INTRODUCTION

The Ph chromosome negative myeloproliferative neoplasms are a class of stem cell derived haematological malignancies that result in increased production in one or more blood cell types. The three main disorders in the group are PV, AMM and ET.

ET is characterized by an increased platelet count. Clinically it is frequently asymptomatic but the thromboembolic events may lead to disease detection. There is a small propensity to progress to myelofibrosis and acute leukemia (1).

Apart from the BCR/ABL rearrangement in chronic myelogenous leukemia originated by a reciprocal translocation between chromosomes 9 and 22, t(9; 22) (q34; q11) information concerning molecular abnormalities of MPN has been scanty until 2005, when a Janus kinase 2 mutation (JAK2V617F) was discovered in the majority of patients with PV and in 50% of those with ET, AMM and the vast majority with PV. The molecular pathogenesis of the (MPN) has been poorly understood until 2005, when an acquired mutation in JAK2 V617F was reported in around 50% of patients with ET, AMM and the vast majority with PV. The molecular pathogenesis of the (MPN) has been poorly understood until 2005, when an acquired mutation in JAK2 V617F was reported in around 50% of patients with ET, AMM and the vast majority with PV. The molecular pathogenesis of the (MPN) has been poorly understood until 2005, when an acquired mutation in JAK2 V617F was reported in around 50% of patients with ET, AMM and the vast majority with PV.

THE AIM OF THE STUDY

In these report we analyzed the JAK2V617F mutation by polymerase chain reactions as well as its clinical and laboratory associations.

MATERIAL AND METHOD

One hundred and four patients diagnosed with ET at Oncological Institute, Hematology Department Cluj Napoca, were included in this study. The diagnosis of ET was made as a result of the presence of thrombocytosis and increased platelet count. The presence of these abnormalities is a major diagnostic criteria (2-6). One hundred and four patients diagnosed with ET at Oncological Institute, Hematology Department Cluj Napoca, were included in this study. The diagnosis of ET was made as a result of the presence of thrombocytosis and increased platelet count. The presence of these abnormalities is a major diagnostic criteria (2-6). One hundred and four patients diagnosed with ET at Oncological Institute, Hematology Department Cluj Napoca, were included in this study. The diagnosis of ET was made as a result of the presence of thrombocytosis and increased platelet count. The presence of these abnormalities is a major diagnostic criteria (2-6). One hundred and four patients diagnosed with ET at Oncological Institute, Hematology Department Cluj Napoca, were included in this study. The diagnosis of ET was made as a result of the presence of thrombocytosis and increased platelet count. The presence of these abnormalities is a major diagnostic criteria (2-6). One hundred and four patients diagnosed with ET at Oncological Institute, Hematology Department Cluj Napoca, were included in this study. The diagnosis of ET was made as a result of the presence of thrombocytosis and increased platelet count. The presence of these abnormalities is a major diagnostic criteria (2-6).
CLINICAL ASPECTS

According to the criteria proposed by the Polycythemia Vera Study Group and/or, since 2002, the WHO criteria. Data regarding demographic data, laboratory parameters (hemoglobin, hematocrit, leukocytes and platelet count), presenting symptoms and clinical course were collected at diagnosis.

JAK2 mutation analysis

Genomic DNA was extracted using Wizard Genomic DNA Purification Kit (Promega Ma, USA). Genomic DNA was amplified by polymerase chain reaction (PCR) and successful amplification was confirmed by electrophoresis on a ethidium bromide – impregnated 3% agarose gel. The PCR amplifications developed in Eppendorf thermocycler in reactions with 25μl volume with the following composition: 12.5μl 2xPCR Master Mix containing Taq-DNA polymerase 0.05U/μl recombinant, MgCl2 4mM, dNTPs mix 0.4mM each (Fermentas MB1, Vilnius, Lithuania), 10 pm of Jak2 gene specific primers, 8 pm of normal allele specific primers and the mutant one, 75ng genomic DNA and nuclease free water up to 25μl. PCR cycling parameters were: one cycle of 94°C for 7 min, 33 cycles of 94°C for 35 s, at 57°C for 40s, 72°C for 45s and 72°C for 7 min

The results were processed using SPSS program.

RESULTS AND DISCUSSIONS

Mutation screening was performed on genomic DNA of peripheral blood from all 104 patients and JAK 2 V617F was detected in 56 (53,8%) patients. (heterozygous in 92,9% and homozygous in 7,1%). JAK2V617F represents a somatic point mutation involving exon 12 of the JAK2 gene that results in the substitution of valine by phenylalanine at codon 617. The highest mutational frequency was reported in PV (97%) but the mutation also occurs in ET (23-57%), AMM (35-50%). In our study the incidence of mutation JAK2V617F was 53,8%. Other groups identified similar rates of the presence of the mutation in ET (6-11): A substantial proportion of patients with PV or AMM (25-33%) are found to be homozygous for the JAK2 mutation in contrast with the situation in ET where homozygosity for the mutation is rare (3-7%), as noted by others (Jones et al 2005, Kralovics et al 2005), (4,11), similar to our JAK2 homozigous (7,1%) patients. The particular observation is consistent with what has been observed in vitro as well as in animal studies where JAK2V617F has been associated with induction of erythropoietin hypersensitivity in cell lines and erythrocytosis in mice. Transplantation of JAK2 V617F mutated cells induced PV–like phenotype in recipient mice, accompanied by leukocytosis and eventually followed by changes suggestive of myelofibrotic transformation. More recently, by manipulating expression levels of the V617F allele, mice with a ET–like phenotype were also generated in the presence of low levels of mutated JAK2. These models suggested that the level of mutated allele may influence disease phenotype.

Results showed that 47 patients (83,9%) with JAK2 mutation had mutant allele proportion lower than 50% (table 2).

The entire cohort of ET patients (n=104) were considered for correlative studies of clinical and laboratory features. We did not identify statistically significant associations between the presence of JAK2 V617F (either homozygous or heterozygous) and age, gender, platelet count, lactate dehydrogenase, palpable spleen size, functional symptoms at diagnosis, thrombosis. In contrast, statistically significant associations were demonstrated linking the presence of a mutant allele with a higher haematocrit (p=0,01) and leucocyte count (p=0,02) at diagnosis (fig1,2). Our analysis confirmed a correlation between JAK2 mutation and leucocyte count and haematocrit level similar to other studies (Wolanskyj) (7).

In contrast with these study we did not find a significant association between the presence of JAK2V617F and an older age at diagnosis but our results were similar with a previous study involving 51 patients with ET (Baxter et al, 2005)(6).

It is possible that these observation that, ET patients with the JAK2 mutation displayed both higher haematocrit level and increased leucocyte count, represents a molecular evidence for the inadequacy of current diagnostic criteria for distinguishing PV from ET.

Table no. 1. Reported incidences of JAK2 mutation in ET

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Wolanskyj</th>
<th>Chim</th>
<th>Green</th>
<th>Vainchenker</th>
<th>Cross</th>
<th>Jelinek</th>
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<tr>
<td>ET (%)</td>
<td>48,7</td>
<td>56,7</td>
<td>57</td>
<td>43</td>
<td>41</td>
<td>30</td>
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Table no. 2. Level of mutated allele

<table>
<thead>
<tr>
<th>Mutant allele proportion (%)</th>
<th>Patients No.(n=56)</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>1-25</td>
<td>27</td>
<td>48,2</td>
</tr>
<tr>
<td>26-50</td>
<td>20</td>
<td>35,8</td>
</tr>
<tr>
<td>51-75</td>
<td>5</td>
<td>8,9</td>
</tr>
<tr>
<td>76-100</td>
<td>4</td>
<td>7,1</td>
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</table>

Figure no. 1. Correlation between JAK2 and haematocrit

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In contrast to other studies we did not find significant associations between the presence of the mutant allele and either the incidence of thrombohaemorrhagic events (7,11-15). However, in many of the studies that showed increased risk of thrombosis in patients with JAK2V617F mutation, the significance of the association has been confounded by variable methodologies, such as inclusion of mycrovascular events, different definitions of thrombotic events and inclusion of thrombotic events before diagnosis of ET (12). Moreover, some studies included relatively small numbers of patients. Other studies did not show any association between JAK2V617F mutation and thrombosis (7,15). Therefore the role of JAK2 mutation on thrombosis remains controversial, particularly because the timing of thrombotic events (before, at or after diagnosis of ET) was variable in different publications.

CONCLUSIONS

JAK2V617F mutation serves as a specific marker of clonal myeloproliferative disease. The availability of this marker facilitates definitive diagnosis of ET. Recently intriguing correlations between genotype and clinical presentation are beginning to be appreciated. It seems reasonable that JAK2V617F mutational status and/or mutant allele burden might merit a role in risk stratification which will require large prospective studies.

REFERENCES