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Clinical significance of Beta-2 glycoprotein I antibodies in BcrAbl (-) myeloproliferative neoplasms.

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Abstract

Background: Beta-2 glycoprotein I antibodies (B2-GPI Ab) are significant markers of thrombosis in otoimmune diseases. No literature has been found in Bcr Abl (-) MPNs to exclude the risk of hereditary thrombophilia and to investigate the association of B2-GPI Ab levels with thrombosis. This study aimed to investigate the relation between levels of B2-GPI Ab and thrombosis in BcrAbl (-) MPN without the risk of hereditary thrombophilia.
Methods: Plasma samples from healthy volunteers and BcrAbl (-) MPN's patients with and without thrombosis were collected after receiving consent. B2-GPI Ab in plasma was quantified by enzyme-linked immunosorbent assay and the data were analyzed to determine whether plasma B2-GPI Ab correlates significantly with thrombosis.
Result: There was a statistically significant difference between the B2-GPI Ab levels of the patients and the control group (p=0.006). However, no statistically significant difference in levels of B2-GPI between patients with and without thrombosis history was determined (p=0.144).
Conclusion: This study supports that no relationship between levels of B2-GPI Ab and thrombosis complications in BcrAbl (-) MPN.

Keywords Myeloproliferative neoplasms, Policythaemia vera, Essential thrombocytosis, Beta-2 glycoprotein I antibody, Thrombosis.

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Introduction

Myeloproliferative neoplasms (MPN) arise from clonal proliferation of hematopoietic stem cells and are characterised with excessive production of functional blood cells [1]. Thrombotic complications develop in PV at 12-39% and in ET at 11-35% [2,3]. The most important factors affecting survival in PV and ET are age, leukocytosis and thrombosis1. Arterial and venous thrombosis is important causes of morbidity and mortality in PV and ET6. Although the risk of thrombosis and haemorrhage in PV and ET are known, the mechanisms are not known yet. The mechanisms of thrombosis in PV and ET include: thrombosis history of the patient, leukocyte-neutrophile activation, JAK2 V617F mutation, JAK2 V617F allogenic burden, platelet activation, microparticules secreting tissue factors and phospholipids, gender and endothelium activation [4]. B2-GP is synthesized by hepatocyte, endothelial cells and trophoblasts. It consists of repeating five large components that are comprised of 326 aminoacids sized 48 kDa. The first component of B-2GP (B2-GPI) binds to anionic phospholipids without the need for calcium ions, resulting in activating thrombocytes and endothelial cells. No literature has been found in Bcr Abl (-) MPNs to exclude the risk of hereditary thrombophilia and to investigate the association of B2-GPI Ab levels with thrombosis. This study aimed to investigate the relation between levels of B2-GPI Ab and thrombosis in Bcr Abl (-) MPN without the risk of hereditary thrombophilia.

Materials and Methods

This study is a type of case-control study. A total of 150 patients were included in the study, 75 patients with PV and ET, 75 controls, above the age of 18, without cardiovascular risk factors, malignancy, active infection, kidney failure. The samples obtained from the patients were in gel tubes with EDTA to measure B2-GPI ab, homocysteine and APCR and were in centrifugated for 5 mins at 3000 rpm. The samples were kept at -80°C until day of analysis. APCR was analyzed using a coagulometric method with a Sysmex CA-1500 (Japan) device after calibration-control procedures using Siemens brand kits. B2-GPI Ab measurements in plasma samples were performed with Rayto-2100C Microplate Reader device using Human B2-GPI IgG Ab enzyme-linked immunosorbent assay

(ELISA) kit (Orgentec, Germany). Homocysteine measurements in plasma samples were analysed using electrospray ion source (ESI) at positive mode on the ABSCIEX API 2100 triple quadruple mass spectrometry (Canada) device. Factor V leiden and prothrombin G20210 measurements in samples were DNA isolated with a Vivantis brand commercial DNA isolatin kit. The obtained DNA was analysed with a Diagen brand commercial kit, a Factor V Leiden (G1691A) PCR RFLP kit, a Factor II (G20210A) PCR RFLP kit and a LightCycler 480 Real Time PCR device. 4 patients and 13 controls were excluded from our study because they did not meet our criteria and had a risk of hereditary thrombophilia.

Statistical analysis

For evaluating the data statistically, the IBM Statistics15.0 (SPSS) statistic package software was used *in silico*. Nominal values were assessed via the Ki-square test. The convenience of the continuous variables to the normal distribution was investigated via the Kolmogorov Smirnov tests. To evaluate the difference between the means of the continuous variables of the 2 groups that don't distribute normally, the Mann-Whitney U test was conducted. For the correlation between 2 continuous variables that don't distribute normally, the Spearman correlation test was used. $P<0.05$ was considered statistically significant.

Results

A total of 133 people (71 patients and 62 controls) participated in our study. The characteristic features are shown on Table 1. The control group consisted of 26 (41.9%) males and 36 (58.1%) females and the median value for age was 49.2 (20-70%).

Table 1. *Characteristic features of the patient group.*

	Patient group N=71
Mean age	62.5
Gender	
Male (%)	32 (45.1%)
Female (%)	39 (54.9%)
P. Vera	26
E. Thrombocytosis	44
P. Myelofibrosis	1
Mean duration of disease (years)	4,4 (1-20)
Mean Hb (gr/dL)	15.7 (8.2-18.1)
Mean platelet (K/uL)	399 (564-869)
Mean leukocyte (K/uL)	8.1 (3.5-19.9)
History of thrombosis	
P. Vera (%)	13 (50%)
E. Thrombocytosis (%)	13 (29.5%)
Total (%)	27 (38%)
Factor V Leiden mutation (number of positive individuals)	4
Prothrombin G20210 mutation (number of positive individuals)	-
Active Protein C Resistance (APCR) (number of positive individuals)	-

There was a statistically significant difference between the B2-GPI Ab levels of the patients and the control group (p=0.006) (Table 2).

The median value of the B2-GPI Ab levels for patients with history of thrombosis was 187 (15-696) U/mL, for patients with no history of thrombosis was 242.5 (34-763) U/mL. When comparing the patients with history of thrombosis to the patients with no history of thrombosis, It was found that these values showed no statistically significant difference (Table 3, p=0.144). 13 (29.5%) of the 44 participants who were included in the group of patients with ET had a history of thrombosis while 31 (70.5%) had not. When comparing the B2-GPI Ab levels in the group of patients with ET according having a history thrombosis, the median value of the B2-GPI Ab levels for the patients with history of thrombosis was 217 (15-373) U/mL, while this value was 250 (34-763) U/mL for the ones without history of thrombosis. It was found that these values

showed no statistically significant difference (P=0.382). While 13 (50%) of the 26 participants who were included in the group of patients with PV had a history of thrombosis, 13 (50%) of them had not. When the B2-GPI Ab levels were compared according to their status of having a history of thrombosis in the group of patients with PV, the median value of the ones with a history of thrombosis was 169 (76-696) U/mL, while it was 235 (129-566) U/mL for the ones without a history of thrombosis. It was found that these values showed no statistically significant difference (P=0.228).

Table 2. *Comparison of B2-GPI Ab levels in patient and control groups*

	Number (%)	Median	B2-GPI Ab (U/ml) (Min-Max)	p-value
Patient	71 (53.4%)	217	(15-763)	0.006
Control	62 (46.6%)	160	(38-630)	
Total	133 (100%)	187	(15-763)	

Table 3. *Comparison of B2-GPI Ab levels in the patients with history of thrombosis to the patients with no history of thrombosis.*

Thrombosis history	Patient (%)	Median	B2-GPI Ab (U/ml) (Min-Max)	P value
Yes	27(%38.0)	187.0	(15-696)	0.144
No	44(%62.0)	242.5	(34-763)	
Total	71(%100.0)	217.0	(15-763)	

Discussion

B2-GPI Abs are important markers of thrombosis in autoimmune diseases. No literature has been found in Bcr Abl (-) MPNs to exclude the risk of hereditary thrombophilia and to investigate the association of B2-GPI Ab levels with thrombosis. The thrombosis in MPN develops with different mechanisms. These include secretion of tissue factors and phospholipid from the microparticules, platelet activation, leukocyte and neutrophile activation, endothelial activation and chronic inflammation mechanisms. The activation of platelets and endothelial cells by the phospholipids secreted in microparticules causes the generation of thrombosis. In our study, it was established that there was a statistically significant difference in the median levels of B2-GPI Abs between the patient group and the control group (P=0.006). This significant difference may be explained with the fact that this is caused by interaction between the phospholipids which are secreted in the microparticules and B2-GPI.

B2-GPI is correlated with atherosclerosis and oxidative stress [5]. The correlation between atherosclerosis and B2-GPI Abs is known since the day when this antibody was found in the athermanous plaques [6]. In the study from 2014 by Berger et al. statistically significant difference between B-2GPI/OxLDL antibodies measured in patients with an arterial or venous disease and the control group was found (P=0.032) [7]. In the study by De Laat et al. the B2-GPI Ab levels in older patients with a risk of myocardial infarction were compared. No difference between the mean B2-GPI Ab plasma levels of patients who had a mycordial infarction (214 ug/ml) and the mean B-2GPI Ab plasma levels of the control group was found [8]. De Laat et al. suggested that the relation between B-GP and thrombosis was due to the antibodies developing against the first segment of this protein and not due to the antibodies that develop against the other segments [8]. In our study, the IgG type antibodies that develop against the first segment of this protein were analyzed. We found no statistically significant difference between the median values of B2-GPI Ab levels in the patients with history of thrombosis and the ones without a history of thrombosis in the patient group in our study (P=0.144). When comparing the B2-GPI Ab levels for patients with a history of thrombosis and for patients without a history of thrombosis in the group of patients with ET and in the group of patients with PV separately, no statistically significant difference was found (P=0.382, P=0.228). In our study, it was concluded that there was no significant difference in B2-GPI Abs binding to phospholipids between patients with thrombosis and patients without because the leukocyte, hemogram and platelet values were not high enough to generate microparticules due to the treatment of the patients and the amount of phospholipids secreted from the available microparticules was not much. In a study by De Laat et al. the difference in B2-GPI Ab levels between the group of individuals who had myocardial infarction and the control group was analysed according to smoking, use of alcohol, age and gender and no statistically significant difference was found [8]. In our study, when comparing the B2-GPI Ab levels in the patient group according to the gender, no statistically significant difference was found (P=0.443), as in the study by De Laat et al. When comparing the B2-GPI Ab levels in the control group according to the gender, no statistically significant difference was found (P=0.443), as in the study by De Laat et al. [8].

Conclusion

Although it is known that the risk for thrombosis and hemorrhage increases in BcrAbl (-) MPNs, the mechanisms aren't clear yet. To our knowledge, our study is the first in literature that evaluates the relation between B2-GPI Abs and thrombosis in Bcr Abl (-) MPN excluding the risk for hereditary thrombophilia. The B2-GPI Ab level is significantly high in patients with BcrAbl (-) MPNs compared to normal individuals, while no difference was detected between BcrAbl (-) MPNs with thrombosis and without thrombosis. Alongside B2-GPI Ab, it might be beneficial to assess the antibodies developing against segment IV and V which bind to the negative burdened sites of B2-GPI Ab.

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