give support for or minimize the leukemic risk.
should be noted that HU is teratogenic. For patients wishing to maintain their fertility interferon therapy may be a more appropriate treatment.

Anagrelide, an inhibitor of cyclic adenosine monophosphate (AMP) phosphodiesterase III, originally designed as an anti-platelet agent was subsequently found to inhibit both megakaryocytic differentiation and proliferation.\textsuperscript{37} At higher doses, anagrelide acts through AMP phosphodiesterase to inhibit platelet aggregation. At lower doses, it also works by decreasing platelet counts.\textsuperscript{38} Anagrelide is generally considered for patients where HU or interferon fails or causes unacceptable toxicities. Studies have evaluated anagrelide as a first-line therapy for ET. However, there is conflicting evidence on the efficacy and safety. One study suggested that anagrelide was not inferior to HU.\textsuperscript{39} In another study, patients receiving anagrelide experienced higher incidences of arterial thrombosis, bleeding complications, and fibrotic progression.\textsuperscript{40} Similarly, non-controlled studies have suggested that more than a quarter of patients receiving anagrelide therapy become anemic while a lesser percentage experience renal insufficiency.\textsuperscript{41} Anagrelide is considered to have a significant side effect profile related to its vasodilator action. Risks reported include fluid retention, headaches, heart palpitations and cardiomyopathy. Cardiac evaluation is recommended before treatment.\textsuperscript{13,38,42}

Interferon-alfa (Interferon-\(\alpha\)) has been used successfully for the treatment of ET for many years, with consistently high hematologic as well as molecular remission rates. Interferon-\(\alpha\) may result in approximately 80% hematological responses as defined by reduced hematocrit, WBC and platelet counts.\textsuperscript{34} Interferon-\(\alpha\) targets the malignant clone to reduce the colony-forming capabilities of erythroid, granulocytic, and megakaryocytic progenitors. It is an effective agent for treating both ET and PV.\textsuperscript{38} Interferon-\(\alpha\) is able to clear not just JAK2- but also CALR-mutated clones, suggesting its potential as a true disease-modifying agent, although there is preclinical evidence that CALR-mutated ET may be less responsive to interferon-\(\alpha\) than JAK2-mutated disease.\textsuperscript{35} Pegylated interferon-\(\alpha\) (P-IFN-\(\alpha\)) treatment in ET, where interferon-\(\alpha\) is conjugated with polyethylene-glycol, is often the second line therapy of choice in patients who are intolerant or refractory to HU. Pegylation allows the interferon-\(\alpha\) to stay in the body longer before it is broken down and eliminated. P-IFN-\(\alpha\) has been shown to be relatively safe and effective and has been associated with both clinical (70-80%) and molecular (10-20%) remissions in some patients, especially in the presence of CALR gene mutations.\textsuperscript{41} However, side effects have been reported with P-IFN-\(\alpha\) and include depression, flu-like symptoms, headache, malaise, fevers, arthralgia, pruritus, injection-site reactions, gonadal toxicity, and thyroid dysfunction.\textsuperscript{13} The average rate of discontinuation due to side effects is approximately 25% with patients younger than 60 years tending to tolerate this agent better than older patients.\textsuperscript{38} Because of the significant side effects, Interferon-\(\alpha\) is usually reserved for younger patients or those who are pregnant.\textsuperscript{34}

While considered a relatively safe treatment, Ruxolitinib is an expensive therapy. It is also an immunosuppressant with an increased propensity for infection and development of skin cancers, especially in those predisposed. The use of Ruxolitinib, not currently licenced for use in ET, needs further evaluation, in part due to the fact ET patients have near-normal life expectancy and exposure to this treatment could be over several decades.\textsuperscript{34}

With an increased incidence of ET in women over men (approximate ratio of 2:1) and 20% of patients receiving a diagnosis at less than 40 years of age, there is the potential for the need to manage pregnancy in female patients with ET.\textsuperscript{18} A study of 234 patients with ET identified 20.49% of females that were fertile at the time of diagnosis.\textsuperscript{18} In these patients, the risk of first-trimester fetal loss (about 3.5-fold) and placental complications (e.g. abortion, pre-eclampsia) is increased if compared to healthy women. Risk factors include previous pregnancy complications and possibly the presence of a JAK2V617F mutation. Venous thrombosis may occur, particularly in the
postpartum period. The risk is also higher in patients with a history of vascular events. Current treatment recommendations in young women wishing to be pregnant or are pregnant include once-daily ASA for “very low-risk” or “low-risk” disease and P-IFN-α for high-risk disease. Both ASA and Interferon-α therapy have been shown to be safe for use during pregnancy and might be associated with lower miscarriage rates in women with ET.41 Interferon-α is not associated with gonadal toxicity and has no teratogenic effects, and therefore is not contraindicated in pregnancy, unlike HU that is teratogenic and requires a recommended 3-6 month washout period for patients wishing to conceive.18 The patient’s clinical history, circumstances, and life events remain the main influences on the treatment of choice for the physician. Table 4 summarizes the potential therapeutic approaches based on the different revised IPSET-Thrombosis model risk categories.

### Conclusion
ET is an acquired MPN characterized by the thrombocythemia but relatively benign prognosis. In the WHO classification, it is defined as a Philadelphia negative MPN. ET is most often caused by JAK2, CALR and MPL mutations. The mutational status and risk of thrombosis dictate the therapeutic regime. Focus of the treatment is to suppress the megakaryocytic proliferation with busulfan, HU, Interferon-α and Anagrelide, and to reduce the risk of thrombosis using antiplatelet therapy such as ASA. Early detection reduces risk of thrombosis and improves prognosis. Despite significant improvement in the diagnostic, prognostic, and therapeutic clinical knowledge about ET, there are still unanswered questions about uncertainties associated with clinical decision making particularly when there is increased incidence of vascular complications and a tendency to progress to pre-PMF, myelofibrosis or acute myeloid leukemia.

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### References


