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The Importance of Evaluating the Frequency of Thrombotic Events in Patients with Polycythemia Vera JAK2 V617F Positive

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Abstract

The polycythemia vera is a myeloproliferative neoplasia whose overall incidence is 0.7-2.6 cases per 100.000 inhabitants a year, what increases with the ages of the patients, independently of sex. PV patients have a higher risk of occurrence of thrombotic events, considering these are the cause of morbidity and mortality of them. The aim of this research was to evaluate the frequency of thrombotic events in patients with polycythemia vera treated in a medical center. We did a molecular analysis of the gene *JAK2*, evaluating the exons 12 and 14 in samples from 26 patients clinically diagnosed with the disease, in the period of March to September, 2013. As we found, 92.3% of the patients were positive to the mutation JAK2 V617F (exon 14), and 7.7% were negative also to the mutations on the exon 12. The frequency of thrombotic events in these patients was evaluated, describing the main clinical characteristics associated with this mutation. Around 29.16% of the JAK2 V617F positive patients had arterial or venous thrombosis, considering that the venous thrombosis was more frequent. The percentage of patients with the mutation JAK2 V617F, and the frequency of thrombosis in PV JAK2 positive patients demonstrated in our study, is according to the data presented in the literature. With this work, we emphasize the role of the research on this mutation into the causes of thrombotic events, especially in unusual site, pointing the myeloproliferative neoplasia as the cause of thrombophilia.

Keywords: Polycythemia Vera, myeloproliferative neoplasias, JAK2 V617F, thrombotic events, venous thrombosis.

Introduction

The Polycythemia Vera(PV) is a myeloproliferative neoplasia which comes from a change in the multipotent hematopoietic stem cells that causes the accumulation of erythrocytes, leukocytes, and morphologically normal platelets, independent of erythropoietin. It can bring about leukocytosis, thrombocytosis, splenomegaly, and an increased risk of thrombotic events (1,2,3). Around 95% of the patients diagnosed with PV have the mutation JAK2 V617F (19).

The PV global incidence is 0.7-2.6 cases per 100.000 inhabitants a year, what increases with the advancing age of the patients, independently of sex (4). Between the main complications of PV are the thromboembolic events that cause the morbidity and mortality of these patients (5).

Even in the absence of thrombotic events, the patients with a type of myeloproliferative neoplasia (MPN) present a hypercoagulable state, which can be identified for an increasing in the concentration of several plasmatic markers on the system of haemostatic activation (5). Besides this, studies suggest that these patients with MPN, who have the mutation JAK2 V617F, are exposed to an increasing risk of thrombotic complications, possibly because of the increased platelet and the leukocyte activation (6).

Thereby, the present work evaluated the frequency of thrombotic events in patients with polycythemia vera (PV), positive JAK2 V617F, of one medical center, describing the main clinical characteristics associated to this mutation, which is one of the most important criteria for the diagnosis according to the World Health Organization - WHO (2008).

Material and Methods

Patients

They were included 31 patients with PV diagnoses according to the established criteria by WHO (2008), between March and September, 2013. Considering this total number, five patients refused to participate of the research. The project was approved by the Human Research Ethics Committee.

The patients were evaluated according to the main clinical and laboratory features, emphasizing the thrombotic events preceding or posteriors to the PV diagnosis.

DNA Extraction

The blood samples were collected by peripheral puncture and routed to the Genetics Laboratory and Sector Advisory from the Federal University of Juiz de Fora/Brazil. The PCR-AE technique was performed according to standard conditions, and the product of PCR was visualized on 2% agarose gel. On the negative samples to the presence of the mutation JAK2 V617F, it was done the automatic sequencing, with the aim of identify possible changes in the exon 12 in the gene *JAK2*.

The sample of blood was collected in an ethylene diamine tetraacitate anticoagulated tube. The Genomic DNA was extracted with standard procedures after the total isolation of the peripheral blood samples, and stored at -20°C until the processing. The DNA was quantified using spectro photometric measurements.

Allele-Specific PCR

All the DNA samples were genotyped for the JAK2V617F mutation situated in exon 14 by an allele-specific (AS) polymerase chain reaction (PCR), exactly as described by Baxter *et al*. (2005) (7). So, 1 µmol/L of a common reverse primer, 0.5 µmol/L of a forward primer specific for the mutant allele (giving a 203-bp product), and 0.5 µmol/L of another forward primer amplifying a 364-bp product from both mutant and wild type of alleles – which also serves as an internal PCR control –, were used. Samples that were positive for the mutation were subsequently analyzed via PCR-restriction fragment length polymorphism (PCR-RFLP), with the restriction endo nuclease BsaXI (New England Biolabs, Hitchin, UK), which allows an estimation between mutated and wildtype alleles. A successful amplification was confirmed by electrophoresis on an ethidium bromide, impregnated 2% agarose gel. The G-T mutation destroys a BsaXI site present in the wild-type JAK2 sequence. This approach allows both normal and mutant alleles be visualized and distinguishes homozygous and heterozygous mutations.

DNA Sequencing

When the PCR was done for the preparation of the template for sequencing, the primers were not labeled with a fluorescentdye. The amplicons were treated with ExoSap (Amersham Biosciences, Piscataway, NJ) to remove the primers and dNTPs, then were sequenced by usingthe PCR as sequencing primers, and Applied Biosystems (ABI, Foster City, CA) Big Dye Terminator version3.1 on chemistry. The sequencing reactions were purifiedby

using the Clean Seq System (Agencourt Bioscience, Beverly, MA), and subsequently resolved by capillary electrophoresison the ABI 3100 Prism Genetic Analyzer. The mutations were determined by comparison with the normal *JAK2* sequence (accession NM-004972) and with a normal control that was included in each run.

Results

The table 1[1]presents some clinico-hematological features of each patient.

Table1. *Clinico-hematological data presented by the patients*

PATIENTS	AGE	GENDER	THROMBOTIC EVENT	HAMATOCRIT %	HOMOGLOBIN g/dL	LEUKOCYTES	PLATELETS	SPLENOMEGALY	JAK2 V617F
C.A.P	63	F	YES	68	20	11.600	481.000	NO	YES
A.A.B	88	F	YES	57	18.2	15.400	1.147.000	NO	YES
F.B.F	55	M	YES	58	19	6.500	480.000	YES	YES
E.S.N.S	63	F	YES	47.2	15.2	7.200	253.000	YES	YES
R.M.A.A.	62	F	YES	57	18.7	16.500	473.000	YES	YES
M.D.L	78	F	YES	54.7	17.6	6.900	744.000	NO	YES
A.S.E	58	M	YES	62.3	21.3	10.000	496.000	NO	YES
M.J.D.J	27	M	NO	58	18.1	10.100	720.000	YES	YES
R.A.B	77	M	NO	60	18.1	86.000	490.000	NO	YES
R.M	68	M	NO	50	17.2	8.200	416.000	NO	YES
M.P.A	82	F	NO	39	13	6.200	280.000	NO	YES
T.G	66	F	NO	58	19	12.000	450.000	YES	YES
F.L.C.T	59	M	NO	55.9	19.4	16.200	356.000	YES	YES
J.C.A	41	M	NO	61	19.5	7.300	220.000	NO	YES
J.S	48	M	NO	56	18.1	26.100	783.000	NO	YES
M.C.L.S	71	F	NO	71.6	21.5	14.600	696.000	YES	YES
L.A.S	69	F	NO	59.1	19	12.000	541.000	NO	YES
M.G.E	67	F	NO	68.4	22.1	12.800	841.000	NO	YES
J.M.S	75	M	NO	37	11.7	5.200	360.000	NO	YES
T.S.M	74	F	NO	63.2	20.3	11.200	390.000	NO	YES
M.R.S.S	64	F	NO	57.9	18.6	10.100	651.000	YES	YES
A.R	72	M	NO	51.9	18.2	13.000	810.000	NO	YES
I.T.S	54	F	NO	60	18.5	14.500	788.000	NO	YES
M.H.G.A	57	F	NO	54	16.9	13.300	1.093.000	NO	YES
G.M.M.P	59	F	NO	55.1	18	9.300	366.000	YES	NO
J.G.F.Y	57	F	NO	60	21	12.000	560.000	NO	NO

Considering the 26 collected samples, 24 (92.3%) were positive for the mutation JAK2 V617F, and two (7.7%) were negative also for the mutations in exon 12. The automatic sequencing of these samples did not reveal alteration (not shown data). The negative patients for the mutations in exons 14 and 12 did not present different clinical signs from the other ones, and also did not have a historical of thrombotic events. Between the patients identified as positive for JAK2 V617F, 7 of them (29.16%) presented arterial and venous thrombosis. The venous thrombosis was more frequent, diagnosed in 4 (57%) of the 7 patients with a medical historic of thrombosis.

1 *M (male); F(female).*

Discussion

The investigation about the mutations in the gene *JAK2* in patients with PV is extremely important to the diagnoses of these ones.

The percentage of patients with the mutation JAK2 V617F found in our study is according to the data presented in the literature, which is around 95-98% (8). The analysis of other possible mutations in exon 12 of the gene *JAK2* was investigated, and it was not detected any alteration.

However, we must not discard the clinical diagnosis of PV, because it is necessary to consider other genes that may be involved in the disease manifestation, particularly those associated with the negative regulation tracts of JAK-STAT (9,10), and other MPN must be considered (11). Howsoever, the lack of mutations in the gene *JAK2,* especially JAK2 V617F in general, is frequently low (8). The frequency of thrombosis in patients with PV varies from 19% to 39%, a value similar to the one we have found (29.16%), but different from our casuistry. The arterial thrombosis is more frequently described in the literature (12-16), as we can see in table 2:

Table2. *Scientific data about the relation between the number of patients with PV and the occurrence of thrombotic events associated with the disease.*

Publications	Patients with PV	Ocurrence of thrombotic events (%)	Arterial Thrombosis (%)	Venous Thrombosis (%)
Passamontiet al. (2002)	163	34%	64%	36%
BarbuiandFinazzi (2003)	1638	38.60%	75%	25%
Passamontiet al. (2003)	70	24.30%	70.60%	29.40%
Marchiolli (2005)	1638	38.60%		
Couceloet al. (2013)	31	39%	75%	25%
Alvarez-Larránet al. (2014)	163	23%		
Edahiroet al. (2014)	66	19%		

In our study, the research for causes of thrombotic events should have been better investigated. Information on the cause of thrombotic events was not found in some patient data. With the data in the table 2, we emphasize the role of the research about this mutation on the investigation about the causes of thrombotic events, especially in unusual site, highlighting the MPN as a cause of thrombophilia (18). This probably occurs due to correlation of the mutation JAK2 V617F presence, and the increasing of platelets and leukocytes and with the hyper coagulable state (6,5).

Although we do not have patients with mutation in the exon 12, their screening can be useful on the identification of patients with MPN. We believe that their absence in our research is because of the small sample we used, what justify new studies about the Brazilian population in order to evaluate its real frequency, and to determine the role of the JAK2 V617F mutation in patients with a historical of thrombosis. On the opposite of what is present in the literature, we have found more relation to the venous thrombosis.

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