The Essential Thrombocythemia in 2020: What We Know and Where We Still Have to Dig Deep

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ABSTRACT: The Essential Thrombocythemia is a Chronic Philadelphia-negative Myeloproliferative Neoplasm characterized by a survival curve that is only slightly worse than that of age- and sex-adjusted healthy population. The criteria for diagnosis were reviewed in 2016 by WHO. The incidence varies from 0.2 to 2.5:100 000 people per year, with a prevalence of 38 to 57 cases per 100 000 people. The main characteristics of ET are the marked thrombocytosis and the high frequency of thrombosis. The spectrum of symptoms is quite wide, but fatigue results to be the most frequent. Thrombosis is frequently observed, often occurring before or at the time of diagnosis. The classification of thrombotic risk has undergone several revisions. Recently, the revised-IPSET-t has distinguished 4 risk classes, from very low risk to high risk. Driver mutations seem to influence thrombotic risk and prognosis, while the role of sub-driver mutations still remains uncertain. Antiplatelet therapy is recommended in all patients aged ≥ 60 years and in those with a positive history of thrombosis or with cardiovascular risk factors, while cytoreductive therapy with hydroxyurea or interferon is reserved for high-risk patients.

KEYWORDS: Myeloproliferative Neoplasms, Thrombocythemia, Platelets

Disease Overview

Essential Thrombocythemia (ET) is a Chronic Philadelphia-negative Myeloproliferative Neoplasm (MPN), characterized by marked thrombocytosis, thrombotic and hemorrhagic risk and constitutional symptoms. ET patients carry a low but known risk of disease evolution into other MPNs (Polycythemia Vera and Myelofibrosis) and/or Acute Leukemia. Thrombotic risk stratification and therapy recommendations were recently reviewed.[1] In 2016, the World Health Organization (WHO) revised the criteria for diagnosing ET, identifying 4 major criteria and 1 minor criterion. The major criteria are: platelet value ≥450 000/μL; bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with increased number of enlarged, mature megakaryocytes with hyperlobulated nuclei, no significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and, very rarely, a slight (grade 1) increase in reticulin fibers; exclusion of WHO criteria for BCR-ABL CML, PV, PMF, myelodysplastic syndromes, or other myeloid neoplasms; presence of JAK2, CALR, or MPL mutations as clonal marker. The only minor criterion identified is the absence of evidence for reactive thrombocytosis or the presence of another clonal marker. Diagnosis of ET requires meeting all major criteria or the first 3 major criteria and the minor criterion.[2] There is no recently published data on the real incidence and prevalence of ET. In a retrospective study involving 801 adult patients with thrombocytosis in a tertiary care hospital, primary thrombocytosis was observed in 5.2% of cases.[3] The available data show that the estimated ET annual incidence in the United States is 2.5 cases per 100 000, whereas the prevalence is estimated to be 24 cases per 100 000. Furthermore, other studies estimated the annual ET incidence in Western countries in 0.2 to 2.5 cases per 100 000 with a prevalence of 38 to 57 cases per 100 000.[4,5] Moreover, the incidence is higher in female than in male patients, with an approximate ratio of 2:1.[6] The median age at diagnosis is 60 years with 20% of patients younger than 41 years old. According to some studies conducted over the last few years, there has been a decrease in the age of ET diagnosis: 56 years versus the previous data of 60 years.[7,8] In our experience, in 253 consecutive patients diagnosed with ET between January 1997 and December 2019, 36.3% were diagnosed under 61 years of age, 25.7% under 51 years and 16.67% under 41 years. ET is characterized by overall favorable prognosis if compared to the other MPNs (but life-expectancy in ET is shorter than the general population) with an expected survival of 18 to 19.8 years (compared to 13.5 years in PV and 5.9 years in PMF). Survival, however, appears significantly better in patients with low risk of thrombosis (26.7 years).[10] The cumulative incidence of blast transformation is lower in ET (3.8%) than in PV (6.8%) and PMF (9.2%). Moreover and similarly, the cumulative incidence of fibrotic transformations is lower in ET (13%) than PV (21%).[7-10]

Mutational Status

During the last 15 years molecular biology advances built up a remarkable knowledge that has improved the ability of diagnosing MPNs. JAK2 (janus activated kinase 2) is a gene located on chromosome 9, locus p24. V617F mutation of JAK2 was first reported in 2005 and represents the most frequent mutation in ET with an estimated frequency of 50% to 60%.[11-14] Furthermore, in 2006 and 2013 further driver mutations have been identified, affecting MPL (myeloproliferative leukemia virus oncogene) and CALR genes (calreticulin). The MPL gene is located on chromosome 1p34, also known as the thrombopoietin (TPO) receptor gene. MPL mutations are present in about 5% of patients with ET, while the CALR gene lies on chromosome 19p13.2 and according to some authors, is closely related to platelets production.[15-18]

JAK2, CALR, and MPL mutations are also found in patients with thrombocytosis other than MPN, such as refractory anemia with ringed sideroblasts and marked thrombocytosis (RARS-t), in which these mutations are usually acquired on a background of SF3B1 mutation. The most frequent CALR mutations are, respectively, a 52 bp deletion (type 1) and a 5 bp insertion (type 2).[19,20] In our series of 253 patients with ET, 72.33% harbored V617F JAK2 mutation, 9.4% CALR mutations, 1.6% MPL mutations. In 16.6% cases, our ET patients were triple-negative.

The clinical significance of JAK2 V617F mutational burden is still controversial, whether quite long investigated. However, it seems that JAK2 burden in ET may be useful to identify cases with a higher risk of evolution to MF, despite a favorable histology (without fibrosis).[21]

Driver mutations were considered to be mutually exclusive. However, Mansier et al revealed that CALR or MPL mutations may co-exist in almost 10% of patients harboring a low burden of JAK2 V617F mutation. Unfortunately, the clinical significance of the coexistence of multiple mutations is still unclear.[22] Simultaneous testing and eventual identification of the 3 driver mutations using targeted next-generation sequencing (NGS) approaches would likely improve the documentation of further cases, and this could allow a better understanding of the multiple mutation phenomenon.[23]

JAK2-mutated ET patients are usually older, show higher hemoglobin levels and white blood cell counts as well as a lower platelet count and serum EPO levels, but more likely to develop thrombosis than patients with wildtype JAK2.[24-26] Furthermore, patients with CALR mutations express a different phenotype than JAK2- or MPL-mutated ET patients. Indeed, they carry a higher platelet and lower hemoglobin values and low absolute leukocyte count, along with lower thrombotic risk.[27,28] Several CALR mutations, related to different pathological phenotypes, have been identified. In particular, the type1 and type1-like mutation variants are associated with a greater risk of MF transformation, while the type2 and type2-like mutations variants are associated with a more indolent clinical course.[29] The mutational landscape of MPNs is actually very complex. Indeed, alongside the aforementioned driver or phenotypic mutations (sufficient to determine the clinical phenotype of the disease), other sub-clonal mutations have been also identified, involving genes already known to be mutated in myeloid neoplasms other than MPNs. Between them, mutations involving TET2, ASXL1, CBL, IDH, and IKZF1 genes have to be considered as diagnostically and prognostically significant. Subclonal mutations can occur more often in conjunction with phenotypic (driver) mutations, but can chronologically either precede or follow them. To date, subclonal mutations do not have a clear diagnostic value, even if the demonstration of their presence is considered in the 2016 WHO revision as the minor criterion for ET diagnosis. Mutations involving these "non-driver" genes are known to occur also in myelodysplastic syndromes as well as in other hematological neoplasms, such as acute leukemia. Interestingly, these mutations have been reported to confer a worse prognosis in patients with diagnosis of primary myelofibrosis (PMF), meaning lower survival and a greater risk of acute leukemia evolution.[30-33] In 2013, the study reported by Nangalia et al highlighted a median of 6.5 mutations in patients with ET if compared to 13 mutations found in patients with PMF. The most frequent sub-clonal mutations were found in DNMT3A, TET2, and ASXL1.[34] Moreover, in a cohort of 181 ET patients, 46% were found to have somatic mutations including TET2 (13%), ASXL1 (11%), DNMT3A (6%), SF3B1 (5%), CEBPA (4%), along with mutations in TP53, SH2B3, EZH2, and CSF3R (2% each). The impact on prognosis is not clear and today the use of next-generation sequencing in ET is still not routine neither recommended by guidelines.[35]

Clinical Features

In MPN symptomatic burden is often severe and affects the majority of patients with the disease. An online survey conducted on 1179 MPN patients aimed at quantifying MPN symptom burden showed that constitutional symptoms and splenomegaly-associated manifestations dominated the clinical picture (70% of patients) and worsened quality of life. Other symptoms reported in the survey were fatigue (81%), pruritus (52%), night sweats (49%), bone pain (44%), fever (14%), and weight loss (13%). Profound fatigue was referred in the MPN patients group in excess of age-matched controls and those patients declared the need to be assisted by caregivers for daily activities or severe disabilities (34.5%). MPN-associated disability accounted for 11.2% between MPN patients in that study.

After that, a 18-item tool (MPN symptoms assessment form [MPN-SAF]) was tested for validation by the same authors' team, in coordination with the Brief Fatigue Inventory, aiming at the evaluation of the symptoms between MF, ET, and PV cohorts in the United States, Sweden and Italy. Patients reported that symptoms associated with MPN disease were severe and frequent among all 3 MPNs.[36]

In a cohort of 161 ET patients the most frequently reported symptoms were fatigue (90.3%), numbness (58.8%), insomnia
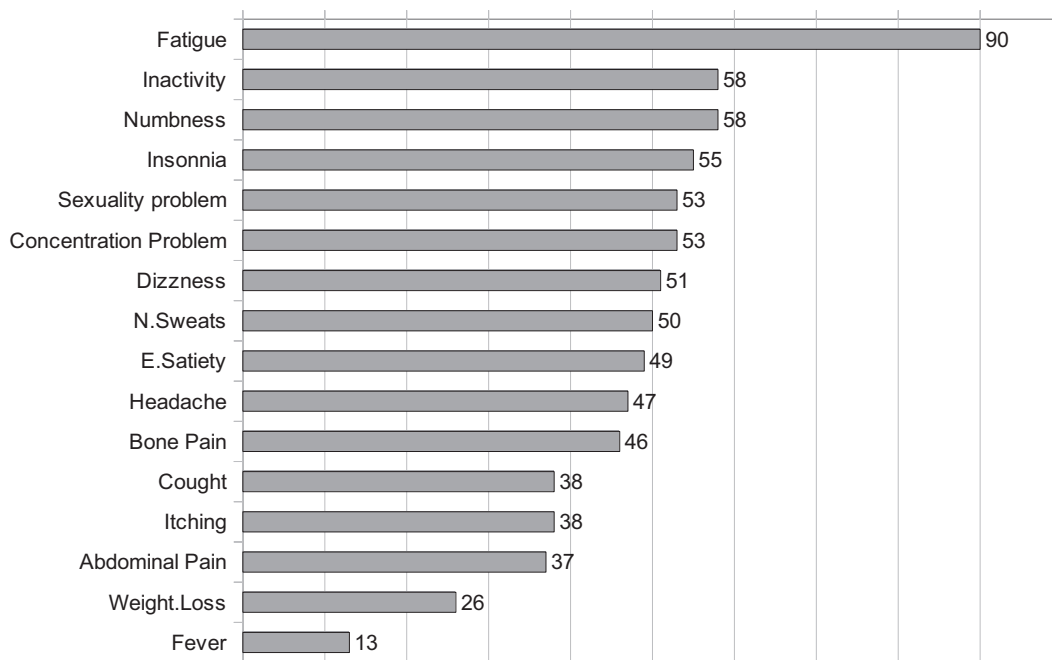
Figure 1. Symptoms in 253 ET patients.

(58%), sad mood (57.3%), vertigo (56,1%), concentration problem (55.8%), inactivity (53.7%), early satiety (53.2%), night sweats (51.3%), sexual activity disorders (51.0%), headache (47.1%), abdominal discomfort (45.3%), bone pain (45.2%), cough (41.4%), itching (40.6%), abdominal pain (38.2%), weight loss (23.4%), and fever (17%). Patients with ET complain with such symptoms with a lower frequency and with a severity than is significantly lower than in PV and PMF patients.[37] Figure 1 shows the frequency of symptoms in the cohort of 253 ET patients visited at our institution between June 1993 and January 2020.

Thrombotic Risk and Bleeding Complications

Thrombotic risk is the main clinical feature of ET, with the risk of a vascular (venous or arterial) event increasing over time after the diagnosis. A study on 1297 WHO-diagnosed ET patients reported 231 cases (17.8%) of thrombosis before or at the time of ET diagnosis.[38] The time from previous thrombosis to the diagnosis of MPN has to be evaluated when critically judging the impact of MPN on the patient's thrombotic history, even if it is hardly discernible if a preMPN thrombosis occurring months before the diagnosis date can be related to the MPN diagnosis itself.

Venous thromboses in atypical sites are more frequent than in the general population, especially involving splanchnic (SVT) or cerebral veins.[39] In this regard, it is reported that over half of the cases of Budd-Chiari Syndrome (supra-hepatic veins thrombosis) occur in the course of an MPN and one-third of portal vein thrombosis are due to MPNs. Of particular note, SVT could be the first and unique sign of an MPN, often presenting without hypercytosis or other complete blood count disorders.[40] For these reasons, several authors recommend

JAK2 V617F and Ex12 mutation, CALR and MPL mutational study in case of SVT, despite the eventual lack of CBC alterations, above all in apparently unprovoked SVT.[41-43]

The conventional thrombotic risk stratification in ET distinguishes patients in 2 risk groups: high-risk group for patients older than 60 years or history of thrombosis, and low-risk group in the absence of both risk factors.[44] The IPSET-t was subsequently validated to improve sub-stratification of thrombotic risk in ET patients: it assigns 2 points each for a positive thrombotic history and the presence of JAK2-V617F mutation and 1 point each for age >60 years and the presence of cardiovascular risk factors (CVR.). Low risk is defined by a score lower than 2, the intermediate-risk by a score equal to 2 and high risk by a score greater than 2.[45] Recently, a revision of IPSET-thrombosis (rIPSET-t) was achieved by the re-analysis of the original IPSET-thrombosis dataset. The rIPSET-t delineate 4 risk categories according to the score obtained by evaluation of 3 variables: age (with 60 years cutoff), thrombosis history and JAK2 mutational asset. Patients younger than 60 years old, negative history of thrombosis and no JAK2-V617F mutation are considered at very low risk; patients with JAK2-V617F mutation but no thrombosis history are considered at low risk; patients with a diagnosis of thrombosis or with the JAK mutation and age over 60 years define the intermediate-risk category; high-risk category is defined by the presence of all 3 risk factors.[46]

In 2017, further enhancement of rIPSET-t was proposed by Tefferi and Barbui adding the negative effect of MPL mutation.[47] Table 1 displays how the 253 ET patients followed at our institution between June 1993 and January 2020 are classified according to the 2 above described scores (IPSET-t and rIPSET-t). It appears of particular note how patients with

Table 1. Distribution of 253 ET patients followed at our institution according to the IPSET-t and revised IPSET-t.

	IPSET-T		REVISED IPSET-T		TOTAL
	V_{LOW}	LOW	INTERMEDIATE	HIGH	
Low	31	5	10	0	46
Intermediate	0	18	12	5	35
High	0	12	7	153	172
TOTAL	31	35	29	158	253

Table 2. Cardiovascular risk factors in 253 ET patients.

CARDIOVASCULAR RISK	N°	%
Smoke	37	14.62
Hypertension	163	64.42
Obesity	22	8.69
Dyslipidemia	66	26.08
Diabetes	35	13.83

mutated JAK2/MPL, aged 60 or less and with the presence of at least one of the designed cardiovascular risk factors, happens to be classified as high-risk with the IPSET-t classification and as low-risk with the revised IPSET-t.[48]

In Table 2 we listed the frequencies of cardiovascular risk factors in our dataset of 253 ET patients. Nowadays, the rIPSET-t does not take into account the impact of CVR, and their eventual presence does not currently influence the choice of whether prescribing cytoreductive therapy or not. However, our experience revealed a close correlation between the presence of one or more CVR (cigarette smoking, hypertension, diabetes, obesity, dyslipidemia) and the occurrence of thrombotic events in MPNs. In fact, the frequency of thrombosis is lower in patients without CVR (11/63) when compared to patients with only one CVR (19/98) and the difference is also bigger when the comparison is made with patients carrying more than one CVR (37/92) (P = .0009). This data confirm previous experiences showing, through retrospective analysis, the correlation between the presence of cardiovascular risk factors and thrombotic events.[49]

The frequency of bleeding complications in patients with ET varies in different studies. In a 2012 international study conducted on 891 ET patients, 55 major bleeding events occurred (6.17%).[50] Previously, several reports reported the incidence of bleeding events as the 36% to 37% of patients with ET per year. In 2005, Elliott and Tefferi reported an incidence of bleeding events equal to 0.33% per person per year.[51] The most frequent hemorrhages concern the gastrointestinal and urogenital apparatus, but literature exists also for other bleeding sites, between which the central nervous system.

Bleeding episodes are related to extreme thrombocytosis (PLT > 1—1.5 million/µL) and may be associated with or due to acquired vonWillebrand Disease (a-vWD). The mechanism that causes the a-vWD consists in the absorption of large vonWillebrand multimers by the platelets' membrane when they exceed the above mentioned concentration limits, determining lack of vonWillebrand Factor's activity and therefore the failure of coagulation Factor VIII stabilization and function. For these reasons, extreme thrombocytosis is today an indication for starting cytoreduction.[52,53]

Disease progression

In ET the frequency of evolution in post ET myelofibrosis is lower compared to polycythemia vera and also the evolution in acute leukemia is a rarer event compared to the other MPNs. The risk of transformation in acute leukemia was reported as 2% to 3% at 10 years and as 5% at 15 years.[54] Recently, the publication of the 2016 revision of WHO criteria for MPN diagnosis that better distinguishes pre-fibrotic MF from ET permitted to better identify cases that previously fell into the diagnosis of ET, but had pre-fibrotic MF features and then carried a higher risk of transformation. In a large international study involving patients with ET or pre-fibrotic MF, the 10-year survival rate accounted for 89% and 76%, leukemic transformation frequency accounted for 0.7% and 5.8%, and progression of fibrosis for 0.8% and 12.3%, respectively.[55]

Risk factors for leukemic transformation in ET patients were supposed: the presence of anemia, platelets count <1 million per microliter, advanced age and leukocytosis as well as reticulin grading (if more than 0) and bone marrow cellularity (if reduced) seem to play a role.[56,57] In 2012 a prognostic model to predict survival in patients with ET (International Prognostic Score for ET: IPSET) was proposed. According to the IPSET score, 2 points are given for age >60 years, 1 point for previous thrombosis and 2 points for WBC > 11 × 10⁹/L. Patients with a score equal to 0 are considered at low risk for transformation, a score of 1 to 2 identifies the intermediate risk, while >2 points define the high-risk category.[58]

Mutation subtype may play a role in the risk of transformation to acute leukemia or post-ET MF, but more data are still needed. In particular, regarding leukemia transformation, a study highlighted that JAK2-mutated patients carry a higher risk of transformation than CALR-mutated cases, but this difference was not of any statistical significance.[59]

Therapy

In 2018, the LeukemiaNet (ELN) consortium, based on retrospective studies, recommended low-dose aminosalicylic acid (LDA) for high-risk patients according to the IPSET-t classification. LDA is also recommended for low and intermediate-risk patients, either in cases with age ≥60 years or uncontrolled CVR or mutated JAK2.[60] The use of LDA in patients with low-risk essential thrombocythemia with CALR mutation can

increase the risk of bleeding without decreasing the thrombotic risk and thus is controversially prescribed and has to be evaluated case by case by the hematologist.[61] According to a panel of Italian experts, the use of LDA in patients with ET for primary thrombosis prophylaxis requires randomized trials investigating the optimal dose and frequency of administration aiming to clinical outcomes in terms of both thrombosis prevention and risk of bleeding.[62] The ELN expert panel recommends cytoreduction for high-risk ET and indicates hydroxyurea and recombinant interferon-alfa (rIFNalfa) as possible first-line therapies. According to the ELN experts, anegrelide is not recommended in this setting, being the evidence of non-inferiority to hydroxyurea of insufficient quality and in the basis that benfit-risk balance was unfavorable. On the contrary, the ELN agrees that the patients falling into the low or intermediate risk with well-controlled cardiovascular risk factors do not need cytoreductive therapy. Early start of cytoreductive therapy is moreover recommended by ELN for patients who switch to the high-risk category when completing the 60 year of age or following a thrombosis or a major hemorrhagic event and/or whenever the platelet count overcomes 1.5 million per microliter. Anegrelide or rINFalfa are recommended as second-line therapies in hydroxyurea resistance or intolerance. Cytoreduction may be also required for progressive myeloproliferation-associated signs (eg, increasing splenomegaly) or uncontrolled ET-related systemic symptoms.[59]

Imetelstat, Vorinostat, Givinostat, and Ruxolitinib have been experimentally tested on ET patients with hydroxyurea resistance or intolerance. A total of 18 patients received the telomerase inhibitor Imetelstat at the dose of 7.5 or 9.4 mg/kg intravenously once a week with target platelet count 250 000 to 300 000 per microliter. The primary endpoint was hematologic response. Hematologic response was reached in all 18 patients, while 16 patients (89%) had a complete hematologic response and in 7 of the 8 patients who were positive for JAK2 V617F mutation, the molecular response was achieved (88%; 95% confidence interval). CALR and MPL mutant allele burden was also reduced by 15% to 66%. However, Imetelstat showed a high risk of grade 3 to 4 neutropenia, thrombosis and abnormal liver function test.[63] The histone deacetylase inhibitors Vorinostat and Givinostat were able to induce some grade of spleen reduction, systemic symptoms relief and hematologic response in a substantial proportion of tested patients.[64,65] Verstovsek et al published the results of Ruxolitinib therapy in patients with hydroxyurea-intolerant/resistant ET, demonstrating to achieve a clinically meaningful and durable reduction in PLT and WBC counts and sensible improvements in ET-related symptoms.[66] On the contrary, results from the MAJIC-ET trial published by Harrison et al on Blood in 2017 showed no difference in complete response (defined as PLT less than 400×10^9 per liter; normal spleen size on imaging; absolute WBC less than 10×10^9 per liter) between treatment with Ruxolitinib and best available therapy (BAT) in HU-resistant/intolerant ET. The same study revealed shorter duration of complete response with Ruxolitinib than BAT.[67] However, considering the almost benign global outcome of ET patients and the low rate of evolution in MF and AML, Ruxolitinib therapy in these patients appears questionable.[68]

Pregnancy

Out of 253 patients with ET visited in our center, 172 were women and 42 of them (24.4%) were capable of child-bearing at the time of diagnosis. Between fertile female ET patients, the risk of first-trimester fetal losses is about 3.5-fold and placental complications (eg, abruption, pre-eclampsia) is increased if compared to healthy fertile women.[69-71] The augmented risk of these events seems to be mediated by thrombosis. Risk factors include previous pregnancy complications and the presence of V617F mutation of JAK2. Venous thrombosis may occur, particularly in the postpartum and the risk is higher in patients with a positive history of vascular events.[72] Furthermore, in ET pregnant patients, the incidence of postpartum hemorrhage is estimated to be around 9%. With regard to fetal complications, the perinatal mortality rate was 17 per 1000 live and stillbirths. Of the newborns, 22% were below the 10th percentile in weight, while 13% of them required admission to the neonatal intensive care unit.[73]

In 2011, the ELN defined treatment approach during pregnancy, though evidence of the impact on outcome is weak. For low-risk patients, low dose aspirin is recommended; moreover, prophylactic doses of low molecular weight heparin (LMWH) is recommended from the day after delivery until 6 weeks postpartum. For high-risk patients with previous major thrombotic events or severe pregnancy complications, prophylaxis with LMWH throughout all the pregnancy is recommended, with LDA withdrawal in case of bleeding complications. If platelet count reaches >1 500 000/microliter, it is suggested to consider rIFNalfa to reduce bleeding risk.[74] In 2014, consensus statement of the Haemostasis Working Party of the German Society of Hematology and Oncology (DGHO), the Austrian Society of Hematology and Oncology (ÖGHO), and Society of Thrombosis and Haemostasis Research offered similar recommendations (GTH e.V.).[74]

Familial ET

Rare cases of ET in the same household are reported. Predisposition haplotypes, including 46/1,[75] and predisposition alleles have been identified and seem to confer an increased risk of developing, not only MPN, but also JAK2-V617F clonal hematopoiesis.[76-78] Maffioli et al, analyzed 22 cases of familial MPNs, for a total of 45 patients. Within this cohort, disease phenotype and genotype are heterogeneously distributed and occurrence has often a vertical distribution.[79] Among our patients, a unique case of familial ET has been identified. The case concerns a brother and a sister who share only 1 parent (the father); in both cases, the diagnosis of ET was made according to the 2016 WHO criteria. Of particular interest, the molecular signature, which is a type2 CALR mutation in the male patient

and the JAK2 V617F mutation in the sister, a previously unpublished data. Unfortunately, it has not been possible to perform further molecular testing on the common father, as he died several years before the occurrence of first manifestations of his sons' diseases. It is advisable to interview all patients with ET diagnosis to determine whether there is a family history of uninvestigated thrombocytosis or already known MPN.[76]

Conclusion

Literature data and clinical experience show that ET is a chronic myeloproliferative neoplasm with a relatively benign prognosis, with overall survival data appearing similar or slightly lower than that of the age-matched healthy general population.

The definition of prefibrotic MF according to 2016 WHO revision, better selected patients with ET and therefore further improved the prognosis of ET between the MPN patients group, being that histological revision sometimes permits to undercover prefibrotic MF where ET was previously diagnosed. The mutational status and the thrombotic risk critically influence therapeutic choices. Moreover, the introduction of very advanced molecular biology techniques has greatly improved the understanding of the pathogenesis and the ability to make diagnosis and estimate prognosis. Despite this improvement, unfortunately, many diagnoses of ET are still performed after the thrombotic event. In our experience, many patients later diagnosed with ET, undergo the first hematology evaluation after a long story of thrombocytosis. In the effort to achieve an early diagnosis, we suggest to improve the divulgation of the new WHO 2016 criteria between general practitioners with the aim of anticipating the specialistic exams that may lead to ET diagnosis. Early detection of ET may further minimize the risk of thrombotic events and globally improve prognosis.

Author Contributions

VA and MS manuscript conception and writing, SM manuscript critical revision, MN, MC, MM, CR, ADS, DS reference collection and study, SS project overview and revision.

Ethical Approval

Data were obtained and analyzed according to the Helsinki declaration. Patients gave appropriate informed consent for this study.

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Data Availability

Data used to support the findings of this study is available from the corresponding author upon request.

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