JAK2 MUTATIONAL STATUS: CLINICAL AND LABORATORY CORRELATES IN 104 PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

LAURA URIAN[1], L. PETROV[2], A. P. TRIFA[3], ANCA BOJAN[4]

[1,2,3,4] *Oncological Institute Ion Chiricuță, Cluj Napoca*

Cuvinte cheie: *Neoplazii mieloproliferative, trombocitemie esențială, JAK2*

Rezumat: *Bolile sau neoplaziile mieloproliferative (BMP) sunt boli heterogene situate la nivelul celulei stem hematopoietice. Se caracterizează prin proliferare celulară şi maturare aparent matură. Trombocitemia esențială (TE), policitemia vera (PV) şi metaplazia mieloidă agnogenică (MMA) sunt în mod curent clasificate ca şi neoplazii mieloproliferative Philadelphia (Ph1)- negative. TE se caracterizează prin trombocitoză persistentă, proliferare în exces a megakariocitelor în măduvă, masă eritrocitară normală şi absența mielofibrozei medulare. Patogeneza moleculară a BMP nu a fost bine înțeleasă până în anul 2005 când s-a raportat existența unei mutații JAK2V617F dobândite la aproximativ 50% din pacienții cu TE, MMA şi la majoritatea pacienților cu PV. Am studiat prevalența mutației JAK2V617F, corelațiile clinice şi de laborator la 104 pacienți într- un singur centru hematologic, în Transilvania.*

Keywords: *Myeloproliferative neoplasms, essential thrombocythemia, JAK2*

Abstract: *The myeloproliferative disorders or neoplasms (MPN) are heterogeneous diseases that occur at the level of a multipotent hematopoietic stem cell. They are characterized by incresed blood cell production and virtually normal cell maturation. Essential thrombocythemia (ET) is currently classified, with polycythemia vera (PV) and agnogenic myeloid metaplasia (AMM) as a classic myeloproliferative neoplasms (MPN) Philadelphia- negative (Ph). ET is characterized by persistent thrombocytosis, excessive proliferation of megakaryocytes in the bone marrow, normal erythrocytic mass and the absence of prominent bone marrow fibrosis. 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. Clinical and laboratory associations and the prevalence of the Jak2 V617F mutational status was studied in 104 patients with ET at a single Hematology Department, in Transilvania.*

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 chacacterized 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 or AMM (2-4). Members of the Janus kinase family (JAK1, JAK2, JAK3 and tyrosine kinase2- TYK2) contain two symmetrical kinase –like domains: the JH1 domain possesses tyrosine kinase function, whereas the immediately adjacent JH2 domain is enzymatically inactive, but is credited with negatively regulating the activity of JH1. The JAK2V617F mutation is a somatically acquired G to T nucleotide shift at position 1849 in exon 12 that results in a valine to phenylalanine substituton at codon 617. The mutation is located in the Jh2 pseudo-kinase domain and is believed to result in the loss of auto-inhibitory control of JAK2. JAK2V617F is a constitutively active tyrosine kinase that activates JAK STAT signalling pathway, independent from the binding of ligand to its receptor and lead to a cytokine hipersensitivity and cytokine – independent growth to hematopoietic cells. In most patients with PV or AMM, as opposed to a minority of those with ET, the mutation is harbored in a homozygous state, which is accomplished by mitotic recombination affecting chromosome 9p. From clinically point of view, the availability of these molecular markers has been resulted in a revision of the WHO diagnostic criteria, in which the presence of the mutation is considered as a major diagnostic criteria (2-6).

THE AIM OF THE STUDY

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

MATERIAL AND METHOD

One hundred and four patients diagnosed with ET at Oncologycal Institute, Hematology Department Cluj Napoca, were included in this study. The diagnosis of ET was made

[1] Corresponding Author: Laura Urian,Oncoplogic Institute Ion Chiricuță, Clinic of Haematology, B-dul 21 Decembrie street, no 93, app 15 Cluj Napoca Romania; e-mail: urianlaura@yahoo.com; tel +40-0745647051
Article received on 22.11.2010 and accepted for publication on 20.04.2011
ACTA MEDICA TRANSILVANICA June 2011; 2(2) 224-226

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, $MgCl_2$ 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 94^0C for 7 min, 33 cycles of 94^0C for 35 s, at 57^0C for 40s, 72^0C for 45s and 72°C for 7 min

The results were processed using SPSS program.

RESULTS AND DISCUTIONS

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. (heterozigous 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 phenilalanine 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 hipersensitivity 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

Investigator	Wolanskyj [7]	Chim[8]	Green[6]	Vainchenker[9]	Cross [11]	Jelinek[10]
ET (%)	48,7	56,7	57	43	41	30

Table no. 2. Level of mutated allele

Mutant allele proportion (%)	Patients No.(n=56)	%
1-25	27	48,2
26-50	20	35,8
51-75	5	8,9
76-100	4	7,1

Figure no. 1. Correlation between JAK2 and haematocrit

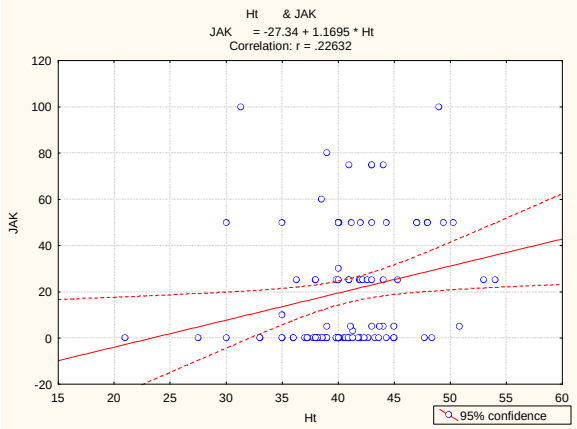
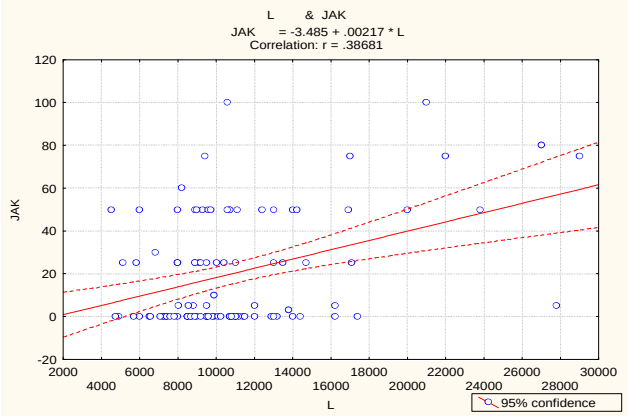


Figure. no. 2. Correlation between JAK2 and leucocyte count



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 relativelly 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, particulary 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 myeloprolyferative disease. The availibility 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 allel burden might merit a role in risk stratification which will require large prospective studies.

REFERENCES

1. Briere JB. Essential Thrombocythemia Orphanet. J Rare Dis 2007;2(3):1-19 .
2. Levine LR, Gilliland DG. Myeloproliferative disorders. Blood 2008 Dec 19;300-305.
3. James C. The JAK2V617F mutation in polycythemia vera and other myeloproliferative disorders; one mutation for three diseases. Hematology 2008 Dec 6:69-73.
4. Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med 2005;352:1779-1790.
5. Zhao R, Xing S, Li Z. Identification of an aquired JAK2 mutation in plolycythemia vera. J Biol Chem 2005 Apr 29; 280: 2788-22792.
6. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, et al. Cancer genome project Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet. 2005; 366(9480): 1054-61. Erratum in: Lancet 2005; 366(9480)122.
7. Wolanskyj AP, Lasho TL, Schwager SM, McClutre RF, Wadleigh M, LeeSJ, Gilliland DG, Tefferi A. JAK2V617F mutation in essential thrombocythaemia: clinical associataion and long-term prognostic relevance. Br J Haematology 2005;131(2):208-213.
8. Chim CS, Sim JP, Chan CC, Kho BC, Chan JC, Wong LG et al. Impact of JAK2V617F mutation on thrombosis and myeloid transformation in essential thrombocythemia: a multivariate analysis by Cox regression in 141 patients. Hematology 2010;15(4):187-191.
9. Vainchenker W, Constantinescu SN. A unique activating mutation in JAK2 (V617F) is at the origin of polycythemia vera and allows a new classification of myeloproliferative diseases. Hematology 2005;1:195-199.
10. Jelinek J,Oki Y, Gharibyan V, Bueso-Ramos C, Prchal JT, Verstovsek S, et al. JAK2 mutation 1849G>T is rare in acute leukemias but can be found in CMML, Philadelphia chromosome-negative CML, and megakaryocytic leukemia. Blood 2005; 106(10):3370-3.
11. Jones AV, Kreil S, Zoi K, Waghorn K, Curtis C, Zhang L, et al. Widespread occurence of the JAK2 mutation in chronic myeloprolifertaive disorders. Blood 2005; 106(6):2162-8.
12. Finazzi G, Rambaldi A, Guerini V, CarroboA, Barbui T.Risk of thrombosis in patients with essential thrombocythemia and polycythemia vera according to JAK2V617F mutation status. Haematologica 2007;92:135-136.
13. Campbell PJ, Scott IM, Buck G, et al.Definition of subtypes of essential thrombocythemia and relation to polycythemia vera based on JAK2V617F mutation status: a prospective study. Lancet 2005;366:1945-1953.
14. Cheung B, Radia D,Pantelidis P, Yadegarfar G, Harrison C. The presence of the JAK2V617F mutation is associated with a higher haemoglobin and increased risk of thrombosis in essential thrombocythemia. Br J Haematol 2006;132:244-245.
15. Antoniolo E, Guglielmelli P, Pancrazzi A. Clinical implications of the JAK2V617F mutation in essential thrombocythemia. Leukemia 2005;19:1847-1849.