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ASSOCIATION BETWEEN *JAK2*V617F MUTATION AND THROMBOTIC COMPLICATION IN PATIENTS WITH CLASSICAL PH-NEGATIVE CHRONIC MYELOPROLIFERATIVE DISEASES

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The main clinical problem of patients with chronic myeloproliferative diseases (CMPD) is venous and arterial thrombosis. *JAK2*V617F mutation was recognized as one of the main thrombogenic factors among this group of patients.

The objective of the study was to assess the effect of clinical and laboratory parameters and *JAK2*V617F mutation on the incidence of thrombotic complications.

Materials and research. The study included 70 patients with confirmed diagnoses of essential thrombocytemia, polycythemia vera and primary myelofibrosis. The analysis included analysis of clinical and laboratory data of outpatient consultatuion and molecular genetic study to detect the *JAK2*V617F mutation by the allele-specific polymerase chain reaction.

Results. During follow-up time thrombotic complications were registered in 28,6% (20/70) patients. Thrombosis of arterial vessels were seen more commonly (80%) – acute myocardial infarction and acute cerebrovascular accident. According to the results of patient genotyping, the *JAK2*V617F mutation was detected in 90,9% of patients with polycythemia vera, 61,3% of patients with essential thrombocythemia and 64,7% of patients with primary myelofibrosis. Thrombotic complications were significantly more often observed in carriers of the *JAK2*V617F mutation and in patients with cardiovascular risk factors.

Conclusion. Carriage of the *JAK2*V617F mutation and the presence of cardiovascular risk factors significantly increase the risk of thrombotic complications. Early detection of *JAK2*V617F mutation will reduce the number of life-threatening complications.

Keywords: chronic myeloproliferative diseases, *JAK2*V617F, thrombosis.

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Introduction. The main clinical problem of patients with chronic myeloproliferative diseases (CMPD) is vascular complications, including microcirculatory disorders, venous and arterial thrombosis, as well as hemorrhagic complications [2]. Currently due to progress of understanding of diseases molecular mechanisms and development of a new class of drugs – janus kinase inhibitors, it has been possible to reduce clinical symptoms of polycythemia vera (PV) and primary myelofibrosis (PMF) who are resistant to hydroxyurea or with drug toxicity [8]. Thrombotic complications are highly prevalent in patients with CMPD, causing a high risk of mortality and disability. According to published data, prevalence of thrombosis in patients with PV is 3.8 per 100 patient-years, in patients with essential thrombocythemia (ET) from 2 to 4 per 100 patient-years, and in PMF, 2.23 per 100 patient-years [1]. The leading place in the structure of thrombotic complications is represented by arterial thrombosis – myocardial infarction, ischemic stroke and transient ischemic attack.

Thrombosis has a complex multicomponent pathogenesis. Genetic abnormalities, qualitative and quantitative disorders of blood cells, endothelial dysfunction make a certain contribution to the formation of a blood clot [10]. The most significant risk factors for thrombotic complications among patients with CMPD include age over 60 years old, previous thrombosis, cardiovascular risk factors, and the presence of the JAK2V617F mutation. Currently, due to the widespread use of a personalized approach to the treatment of many diseases, the study of molecular markers of thrombogenic risk is gaining particular relevance. It has been proven that carriers of the JAK2V617F mutation are characterized by an increase in the pool of activated leukocytes and platelets with a more thrombogenic potential and an increase in their aggregation ability. In addition, muatation plays role in development of endothelial dysfunction and coagulation link of hemostasis [6,10].

The aim of study was to evaluate the effect of clinical and laboratory parameters and JAK2V617F mutation on the incidence of thrombotic complications.

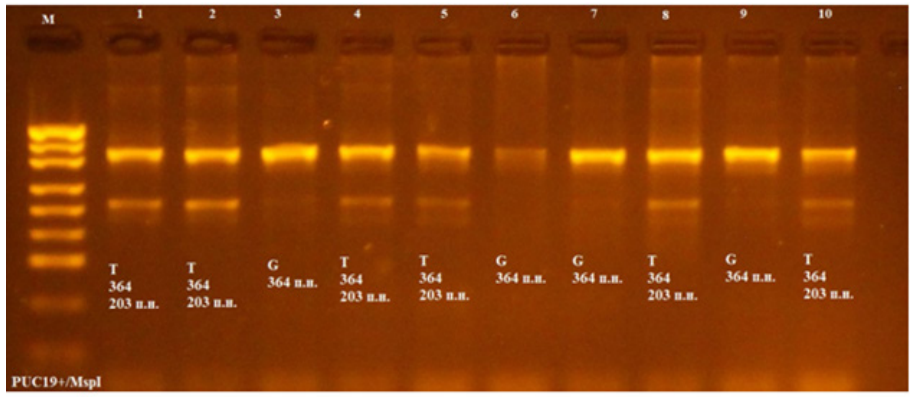
Materials and methods of the research. The study included 70 patients with previously confirmed diagnoses of ET (n = 31, 44,3%), PV (n = 22, 31,4%) and PMF (n = 17, 24,3 %). The median follow-up time was 48 months (from 2 to 252 months). All patients underwent a molecular genetic study to identify the JAK2V617F mutation (rs77375493) by

allele-specific polymerase chain reaction (AS-PCR) using standard pairs of primers manufactured by SybEnzime, Novosibirsk [4]. The reaction mixture with a total volume of 25 µl per 1 sample included: direct allele-specific primers (AGCATTTGGTTTTAAATTATGGAGTATATT), direct (ATCTATAGTCATGCTGAAAGTAGGAGAAAG) 0.5 µl each and reverse (CTGACACCTAGCTGTGATCCT); Dream Taq PCR master mix – 12,5 µl; 9,5 µl of deionized water and 1 µl of DNA. Optimized PCR temperature conditions are presented in Table 1.

Table 1

Condition for AS-PCR

№	Step	t, °C	Time	Number of cycles
1	Initial denaturation	95	10 min.	1
2	Denaturation	95	30 sec.	36
3	Annealing	56	30 sec.	
4	Elongation	72	1 min.	
5	Final elongation	72	10 min.	1



PCR results electrophoregram

Detection of PCR products was performed using electrophoresis on 3% agarose gel stained with ethidium bromide and standard Tris-acetate buffer at 120 V for 45 minutes. The amplification length for the G allele was 364 nucleotide pairs (bp), T - 364 and 203 bp (Figure).

When analyzing the prevalence of thrombotic complications and risk factors, clinical and laboratory data obtained from outpatient patient counseling were used. The statistical significance of the differences was assessed using the χ-square test with Yates correction. Differences were considered significant at $p < 0.05$. To assess the impact of risk factors on the incidence of thrombotic complications, odds ratios (OR) were calculated. Results are presented as OR and 95% confidence interval (CI).

Results and discussion. During the observation period, thrombotic complications were registered in 28,6% (20/70) patients, of which 7,14% (5/70) had two or more episodes. The prevalence of thrombosis among patients with PV was 36,4%, ET - 16% and PMF – 35,2%. 55% (11/20) of thrombotic events were observed before diagnosis. The median time of follow-up from the development of thrombosis to diagnosis was 9 months (from 1 to 78 months). It is known that patients with PV are characterized by more thrombogenic potential associated with an increase in hematocrit and blood viscosity. Estimated prevalence of thrombosis in this group of patients is 12-39% at the time of diagnosis and 10-25% during follow-up time. For patients with ET, microcirculatory disorders are more common than thrombosis of large vessels, and in patients with PMF, thrombotic complications occur with a frequency of 4–7% in the onset and 2–4% during follow-up time [3, 9]. In most cases, thrombosis was localized in the arterial vessels (80%) – acute myocardial infarction (58,8%), stroke (35,3%) and deep femoral artery (5,9%). Venous thrombosis were less common (20%) and represented by deep vein thrombosis (DVT) of lower extremities (10%) and portal system veins (10%).

According to the results of genotyping, the T allele of JAK2 gene (JAK2V617F) was detected in 90,9% of patients with PV, 61,3% of patients with ET, and 64,7% of patients with PMF. When comparing JAK2V617F-positive and JAK2V617F-negative patients, thrombosis were significantly more often observed in carriers of JAK2 mutations – 45% and 10%, respectively ($p = 0.008$).

Next we analyzed the presence of main (age 60 years and over, the presence of JAK2V617F mutation, cardiovascular risk factors) and additional (hypertrombocytosis, leukocytosis) risk factors for thrombotic complications (Table 2). Cardiovascular risk factors included hypertension, diabetes mellitus, and smoking.

Thrombotic complications were significantly more common in carriers of the JAK2V617F mutation ($p = 0,036$) and in patients with cardiovascular risk factors ($p = 0,014$). The OR of the development of thrombotic complications in carriers of the JAK2 mutation was 11,645 (95% CI 1,440-94,191), and 3,605 in patients with cardiovascular risk factors (95% CI 1,213-10,715). There is no significant difference in the frequency of thrombosis between patients aged older 60 years and younger ($p = 0.242$) which is probably related to the fact that the share of those examined over 60 years was only 30,4%.

According to most authors, the most significant independent risk factors for thrombosis among patients with PV, ET, and PMF are age and thrombosis [5]. However, with the gaining of new knowledge about molecular pathogenesis of diseases, prognostic scales based on the mutational status of patients become more relevant. Currently, it has been proven that carriage of JAK2V617F mutation increases the risk of thrombosis among ET patients by up to 45%, and also can be a predictor of recurrent thrombosis. Similar results were obtained in many large studies, which allowed to include presence of JAK2V617F mutation in IPSET-thrombosis scale (a universal tool for assessing the risk of developing ET patients) as an independent thrombogenic risk factor for ET patients [10]. Knowledge about effect of JAK2 mutational status on frequency of thrombosis in patients with PV are contradictory. Some studies have shown that a high allele burden is associated with an increased risk of thrombotic complications. In group of patients with PMF, the greatest risk of thrombosis is associated with JAK2V617F mutation and leukocytosis [7].

In performed research, more than half of thrombotic complications were observed before the diagnosis of the disease. Cases of prolonged latent course of CMPD, when the clinical feature is represented only by thrombosis, are difficult to diagnose. An early molecular ge-

Table 2

Association of thrombosis with risk factors

Risk factor	Patients with chronic myeloprolyferative diseases, % (abs. n)		χ2 criteria	significance, p	OR (95% CI)
	with thrombosis (n=20)	without thrombosis (n=50)			
Age 60 +	65 (13)	46 (23)	1.374	0.242	2.180 (0.745-6.382)
Cardiovascular risk factors	65 (13)	34 (17)	4.411	0.036	3.605 (1.213-10.715)
JAK2V617F mutation	95 (19)	62 (31)	6.092	0.014	11.645 (1.440-94.191)
Leukocytosis >11*109/l	45 (9)	36 (18)	0.182	0.67	1.455 (0.507-4.171)

Note: χ2 - χ-square criteria with Yates correction, p – significane, OR – odds ratio, CI – confidence interval

netic study among people with borderline polycythemia will improve the diagnosis of masked forms of CMPD and prevent the development of fatal complications, and the determination of indications for the detection of the JAK2V617F mutation among patients with thrombosis requires further study.

Conclusion. The occurrence of thrombotic complications is an important factor affecting the survival and quality of life of patients with CMPD. In most cases, thrombosis is the first clinical symptom of disease or manifests before the verification of diagnosis. Carriage of JAK2V617F mutation and the presence of cardiovascular risk factors significantly increase the risk of thrombotic complications. An early molecular genetic study will improve the diagnosis of masked forms of CMPD and prevent the development of fatal complications.

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