Clinical, laboratory and molecular features in essential thrombocythemia

Aspecte clinice, moleculare şi de laborator în trombocitemia esenţială

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Abstract

Essential thrombocyhtemia is a BCR-ABL negative myeloproliferative disorder (MPD) distinct from polycytemia vera and idiopathic myelofibrosis and characterized by persistent thrombocytosis, excessive proliferation of megakariocytes in the bone marrow, normal erythrocytic mass and the absence of prominent bone marrow fibrosis. In the last few years new information on pathogenesis of myeloproliferative disorders became available: the discovery of an acquired recurrent molecular abnormality in the 14th exon of the JAK2V617F gene resulting in a substitution of valine for phenylalanine at position 617 in the JAK2 protein (V617). We analyzed the recently identified JAK2V617F mutational status, the relevance of mutated allele burden for clinical presentation, thrombosis and hemorrhagic complications.

Keywords: *essential thrombocytemia, mutation JAK2 V617F, thrombosis and hemorrhagic complications.*

Rezumat

Trombocitemia esenţială (TE) este o boală mieloproliferativă BCR-ABL negativă, entitate distinctă faţă de policitemia vera şi mielofibroza idiopatică, caracterizată prin trombocitoză persistentă, proliferare predominant megakariocitară în măduvă, masă eritrocitară normală şi absenţa fibrozei. În ultimii ani, noi informaţii cu privire la patogeneza bolilor mieloproliferative s-au datorat descoperirii în anul 2005 a unei mutaţii la nivelul genei JAK2V617F (la nivelul exonului 14), avand ca rezultat inlocuirea valinei cu fenilalanina in pozitia 617. S-a urmărit detecţia mutaţiei JAK2V617F, relevanţa prezenţei alelei mutante în caracteristicile clinice, complicaţiile trombotice şi hemoragice.

Cuvinte cheie: *Trombocitemie esenţială, Jak2V617F, complicaţii trombotice, hemoragice.*

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Introduction

Essential thrombocythemia (ET), polycythemia vera (PV) and myelofibrosis with myeloid metaplasia (MMM) are BCR/ABL negative classic myeloproliferative disorders (myeloproliferative neoplasms – WHO 2008 classification) characterized by the clonal expansion of an abnormal stem/progenitor cell [1]. Essential thrombocythemia is a myeloproliferative disorder characterized by a persistent thrombocytosis associated with megakaryocyte hyperplasia in the absence of erythrocytosis and leukoerythroblastic blood picture, acknowledged as clinical syndrome for the first time in 1934 by Emil Epstein and Alfred Godel. In 1951 William Dameshek settled the term of myeloproliferative disorder to characterize the clinical and pathogenetic interrelation between ET, PV and MMM. In 2005, five researchers groups identified an acquired mutation in *JAK2* gene (Janus kinase 2) present in almost all subjects with PV and in approximately half of the subjects with ET and MMM. This mutation consists of the substitution of guanine with thyamine at position 1849 of *JAK2* gene (1849 G > T), resulting the substitution of valine with phenylalanine at position 617 of JAK2 (V617F) protein. The protein JAK2 V617F was proven to have a constitutive tyrosine kinase activity leading to non-stimulated hematopoietic progenitors' proliferation especially on the erythroid and megakariocyte lines. This finding had significant implications in the understanding of molecular pathogenesis, genotype-phenotype interrelation (a single mutation generates three different disorders), clinical characteristics, leukemic transformation of the disorder and in diagnostic criteria revision [2- 4].

Essential thrombocythemia incidence is of 0.2 – 2.5/100.000, the average age is of 55 years old and 20% of the subjects have less than 40 years of age. It is extremely rare in children and more frequent in women (1.5-3:1) [5]. The symptoms of essential thrombocytemia subjects are variable. The disorder is accidentally discovered on routine blood test in 12-67% of the subjects. Other subjects are diagnosed when investigated for thrombotic complications (small or large vessels thrombosis) or for minor bleeding. Major bleedings are a rare diagnostic situation[6]. After being diagnosed with ET, 13-37% of patients have symptoms related to hemorrhagic disorders and 22-84% have thrombotic accidents [6]. The microvascular symptoms are also frequent due to microcirculation ischemia: headaches, paresthesia, visual disorders, erythromelalgias [7]. The paradox of the hemorrhagic-thrombotic complications (the association of thrombotic and hemorrhagic accidents in the same subject) is due to an acquired von Willebrandt syndrome in subjects with extreme thrombocytosis caused by the increase of von Willebrandt factor multimer proteolysis by the ADAMTS 13 protease dependent on the high level of thrombocytes.

The positive ET diagnostic requires the presence of a persistent, non-reactive thrombocytosis and the exclusion of other myeloid disorders which may resemble ET (chronic myeloid leukemia, PV, MMM, myelodysplastic syndromes). The *JAK2* V617F mutation discovery in 2005 which is present in 50% of ET cases and also in other myeloid diseases had impact over the re-definition of diagnostic criteria for ET and other myeloid diseases. The presence of *JAK2* V617F mutation represents a useful molecular test proving the presence of a myeloproliferative disease and the absence of the reactive thrombocytosis, but the diagnosis of ET also requires other clinical, anatomic, pathological and laboratory data to exclude PV, MMM, MCL or other myeloid diseases [7-8]. The optimal therapeutic strategy intended to prevent vascular events is dependent on the presence or absence of thrombosis risk factors, requiring prognostic stratification in risk groups depending on age, thrombotic history, cardiovascular risk factors and thrombocyte count. The therapeutic recommendations for ET are based on results obtained in international clinical trials: "Polycythemia

Table 1. Risk classification of essential thrombocythemia [2]

Risk category	Age over 60 or thrombosis history	Cardiovascular risk factors (HTA, hypercholesterolemia, diabetes, smoking)
Low	No	No
Intermediary	No	Yes
High	Yes	-

Table 2. Risk classification and treatment algorithm for patients with essential thrombocythemia

Risk category	Age under 60	60 years or over	Women at procreation age
Low	Low doses of aspirin	-	Low doses of aspirin
Intermediary	Low doses of aspirin	-	Low doses of aspirin
High	Hydroxiurea + Low doses of aspirin	Hydroxiurea + Low doses of aspirin	Interferon alfa + Low doses of aspirin

vera study group (PVSG)", "European collaboration on low dose aspirin in polycythemia (ECLAP)", "Bergamo Trial" and "Primary thrombocythemia 1 study (PT1)" [9, 10].

The present paper aims to identify the clinical and histopathological particularities of therapeutic and evolutive aspects in subjects with ET, determination of *JAK2* V617F mutation status, identification of new possible thrombohemorrhagic risk factors, setting some molecular defined clinical subsets, including subjects in risk categories. At the same time, a new risk category elaboration necessary to settle the therapeutic strategy intending the minimization of the thrombohemorrhagic events, taking into account the new risk factors and also a a new diagnostic algorithm for ET in the light of the new scientific discovery: the mutation of *JAK2* V617F.

Material and method

The presented study is of clinical - static, retrospective-prospective type including 66 subjects diagnosed with ET at "Ion Chiricuţă" Oncology Institute, Hematology Clinic of Cluj-Napoca between June 1975 – September 2009. The evaluation was based on anamnestic, clinical-biological and therapeutic data in subjects observation sheets. The diagnostic criteria were those settled by the Polycythemia vera study group (1986), 2001 WHO criteria and 2008 revised WHO criteria using as molecular test the detection of *JAK2* mutation. Major vascular events considered were: transient ischemic attack, acute myocardial infarction, arterial and venous thrombosis, hemorrhages. The treatment was done complying with the subjects' prognostic stratification (*Table 1, 2*).

JAK2 V617F mutation analysis was performed in most cases at Medical Genetics Department by tetra primer PCR, on DNA obtained from peripheral blood.

In order to analyze *JAK2* V617F mutation, genomic DNA was obtained from leukocytes from 300 µl peripheral blood using Wizard Genomic DNA Purification Kit (Promega, Ma, USA). Genotyping for *JAK2* V617F mutation was performed essentially as described initially by Jones et al [11], by a tetra-primer PCR technique, with some modifications described by Trifa et al [12]; this technique allows the study of the normal and the mutant alleles in the same reaction, using 4 primers: 2 of them are gene-specific, while 2 of them are allele-specific; the control fragment, which is obtained in all the samples and is gene specific has 463 bp; the normal allele specific

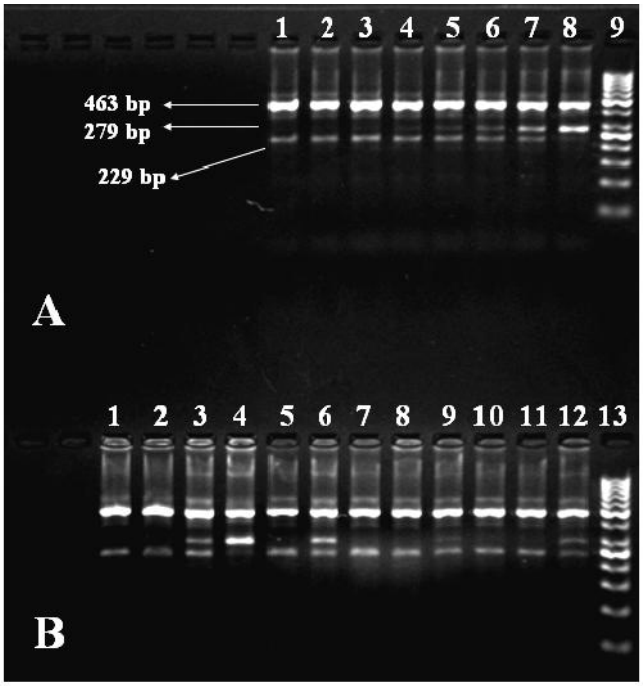

Figure 1. A) Dilution-based scale employed for estimating the mutant allele burden. 1 - *JAK2* V617F negative sample; 2 - 1/64 proportion of mutant allele; 3 - 1/32 proportion of mutant allele; 4 – 1/16 proportion of mutant allele; 5 – 1/8 proportion of mutant allele; 6 – 1/4 proportion of mutant allele; 7 – 1/2 proportion of mutant allele; 8 – almost 100% mutant allele sample; 9 – 50 bp DNA marker. **B) *JAK2* V617F negative and positive patients.** 1, 2, 5, 7, 8 – *JAK2* V617F negative patients; 3 – *JAK2* V617F positive patient, allele burden 25-50%; 4 – *JAK2* V617F positive patient, allele burden 75-100%; 6, 12 – *JAK2* V617F positive patients, allele burden around 50%; 9 *JAK2* V617F positive patient, allele burden around 15%; 10,11 *JAK2* V617F positive patients, allele burden weak (<5%); 13 50 bp DNA marker

fragment has 229 bp, while the mutant allele specific fragment has 279 bp. Even though an exact quantification of the mutant allele could not be performed, the mutant allele levels were estimated using a semiquantitative approach, described in detail by Trifa et al elsewhere [12]. Briefly, a sample which contained almost exclusively mutant allele was serially diluted in a DNA sample negative for JAK2 V617F mutation, using a dilution factor of 1/2; both samples had the same DNA concentration of 75 ng/µl. In this way, 6 aliquots were obtained in which the proportion of the mutant allele was 1/2, 1/4, 1/8, 1/16, 1/32 and 1/64 respectively, all of them with a constant DNA concentration of 75 ng/µl. The scale was used as a reference each time when the patients' samples were amplified and run in electrophoresis gel. The proportion of *JAK2* mutant allele, which corresponds approximately to the degree of the clonal expansion, was estimated visually by comparing the intensity of the signal with the references from the scale. The results were expressed as proportion of mutant alleles from total *JAK2* alleles. *Figure 1* presents an electrophoresis gel analyzing the *JAK2* V617F mutation status in several patients, as well as the reference, dilution-based scale.

The results were processed in statistic SPSS program. Descriptive and statistically analyzed data were obtained from the patients both at diagnosis and throughout the clinical course. Comparison between categorical variables was performed by chi – squared statistics. Comparison between categorical and continuous variables was performed by *t* test. P values < 0.05 were considered statistically significant.

Results

Sixty six subjects diagnosed with ET in the Hematology Clinic, "Ion Chiricuţă" Onchology Institute during June 1975 – September 2009 were evaluated (*Table 3, 4*).

Sixty two subjects (95.64%) were alive out of 66; 4 subjects (4.54%) deceased when the study ended. The majority of the subjects, 42 (63.63%) were females. The average age was 54.4 years old. Fourteen subjects were diagnosed when they were less than 40 years old, mostly women (78.5%). Essential thrombocythemia incidence was greater in women under 40 years old on the sample studied (3-1) and in the group of age 60 – 80 years (2-1) comparing with males. Seven subjects (10.6%) had a family his-

Table 3. Clinical and laboratory characteristics of patients with essential thrombocythemia at diagnosis

Order no.	Characteristics	Data
1.	**Gender**	**No (%)**
	Male	24 (36.37%)
	Female	42 (63.63%)
2.	**Age at diagnosis**	**Years**
	Male	57.41 years
	Female	52.64 years
	Total subjects	54.37 years (22-81)
3.	**Average value of Leucocytes (L) x 10^9/L**	10.115 (4.500 – 75.000)
4.	**Average value of Hemoglobin (Hb), g/dL**	13.81 (5.00 – 33.9)
5.	**Average value of Hematocrit (Ht), %**	41 (21 – 54)
6.	**Average value of Thrombocytes (Tr), x 10^9/L**	1072.78 (489.00-7500.00)
7.	**Average value of MPV**	8.95 (5.90– 11.00)
8.	**Average value of LDH**	424 (241 – 1364)
9.	**Cholesterol**	
	Normal	38 subjects (57.57%)
	High	20 subjects (30.30%)
	Undetermined	8 subjects (12.13%)
10.	**Smokers**	
	24 smokers	(36.36%)
	37 nonsmokers	(56.07%)
	in 5 subjects was not established	(7.57%)
11.	**Diabetes**	2 (3.03%)

tory of vascular events (AMI, ischemic attack) and 2 of them (28.5%) developed similar to one of their parents, arterial thrombosis; 2 (3.03%) had a history of other neoplasias (laryngeal cancer, prostate). The clinical symptoms of ET were variable. In 36 subjects (54.54%) the disease was discovered on a routine blood test. Many of subjects were diagnosed when having thrombotic complications or minor bleedings. Nineteen of the subjects (28.78%) presented a history of vascular events and 11 of the subjects with symptoms (16.6%) had thrombohemorrhagic attacks at diagnostic (*Table 5*). Three subjects (4.54%) had general symptoms (asthenia, weight loss), 14 subjects (21.21%) had microvascular symptoms and one subject (1.51%) presented itching. One subject (1.51%) presented exacerbated symptoms due to chronic obliterating arteriopathy of the lower limbs most probably also due to thrombocytosis. Clinical examinations were scarce, splenomegaly was found in 10 subjects (15.15%), and only one subject had a spleen over 16 cm on ultrasound.

After establishing the diagnosis of ET, more than half of the subjects, 62.12% had no complications concerning this disease (vascular events and the aforementioned microvascular symptoms); 14 (21.21%) had major vascular events, 11 (16.60%) had microvascular symptoms, 3 of them presented severe erytromelalgia evolving to gangrene. Most of the vascular events (92.85%) were present in high risk subjects. The thrombotic episodes were associated with leucocytosis, the leucocyte count being

Table 4. The follow-up period of the patients with essential thrombocythemia: from diagnosis till 2009

Timelapse since diagnosis	Subjects number	Percentage
0 – 1 year	12	18.1%
1 – 2 years	11	16.6%
2 – 3 years	14	21.1%
3 – 4 years	7	10.1%
4 - 5 years	6	9.1%
5 – 6 years	3	4.5%
6 – 7 years	6	9.1%
7 – 8 years	1	1.5%
8 – 10 years	3	4.5%
Over 10 years	3	4.5%

Table 5. Distribution of the vascular events in patients with essential thrombocythemia

Vascular events	In personal history (no, %)	At diagnosis (no, %)	Total (no, %)
Acute Miocardial infarction	3 (4.45%)	-	3 (4.54%)
Stroke	4 (6.06%)	2 (3.03%)	6 (9.09%)
Arterial thrombosis	1 (1.51%)	1 (1.51%)	2 (3.03%)
Venous thrombosis	5 (7.57%)	1 (1.51%)	6 (9.09%)
Bleedings	6 (9.09%)	7 (10.6%)	13 (19.69%)

higher in subjects with thrombotic complications (88.9%) with an average value of 10.7 x 10^9/L compared to the ones with no thrombotic events (23.7%), with average value of the leucocytes of 9.2 x 10^9/L. Four subjects (6.06%) of the studied sample were deceased: 2 by large bleedings at 4 and 9 years from diagnosis. One subject was diagnosed with pulmonary cancer at 3 years from ET diagnosis (presented with erytromelalgia, JAK2 25%, megakaryocyte proliferation with large and mature morphologic aspect characteristic of ET), dying at 6 months after establishing second neoplasia diagnosis and the fourth subject evolved to post-thrombocythemia myelofibrosis after 23 years of evolution, dying after 34 years due to cerebral hemorrhage (on account of severe thrombocytopenia). The subject represents the only case of clonal expansion to post-thrombocythemia myelofibrosis of the studied sample (1.51%) after 32 year of evolution and being treated with alkeran, ciclophosphamide and hydroxycarbamide (HC) in the last period.

The osteomedullar biopsy (OMB) was performed in 44 subjects (66.66%), 22 (33.33%) did not undergo this investigation. Out of the 44 biopsies 5 were inconclusive (small fragment of osseous cortical). Hyperplasia of megakaryocytic series with voluminous megakaryocytes and hyperlobulated nuclei was present in 39 osteomedullar biopsies. Three of these (3.79%) presented a degree of reticular hyperplasia (one belonged to the subject with clonal expansion to post-polycythemia myelofibrosis). The erythrocyte lineage was slightly hyperplasic in 4 subjects, as was the granulocytic lineage (4-10.25% of the 39 OMB). The histopathological aspect in one case is noteworthy: megakaryocytic hyperplasia with large megakaryocytes and hyperlobulated nuclei, granulocyte lineage well represented, reduced erythrocytic precursor number, aspect compatible with ET or prefibrotic MMM.

Table 6. Distribution of *JAK2* mutant allele percentage in JAK2 mutated patients with essential thrombocythemia

% mutant allele	Homozygotes	Under 25%	25 – 50%	50 – 75%
Subjects number (%)	2 (4.1%)	9 (27.2%)	18 (54.5%)	4 (12.1%)

Table 7. Laboratory and clinical characteristics of JAK2 mutated patients compared with JAK2 wild type patients

Essential thrombocythemia n = 48	***JAK2* V617F negative**	***JAK2* V617F Heterozygotes**	***JAK2* V617F Homozygotes**
Subjects, no,%	15 (31.2)	31 (64.5)	2 (4.1)
Women, no, %	12 (80)	20 (64.2)	1 (50)
Age, years	49.33	56.66	53.5
L x 10^9/L	9.7 (6.5 – 16.2)	11.8 (5.1-45.0)	42.8 (10.6-75.0)
Hb, g/dL	12.8 (10.3-15.9)	13.9 (10.3-18)	17.9
Ht %	38.2 (27.5 – 47.7)	40.9 (30-54)	49
Tr, x 10^9/L	1163.3 (636-2271)	943.7 (489-2800)	1633 (767-2500)
Splenomegaly	-	4 (12.1%)	-
Itching	-	1 (2.08)	-
Symptoms	8 (16.66)	5 (10.41)	-

The analysis of *JAK2* V617F mutation was performed on 54 subjects (81.8%). Six determinations are under analysis in the moment of study ending, and 48 results are available. The *JAK2*V617F mutation was found in 33 (68.75%) of cases. As represented in *Table 3*, 2 patients (6%) were homozygous for the mutant allele (> 75%), the rest of 31 patients (94%) being heterozygous. 25-50% *JAK2* positive subjects (54.5%) prevailed over *JAK2* heterozygotes (*Table 6*).

As seen in *Table 7*, the leucocytes count was higher in subjects with JAK2 mutation (24 out of 33 or 72.7%) compared to those without *JAK2* mutation (7 out of 15 or 46.6%, $p<0.001$). The presence of *JAK2* mutation was associated with an older age at diagnosis and higher level of hemoglobin and splenomegaly.

Major cardiac and vascular events (arterial and venous thrombosis) present in history or at diagnosis were registered in 9 subjects, all *JAK2* heterozygous; arterial thrombosis prevailed in 88.8%. Starting from the moment of ET diagnosis 5 patients (10.4%) all heterozygous *JAK2* had thrombotic complications, 80% were arterial thrombosis. The arterial thrombosis prevailed both at diagnosis and during the evolution in heterozygous *JAK2* subjects group. The diagnostic parameters at univariate analysis associated with thrombosis events were: the thrombotic history and the age over 60 years ($p = 0.01$) and the leucocytes count above the average value of $8.89x10^9$/L ($p=0.02$). With no significance regarding the thrombotic risk were, separately: gender, the average level of the hemoglobin, the hematocrit and the thrombocyte count. The multivariate analysis was done using the values in diagnostic and in evolution before the vascular events (*Table 8*).

Hemorrhages in history or at diagnosis were present in 4 subjects (8.35%), one was *JAK2* negative, 3 were *JAK2* heterozygous. Two subjects had hemorrhages during the study period; one was *JAK2* negative and the other *JAK2* heterozygous.

The subjects were included in three risk groups for thrombosis depending on risk standard

Table 8. Risk factors for thrombosis in the follow-up (multivariate analysis)

Risk factors	Association with thrombosis at diagnosis (*p*)	Prognostic value for thrombosis in the follow-up (*p*)
Sex	0.10	0.20
Standard risk factors	0.004	0.04
Hydroxiurea	-	0.02
Antiplatelets	-	0.02
L over 8.89 x 10^9/L	0.01	0.06
Hb over 14 g/dL	0.07	0.07
Ht over 42%	0.60	0.40
Tr over 700 x 10^9/L	0.10	0.70

factors (age over 60 and/or thrombosis history). Over half of the studied sample, meaning 36 subjects (54.54%), were in the high risk category and the rest in equal shares – 22.72% in the medium or low risk. We found that 9 subjects of 15, 60% of the low risk group were treated with hydroxycarbamide (HC), one with anagrelid, 4 (44.44%) under 25 years of age and no indication for cytoreduction. In the group with high risk of thrombosis and with definite cytoreduction indication, 3 subjects (8.33%) of 36 were undertreated with aspirin 100 mg, of which 1 older than 60 with thrombosis history. HC was also administered to 3 subjects (20%) of the low risk subjects with extreme thrombocytosis subjects (over 1.500 x 10^9/L) to prevent the hemorrhagic complications and in 5 subjects (33.33%) of the same risk category which had persistent microvascular symptoms with aspirin treatment. HC was administered in 3 subjects (20%) of the intermediary risk group with no indication of cytoreduction. In the studied group, 18.18% of the subjects were over-treated without indication.

Discussion

The current study represent the largest group of patients with ET (n=66) that was studied for the presence of the *JAK2*V617F mutation, clinical and laboratory correlates in our country. This group showed features typical of ET, with a female predominance (63.6%) and median age, 54.4 years old, comparable with other studies. [5,6,13]. In literature 20% of the subjects are less than 40 years old [5]. Fourteen patients (21.2%) in our study were diagnosed when they were less than 40 years old. In the group of age 60-80 years old ET incidence was greater in women comparable with males probably because of the longer life expectancy of women. The mean platelet, leukocyte, and hemoglobin values at diagnosis of ET in our study were similar to those in other studies [5, 13, 14].

A high percentage (54.5%) of our patients were asymptomatic at diagnosis, compared with 27% to 52 % in other 3 studies [6, 13]. Functional symptoms such as headache, erytromelalgies, dizziness, hazy vision were present in 21.2 % of patients. Some authors have attributed these symptoms to microcirculation ischemia induced by in vivo platelet activation and arteriolar microthrombosis. The beneficial effect of antiaggregant therapy in patients supports this hypothesis [10].

Thrombotic and hemorrhagic complications are the main causes of morbidity and mortality in ET. The frequency of thrombotic and hemorrhagic complications, compared with other studies was 16.6%, similar to that observed by Cortellazo (23%) [6], Jensen (19%) [15], but lower compared with other studies, 36.8% [14]. Similar to these studies, arterial thrombosis was more frequent than venous thrombosis. After diagnosis, the incidence of the thrombotic events was

low (21.2%) compared to literature data (between 30 and 48%), with prevailing arterial thrombosis [6, 16]. The osteomedullar biopsy showed in all cases (39 patients) hyperplasia of megakaryocytic series with enlarged to giant megakaryocytes and deeply lobulated (staghorn-like) nuclei. Reticulin myelofibrosis is extremely rare in true ET at presentation similar to our studies, 3.7% [8]. It seems that the megakaryocytic hyperplasia pattern is more important in diagnosis than the presence of a level of fibrosis or other lineage hyper/hypoplasia. The *JAK2*V617F mutation was performed on DNA obtained from peripheral blood by a tetra primer PCR technique described initially by Jones et al [11], with some modifications described by Trifa et al [12]. *JAK2* V617F was detected in 68.75% (n=48) and the observed incidence was similar to those cited by previous studies with smaller number of cases: 57% (n=51, James et al), 43 % (n=21, Baxter et al) [17, 18]. The incidence of mutation is higher compared with 23% (n=93) and 32% (n=115) found in 2 other studies (Kralovics et al., Levine et al.) [13, 19]. The occurrence of homozygous mutation was 6% as noted by previous studies. Nevertheless, all vascular events were present in patients with *JAK2* mutation and there were no significant association among mutation and thrombosis, similar to other studies [18, 19]. In contrast, in other study *JAK2*V617F mutation is strongly associated with a higher rate of thrombosis at diagnosis (p=0.006) [20]. The role of *JAK2*V617F mutation on thrombosis remains controversial, particularly because the timing of thrombotic events (before, at, or after diagnosis of ET) was variable in different publications. Moreover, some studies included relatively small numbers of patients [17, 18]. ET patients with the *JAK2* mutation displayed both higher hemoglobin level and increased leukocyte count, in contrast to other study [18]. The *JAK2* V617 F mutation was associated with an advanced age, an observation that contrasts with the results of a previous study involving 51 patients with ET [18]. The link between age and *JAK2* might represent yet another example of the influence of age and genetic instability. Nevertheless, the *JAK2* V617F mutation was not a thrombotic risk factor predisposed to thrombosis through its positive association with age, hemoglobin and leukocyte count. There was a tendency to overtreat patients with ET, without indication (18.1%). "There is a tendency in medical practice – by no means limited to hematologists- to treat almost any condition as vigorously as possible. In hematology, this consists in attempting to change an abnormal number - whether this number is hemoglobin, white cell count or platelet count to get normal values, whether the patient needs it or not", William Dameshek, 1968. The results of the current study did not attach any treatment-relevant information from mutation screening for *JAK2* V617F in patients with ET although its diagnostic utility in terms of positive predictive value for clonal thrombocytosis was obviously recognized.

Conclusions

ET may be defined as a clonal disorder of thrombocytes production, the multiple social and economic implications justifying the preoccupation of many research groups for the biological mechanisms, diagnostic criteria revision, risk group stratification and therapeutic principles in the light of *JAK2*V617F mutation discovery. In the thrombocythemia diagnostic algorithm (in the absence of signs or symptoms which suggest a reactive thrombocytosis), determination of *JAK2* is indicated as a molecular test to demonstrate the existence of a myeloid disease. Mutation presence or absence does not exclude the mandatory, osteomedullar biopsy, the anatomopathologist having the main role in the differential diagnosis of ET with other myeloid diseases. In subjects with *JAK2* mutation, we noted a higher leukocyte count, contributing probably to the high incidence of thrombotic complications in this group. The risk prognostic score may improve to include besides the known risk factors (age, thrombotic history) the leukocyte count and the *JAK2* V617F mutation status in the light of JAK2 V617F discovery.

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Abbreviations

AMI – acute myocardial infarction
CML – chronic myeloid leukemia
DNA – dezoxiribonucleic acid
ECLAP – European collaboration on low dose aspirin in polycythemia
ET – essential thrombocythemia
HC – hydroxycarbamide
MMM – myelofibrosis with myeloid metaplasia
MPD – myeloproliferative disorder
OMB – osteomedullar biopsy
PV – polycythemia vera
PVSG – polycythemia vera study group
WT – wild type

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