MEDICAL UNIVERSITY - PLEVEN

FACULTY MEDICINE

DEPARTMENT OF NEPHROLOGY, HEMATOLOGY AND GASTROENTEROLOGY

Doroteya Kostadinova Todorieva-Todorova, MD

Clinical significance of genetic, immunological and comorbidity factors on thrombotic risk in patients with myeloproliferative neoplasms

ABSTRACT

on a dissertation work for acquiring the educational and science degree "MEDICAL DOCTOR"

Doctoral program "Hematology and blood transfusion"

Scientific supervisors:

Prof. Liana Todorova Gercheva-Kyuchukvova, MD, PhD

Prof. Katya Stefanova Kovacheva-Kotseva, MD, PhD

Scientific jury:

Assoc. prof. Vanya Slavcheva Popova, MD, PhD

Assoc. prof. Yavor Yordanov Ivanov, MD, PhD

Prof. Veselina Stefanova Goranova-Marinova, MD, PhD

Prof. Margarita Lyubenova Genova, MD, PhD

Prof. Georgi Nikolaevich Balatzenko, MD, PhD

PLEVEN, 2024

The dissertation work has been developed on 170 pages and contains 26 figures, 47 tables and 5 annexes. Bibliography consists of 223 sources, 2 of them in Cyrillic and the rest – in Latin, most of them published in the past 10 years, 49 of them – in the past 5 years.

Data presented is a result of scientific projects, financed by Medical university – Pleven: project N 13/2013, N 2/2015, N 9/ 2017 and N 9/ 2019.

The author is a doctoral student in a self-preparatory form of education, department of Nephrology, hematology and gastroenterology, Faculty Medicine, Medical university – Pleven.

The dissertation work has been discussed and defined to be defended by an extended Department council of Nephrology, hematology and gastroenterology Department, Faculty Medicine, Medical university – Pleven, hold on 9th Apr, 2024.

Materials on the dissertation defence are available at the Scientific department of Medicine Faculty, Medical university – Pleven, as well as on university site - www.mu-pleven.bg

Scientific jury:

Chairman:

Assoc. prof. Vanya Slavcheva Popova, MD, PhD

Members:

Assoc. prof. Yavor Yordanov Ivanov, MD, PhD

Prof. Veselina Stefanova Goranova-Marinova, MD, PhD

Prof. Margarita Lyubenova Genova, MD, PhD

Prof. Georgi Nikolaevich Balatzenko, MD, PhD

Standby members:

Assoc. prof. Ivan Angelov Shtarbanov, MD, PhD

Assoc. prof. Ivanka Slaveykova Nenova-Chilova, MD, PhD

The dissertation defence will take place on 22nd Jul, 2024, 11.00 a.m. in 113 auditorium – Louis Pasteur, Pharmacy Faculty, Medical university – Pleven.

CONTENT

ABBREVIATIONS

AH – arterial hypertension

CG1/CG2 – control group ½

CI – confidence interval

CML – chronic myeloid leukemia

DM – diabetes mellitus

DNA - deoxyribonucleic acid

ET – essential thrombocythemia

FBC – full blood count

FVL – factor V Leiden

G20210A – mutation G20210A in prothrombin gene

HF – heart failure

IHD – ischemic heart disease

IQR - interquartile range

IS – ischemic stroke

JAK – Janus kinase

MF – myelofibrosis

MI – myocardial infarction

Min/max – minimum/maximum

MPN – myeloproliferative neoplasm

OR – odds ratio

p – statistical significance

PG – patient group

Ph – Philadelphia chromosome

PLA1/A2 – platelet glycoprotein IIb/IIIa polymorphism

PTE – pulmonary thromboembolism

PV – polycythemia vera

RR – relative risk

SD – standard deviation

TE – thrombotic event

TKI – tyrosine kinase inhibitor

I. INTRODUCTION

Chronic myeloproliferative neoplasms (MPNs) are clonal hematological diseases, in which autonomous and increased proliferation of cell precursors is present in the bone marrow. The grounds for their group classification are: a clonal marker and a genetic mutation presence, that stimulate hyperactivity of a pathological tyrosine kinase; uncontrolled production of malignant cell clone; possible transformation between diseases and their evolution to blast phase. Nowadays, these diseases have been diagnosed in patient under 60 years of age, who are supposed to have longer disease course, require ongoing therapy, often medication changes, prophylaxis of the direct and long-term complications. The reasons for increased morbidity and mortality in MPN patients are thrombotic complications and their frequency is approximately 40% in some of them. Thrombogenesis may be stimulated by different factors: age, sex, smoking, history of thrombotic complications, comorbidities, genetic factors (genetic thrombophilia carriership, JAK2V617F carriership), abnormal coagulation (clotting system hyperactivity, suppressed fibrinolysis, inflammatory cytokines presence), blood cells abnormalities (erythrocytosis, leukocytosis, thrombocytosis), as well as blood cell dysfunction (platelet structure abnormalities and membrane receptors changes, endothelial dysfunction, leukocytic activation, platelet activation and thrombotic microparticles presence, leukocyte-platelet aggregates). Given the multifactorial genesis of thrombotic complications in these patient population an exhaustive knowledge on them is important in order to optimize prophylaxis and therapy.

II. AIM

To investigate the role of some genetic [factor V Leiden (FVL); prothrombin G20210A mutation (G20210A); PLA1/A2 polymorphism of glycoprotein IIIa (PLA1/A2); JAK2V617F carriership], immunological (CD11b/CD18 expression) and comorbidity factors (medical history) in the thrombogenesis of MPN patients.

III. TASKS

1. To define frequency of genetic defects carriership for thrombophilia in MPN patients and compare it with a control group of healthy volunteers.
2. To compare the frequency of thrombophilia defects carriership between patients with and without thrombotic complications.
3. To study the association between genetic thrombophilia carriership and thrombotic risk in patients with different MPN entities.
4. To define the frequency and clinical significance of thrombotic risk in patients with JAK2V617F mutation and combined carriership of different genetic mutation
5. To study the significance of full blood count parameters for the thrombotic risk in the patient population.
6. To define the level of CD11b/CD18 expression on neutrophils of patients, compare it with control group level and study the role between expression and genetic defects carriership in patients with and without thrombotic events in different diseases subgroups.
7. To study the role of comorbidity factors on the thrombotic risk in MPN patients.

IV. MATERIAL AND METHODS

1. Material

To accomplish the aim and tasks we performed a prospective case-control study by selecting the participants, according to disease, age and sex. Information about comorbidity/risk factors, anamnesis for thrombotic events (TEs), blood cell counts and carriership of JAK2V617F was gathered retrospectively, via a documentation method.

The study covered the period from 2013 to 2019. Patients (N=138) with confirmed MPNs were studied, aged between 23 and 90 years, treated in Hematology Clinic, UMHAT "Georgi Stranski", Pleven or observed in outpatient clinic. They were divided into 4 subgroups, depending on the type of disease. There were two control groups.

- **Patient group (PG)**

Patient group consisted of 138 people (63 women and 75 men), average age 63.18 ± 14.03, diagnosed with MPNs according to WHO (World health organization) criteria from 2008 to 2016. Patients were selected according to inclusion and exclusion criteria, presented in table 1.

Table 1. Inclusion and exclusion criteria for patient group selection

Inclusion criteria	**Exclusion criteria**
• MPN diagnosis according to the WHO criteria	• A diagnosis, other than CML (chronic myeloid leukemia), PV (polycythemia vera), ET (essential thrombocythemia), MF (myelofibrosis) according to the WHO criteria
• Age above 18 years	• Age below 18 years
• **Voluntarily signed** informed consent from for study participation (annex 1)	• **A refusal** to sign an informed consent form

All patients were tested for genetic thrombophilia carriership. Since the study is a result of several scientific projects and while performing them, the scientific idea was developing in

perspective, first patients included were not tested for surface CD11b/CD18 granulocytic markers. Therefore, this parameter was tested in 113 patients.

- **First control group (CG1) – for genetic thrombophilia**

The first control group consisted of 108 healthy volunteers (53 women and 55 men), average age 31.57±0.95 with no MPN diagnosis and no TEs present. Healthy volunteers were selected according to inclusion and exclusion criteria, presented in table 2.

Table 2. Inclusion and exclusion criteria for CG1 selection

Inclusion criteria	**Exclusion criteria**
• Age above 18 years	• Age below 18 years
• Absence of MPN diagnosis according to WHO criteria	• A MPN diagnosis according to WHO criteria
• Absence of TEs anamnesis	• TEs anamnesis
• **Voluntarily signed** an inform consent form for study participation (annex 2)	• **A refusal** to sign an informed consent form

- **Second control group (CG2) – for the expression of CD11b/CD18 on neutrophils**

Second control group consisted of 46 healthy volunteers (13 women and 33 men), average age 62.63±12.90. There was no anamnesis for MPN or TEs. Inclusion and exclusion criteria for CG2 were the same as for CG1 (table 2).

2. Methods

2.1. Survey method – information was gathered through a questionnaire about passport data, disease history and current therapy, medical history and comorbidities [myocardial infarction (MI), ischemic heart disease (IHD), arterial hypertension (AH), heart failure (HF), diabetes mellitus (DM), obesity, hyperlipidemia, liver disease or other neoplasms], TEs anamnesis before and after diagnosis, family history for TEs, total thrombosis provoking factors – recent operation, trauma, continuous immobilization, malignancies, hormone replacement therapy, smoking. Additional information was gathered in women for miscarriages and pregnancy complications.

2.2. Laboratory methods – parameters in full blood count (FBC). Hemoglobin, erythrocytes, leukocytes and platelet values were defined automatically. The reference count is presented in annex 4.

2.3. DNA (deoxyribonucleic acid) **analysis** in stages:

- DNA extraction from venous blood via saline extraction with commercial kits "AccuPrep Genomic DNA Extraction Kit" – BIONEER, using approved laboratory protocols and manufacturer recommendations.
- FVL, G20210A and PLA1/A2 mutation genotyping via a restriction analysis, using PicoReal 96 platform – Real-time PCR (reverse transcription polymerase chain reaction) – Thermoscientific.
- Allele profile analysis, reported on agarose electrophoresis.

2.4. Immunophenotyping of peripheral blood – leukocytes were tested from whole venous blood 2 hours after obtaining it through immunophenotyping. A 2-laser cytometer was used, FACS Calibur cytometer (Becton Dickinson, Heidelberg, Germany). Result analysis was performed on Cell Quest computer software. Blood cells were processed with a combination of two monoclonal antibodies, marked with two different fluorochromes. After erythrocytes lysing (Lysis buffer; Becton Dickinson) and two-times ablution, leukocytes bound to monoclonal antibodies were resuspended and fixed (CellFIX, BD Biosciences). After obtaining 10 000 cells for each test, cell size and cell granularity were tested through forward and side scatter (FSC/SSC) to define the population of interest (lymphocytic gating). The flowcytometer was calibrated everyday with calibration beads and the results were analysed with FACS Comp software©2007 Becton Dickinson. Cell subpopulations were identified through fluorescence of corresponding monoclonal antibodies.

2.5. RT-PCR (reverse transcription polymerase chain reaction**) –** information about JAK2V617F carriership of patients was obtained through a documentary method. The tests were performed in Cytogenetic and molecular biology laboratory at the Specialized hospital for active treatment of hematological diseases.

2.6. Statistical methods – the collected data were processed with software statistical packages: STATGRAPHICS, SPSS and EXCEL for Windows. Two calculators were used, available online:

- *Georgiev G.Z., "Odds Ratio Calculator", [online] Available at: https://www.gigacalculator.com/calculators/odds-ratio-calculator.php URL [Accessed Date: 01 Feb, 2024]*

- *MedCalc Software Ltd. Odds ratio calculator. https://www.medcalc.org/calc/ (Version 22.019; accessed February 1, 2024)*

Results are described in tables, graphics and numeric indicators for structure, frequency, average values, correlations, etc.

Parametric tests to check hypothesis in normal and close to normal case distribution (t – test, ANOVA c post hoc tests Tukey, Scheffe, Bonferroni, Newman-Keuls, Duncan) and non-parametric tests in different than normal case distribution (Pearson x^2 - test, Mann-Whitney, Kruscal-Wallis H-test) were used.

To model and prognose correlations, regression models were used. To model and compare time-event data, a Kaplan-Maier test was applied.

Significance of results and conclusions was defined at $p<0.05$.

Presented data was a result of projects, financed by Medical university – Pleven: project N 13/2013, project N 2/2015, project N 9/ 2017 и N 9/ 2019 (annex 4).

V. RESULTS AND DISCUSSION

1. Characteristics of investigated patients and control groups

1.1. Age distribution

In this study *138 patients were included, average age 63.18±14.03 years (ranging from 23 to 90 years)*, selected randomly, treated in Hematology Clinic, UMHAT "Georgi Stranski" and in outpatient clinic, for the period of 5 years – from March, 2013 to March, 2019. Patients were included in the study at different periods after their diagnosis.

Healthy individuals in control groups were also selected randomly.

The difference in age between PG and CG1 was statistically significant (**$p<0.05$**). The obtained results were not discussed in the context of factor "age" when comparing with control groups. The age difference between PG and CG2 was not statistically significant ($p=0.96$) (table 3).

Table 3. Age distribution of investigated groups

	PG	**CG1**	**CG2**
Average age (years)	63.18	31.57	62.63
Standard deviation (SD)	14.03	0.951	12.90
Statistical significance (p) when comparing with PG		**p<0.05**	p=0.96

1.2. Sex distribution

The ratio between women and men in PG was 1:1.19 – 63 women (45.65%) to 75 men (54.35%). The distribution of healthy volunteers in CG1 was 1:1.03 – 53 women (49.07%) to 55 men (50.93%). The difference was not statistically significant (p=0.79). The distribution of healthy volunteers in CG2 was 1:2.54 - 13 women (28.26%) to 33 men (71.74%). The difference was statistically significant (**p < 0.05**) (figure 1).

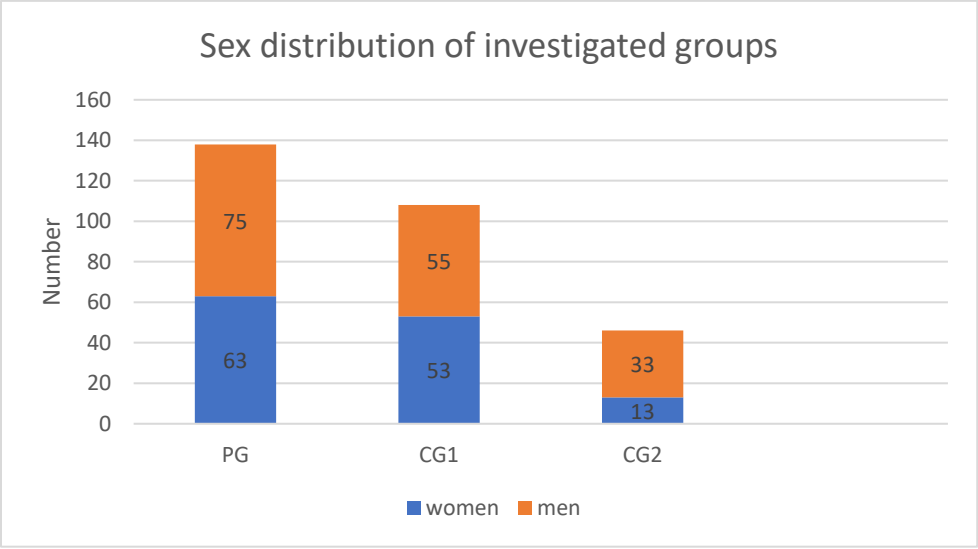


Figure 1. Sex distribution of individuals from PG, CG1 and CG2.

Following result analyses were not associated with and dependent on the "sex" parameter. To confirm this, no statistically significant difference was found in TEs between women and men (χ^2=2.25, df=1, N=138, p=0.13, φ=0.13) (table 4).

Table 4. Sex distribution in investigated groups.

Sex	**PG**	**CG1**	**CG2**
Women (number)	63	53	13
Men (number)	75	55	33
Statistical significance (p)	p=0.15	p=0.79	**p<0.05**

1.3. Diagnosis distribution

According to MPN subtype patients were divided into 4 groups: with confirmed diagnosis of *PV – 49 (35.51%), ET – 20 (14.49%), MF – 39 (28.26%) and CML – 30 (21.74%)* (figure 2). The difference between these subgroups was statistically significant (χ^2=13.36, **p=0.004**). To avoid misinterpretation, analyses were performed on the whole group and separately on every single subgroup, considering patient count.

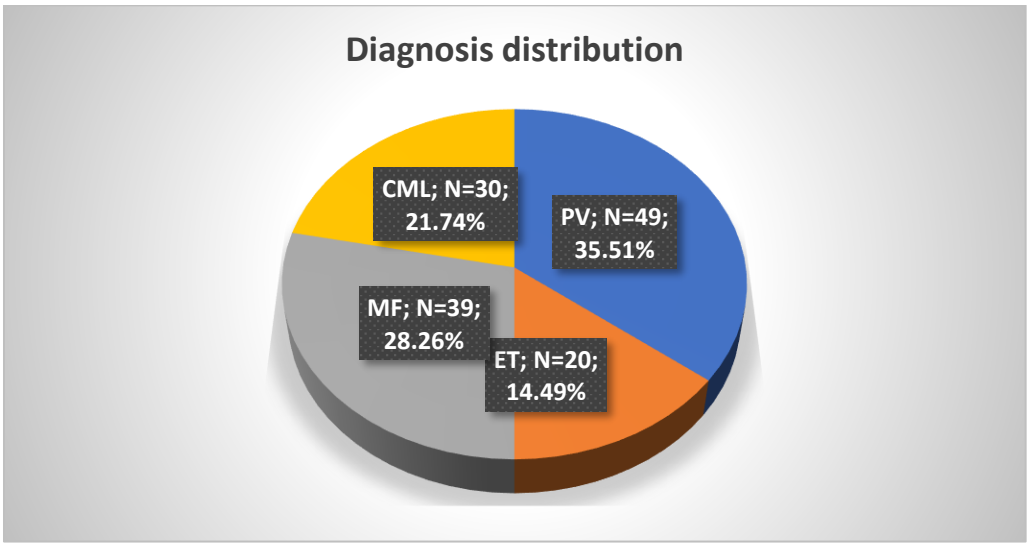


Figure 2. Patient distribution, depending on the diagnosis

2. Thrombotic events in patient group

The term “thrombotic event” consists of venous and arterial thrombosis. Patients reported following types of TEs: myocardial infarction (MI), ischemic stroke (IS), deep vein thrombosis (DVT), pulmonary thromboembolism (PTE), splenic infarction, miscarriages in women.

Frequency of TEs in PG was 28.26%. Of all patients 39 had anamnesis for TEs, 3 of them reported two vascular events and the total number of events was 42. In the rest of 99 (71.74%) patients no TEs were registered (figure 3). In the healthy volunteers of CG1 and CG2 no vascular events were registered before and during the study.

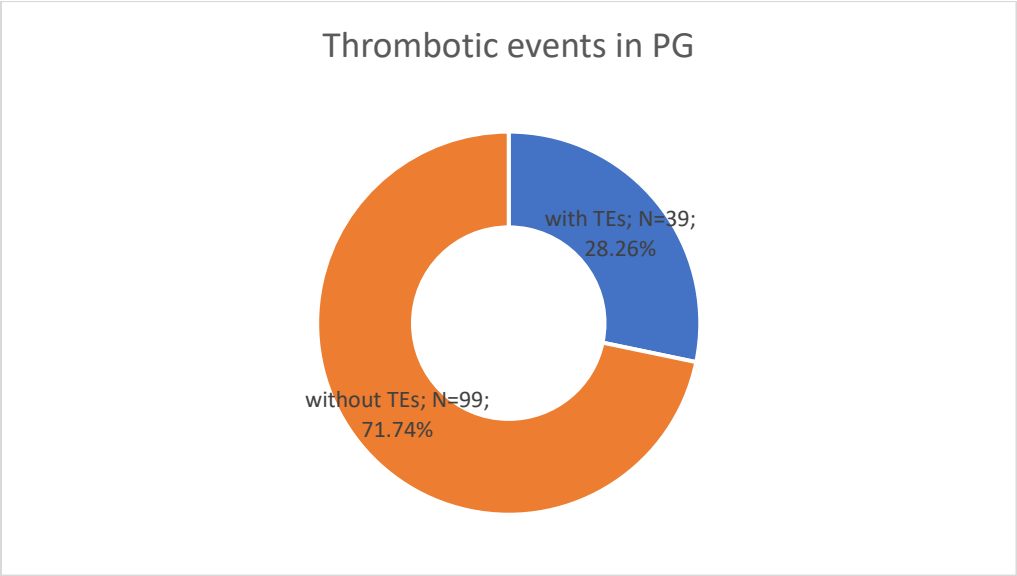


Figure 3. Thrombotic events in patient group

The frequency of 28.26% for TEs, found in our study, was comparable to those reported by other authors for MPN patients – about 20%, but there are data, documenting even higher rate – more than 40%. Most of the available resources report data on the separate subgroups, but not the whole MPN group.

3. Genetic thrombophilia

3.1. Total genetic thrombophilia frequency in patient group and control group 1 – results of task 1 completion

All 138 patients were tested for genetic thrombophilia. Of them *48 (34.78%) were confirmed to carry some of the investigated factors* (FVL, G20210A, PLA1/A2) in heterozygous or homozygous genotype. In CG1 25 (23.15%) volunteers were carriers. The difference was statistically significant (odds ratio - OR=1.77; 95% confidence interval - CI [1.00-3.13]; **p=0.02**; t=1.97). Our results are in accordance with literature data but mostly for PV and ET patients. No data was found available on the whole MPN group – Ph (Philadelphia)-positive and negative.

Frequency of genetic thrombophilia carriership is presented in table 5 – for patients and healthy volunteers, depending on TEs presence.

Table 5. Carriership of genetic thrombophilia and TEs presence in PG and CG1.

Genetic thrombophilia defects	**PG (N=138 patients)**		**CG1, N=108 volunteers, (% of 108)**
	With TEs, N=39 (% of 138)	*Without TEs, N=99 (% of 138)*	
Factor V Leiden (FVL)			
Homozygous for wild allele	39 (28.26%)	95 (68.84%)	101 (93.52%)
Heterozygous for mutant allele	0 (0.0%)	4 (2.90%)	7 (6.48%)
Homozygous for mutant allele	0 (0.0%)	0 (0%)	0 (0.0%)
Prothrombin gene mutation (G20210A)			
Homozygous for wild allele	35 (25.36%)	93 (67.39%)	105 (97.22%)
Heterozygous for mutant allele	4 (2.90%)	6 (4.35%)	3 (2.78%)
Homozygous for mutant allele	0 (0.0%)	0 (0.0%)	0 (0.0%)
Mutation in the gene for glycoprotein IIb/IIIa (PLA1/A2)			
Homozygous for wild allele	30 (21.74%)	71 (51.45%)	93 (86.11%)
Heterozygous for mutant allele	8 (5.8%)	25 (18.12%)	14 (12.96%)
Homozygous for mutant allele	1 (0.72%)	3 (2.17%)	1 (0.93%)

Total carriership of genetic thrombophilia			
Carriers	12 (8.7%)	36 (26.09%)	25 (23.15%)
Non-carriers	27 (19.57%)	63 (45.65%)	83 (76.85%)

3.2. FVL carriership frequency

In PG 4 (2.90%) heterozygous carriers of FVL were found – 2 patients with PV and 2 with CML. In CG1 carriers were almost 2 times more - 7 (6.48%). Non-carriers of FVL (homozygous for wild allele) in PG were 134 (97.10%) and in CG1 - 101 (93.52%).

No significant difference in FVL frequency was found between PG and CG1 (OR=0.45, 95%CI [0.13-1.49], p=0.09, t=1.31; $\chi 2$=1.82, p=0.18). The genetic defect was not more frequently found among MPN patients than in CG1 volunteers, confirmed by other sources as well.

3.3. Polymorphism G20210A in prothrombin gene carriership frequency

In PG 10 (7.25%) heterozygous carriers of G20210A were found – 5 patients with MF, 3 with CML, 1 with PV and 1 with ET. In CG1 the carriers were about 3 times less – 3 (2.78%). Non-carriers of G20210A in PG were 128 (92.75%) and in CG1 - 105 (97.22%).

Statistically significant difference in G20210A frequency between PG and CG1 was not found (OR=2.61, 95%CI [0.74-9.25], p=0.07, t=1.49; $\chi 2$=1.81, p=0.18). *The genetic defect is a risk factor but is not more frequently found among MPN patients than in CG1.*

3.4. Polymorphism PLA1/2 in glycoprotein IIb/IIIa (PLA1/A2) gene carriership frequency

There were 37 (26.81%) carriers of PLA1/A2 in PG – 4 (2.89%) of them homozygous and 33 (23.92%) heterozygous. Homozygous carriers were 3 patients with PV and 1 with MF, heterozygous carriers were 9 with PV, 9 with CML, 7 with ET and 8 with MF. In CG1 the frequency was significantly lower – 15 (13.89%) carriers, 1 (0.93%) of them homozygous and 14 (12.96%) – heterozygous. The non-carriers of PLA1/A2 in PG were 101 (73.19%) and in CG1 - 93 (86.11%) (figure 4).

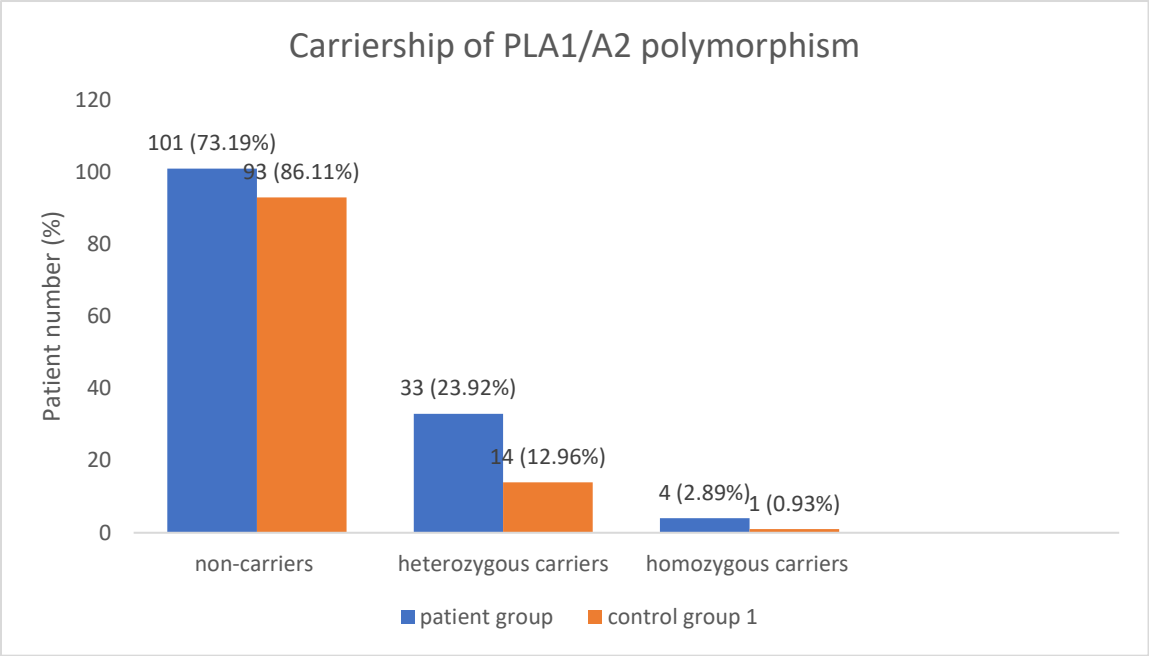


Figure 4. Carriership of PLA1/A2 in PG and CG1.

A statistically significant difference in PLA1/A2 frequency was found between PG and CG1 (OR=1.93, 95%CI [1.12-3.33], **p=0.009**, t=2.37; $\chi 2$=6.04, **p=0.01**). *The genetic defect is more commonly found in PG than in CG1 and is considered to be a risk factor.* Other results, reported by Bulgarian and international authors, are in accordance with our results. There are no specific literature data available on PLA1/A2 frequency of PLA1/A2 carriership for the whole MPN group. Most authors only comment on quantitative and qualitative changes in these receptors, that increase thrombotic risk.

4. Association between genetic thrombophilia carriership and TEs presence – results of task 2 completion

4.1. Association between FVL carriership and TEs presence

No TEs were registered in patients who are carriers of FVL. *No significant difference was found in TEs frequency between carriers and non-carriers of FVL in PG* (OR=0.27, 95%CI [0.01-5.11], p=0.38; $\chi 2$=1.51, p=0.28), as well as between carriers in PG and CG1. There was no change in thrombotic risk. There are few publications in literature with data, opposite to ours, most authors confirm our results.

4.2. Association between G20210A carriership and TEs presence

In PG there were 10 (7.25%) patients, carriers of G20210A, and in CG1 – they were 3 (2.78%). All of them were heterozygous for the mutant allele. In 4 (2.90%) carriers of PG there were TEs registered. There was no statistically significant difference in TEs frequency between patients and healthy volunteers from CG1, carriers of this mutation (OR=2.73, 95%CI [0.73-10.19], p=0.07, t=1.50; $\chi 2$=3.17, p=0.08; relative risk - RR=2.61, 95%CI [0.74-9.25], p=0.07, t=1.49), as well as in PG between carriers and non-carriers of G20210A (OR=1.77, 95%CI [0.47-6.65], p=0.20, t=0.85; $\chi 2$=0.73, p=0.39; RR=1.46, 95%CI [0.65-3.29], p=0.18, t=0.92). *Carriership of G20210A is a risk thrombotic factor but the thrombotic risk in MPN patients is not higher than in CG1.* Most authors also do not report higher thrombotic risk in MPN carriers of this mutation.

4.3. Association between polymorphism PLA1/A2 carriership and TEs presence

There were 37 (26.81%) patients and 15 (13.89%) volunteers in CG1, who were carriers of polymorphism PLA1/A2. In 8 (5.80%) heterozygous carriers and in 1 (0.72%) homozygous carrier of PG TEs were registered, for the rest of 25 (18.12%) heterozygous and 3 (2.17%) homozygous there were no TEs present. Statistically significant difference in thrombotic frequency between patients and healthy volunteers, both carriers of the mutation, was registered (OR=10.33, 95%CI [0.56-189.76], **p=0.04,** t=3.45; $\chi 2$=4.33, p=**0.04)**, as well as a thrombotic risk increase (RR=8.00, 95%CI [0.49-129.37], p=0.14). When comparing thrombotic frequency between patient carriers and non-carriers of the mutation, no significant difference was confirmed (OR=0.76, 95%CI [0.32-1.81], p=0.27, t=0.62; $\chi 2$=0.38, p=0.54) or risk increase (RR=0.82, 95%CI [0.43-1.56], p=0.27). According to other authors, 27.9% of MPN patients, who carry the polymorphism, experience a TE. In our PG we found similar percentage – 37 carriers and 9 of them with thrombosis - 24.32%.

Carriership of PLA1/A2 polymorphism is a significant risk thrombotic factor in the context of confirmed MPN and the risk in these patients is 8 times higher than in CG1 carriers.

5. Genetic thrombophilia carriership and thrombotic risk in patient subgroups according to the disease type – results of task 3 completion

5.1. Polycythemia vera

There were 49 (35.51%) patients with PV diagnosis in PG. In 14 them 15 TEs were registered, they represented 35.9% of patients with TEs and 28.57% of all PV patients.

According to the type of event they are divided into: 3 with MIs, 4 with ISs, 6 DVTs, 2 PTEs. *Thrombotic frequency of investigated MPN patients (28.57%)* corresponds to literature data - 28.6% (30.0-41.0%). Results for PV subgroup are shown in table 6.

Table 6. Frequency of genetic defects in PV subgroup and CG1 according to TEs presence

Genetic defects	**PV subgroup (N=49 patients)**		**CG1, N=108 volunteers, (% of 108)**
	With TEs, N=14, (% of 49)	*Without TEs, N=35, (% of 49)*	
Factor V Leiden (FVL)			
Homozygous for wild allele	14 (28.57%)	33 (67.35%)	101 (93.52%)
Heterozygous for mutant allele	0 (0.0%)	2 (4.08%)	7 (6.48%)
Homozygous for mutant allele	0 (0.0%)	0 (0%)	0 (0.0%)
prothrombin gene mutation (G20210A)			
Homozygous for wild allele	14 (28.57%)	34 (69.39%)	105 (97.22%)
Heterozygous for mutant allele	0 (0.0%)	1 (2.04%)	3 (2.78%)
Homozygous for mutant allele	0 (0.0%)	0 (0.0%)	0 (0.0%)
Mutation in the gene for glycoprotein IIb/IIIa (PLA1/A2)			
Homozygous for wild allele	9 (18.37%)	28 (57.14%)	93 (86.11%)
Heterozygous for mutant allele	4 (8.16%)	5 (10.20%)	14 (12.96%)
Homozygous for mutant allele	1 (2.04%)	2 (4.08%)	1 (0.93%)
Total thrombophilia carriership			
Carriers	5 (10.20%)	10 (20.41%)	25 (23.15%)
Non-carriers	9 (18.37%)	25 (51.02%)	83 (76.85%)
Carriership of JAK2V617F mutation			
Homozygous for wild allele	1 (2.04%)	12 (24.49%)	
Heterozygous for mutant allele	4 (8.16%)	3 (6.12%)	
Homozygous for mutant allele	2 (4.08%)	2 (4.08%)	
Data missing	7 (14.29%)	18 (36.73%)	108 (100%)

We found statistically significant differences when comparing thrombotic frequency between PV patients and healthy volunteers of CG1. Among PV patients there were 15 carriers of genetic thrombophilia (30.61%) – 5 of them with anamnesis for TEs, and in CG1 there were 25 (23.15%) carriers with no TEs. This significance (OR=26.71, 95%CI [1.35-527.52], **p=0.03**, t=2.74; RR=17.88, 95%CI [1.06-302.09], **p=0.05**) confirms *a 17-times increase in thrombotic risk in PV patients, who are genetic thrombophilia carriers.* Bulgarian authors also reported similar data in 2007 for PV/ET patients.

We found a statistically significant difference when comparing TE frequency between PV subgroup patients and CG1 healthy volunteers (OR=22.73, 95%CI [1.11-467.48], **p=0.04**, t=2.93), as well as a thrombotic risk increase (RR=13.54, 95%CI [0.82-222.88], p=0.07). *We confirm a significantly (14 times) higher risk for thrombosis in PLA1/A2 polymorphism carriers with PV diagnosis.* Similar to our data are conclusions by Bulgarian and foreign authors. The risk we found was higher than already reported and was close to the thrombotic risk for all PV patients, who were carriers of genetic thrombophilia defects.

Statistical significance was found when comparing TEs frequency between PV patients, who were carriers and non-carriers of JAK2V617F mutation – 6 TEs registered in 11 carriers and 1 TE in 13 non-carriers (OR=14.40, 95% CI [1.36-152.53], **p=0.01**, t=2.22), the risk was also increased (RR=7.09, 95% CI [1.00-50.28], **p=0.03**). *Carriership of JAK2V617F mutation associates with a significant (7 times) increase in thrombotic risk for PV patients.* Our result for increased thrombotic risk in PV patients, who were JAK2V617F carriers, as compared to non-carriers, was categorically confirmed in literature also.

5.2. Essential thrombocythemia

There were 20 patients (14.49%) with confirmed ET diagnosis in PG. In 6 of them TEs were registered - 15.38% of all patients with TEs and 30.00% of all ET patients. *Thrombotic frequency in the investigated ET subgroup (30.00%) is similar to literature data – 20.7%* (19.00-32.00%), but rather higher. Registered events consisted of: 1 IS, 4 DVTs (66.67% of TEs), 1 splenic infarction. The results for ET subgroup patients are shown in table 7.

Table 7. Frequency of genetic defects in ET subgroup and CG1 according to TEs presence

Genetic defects	**ET subgroup (N=20 patients)**		**CG1, N=108 volunteers, (% of 108)**
	With TEs, N=6, (% of 20)	*Without TEs, N=14, (% of 20)*	
Factor V Leiden (FVL)			
Homozygous for wild allele	6 (30.00%)	14 (70.00%)	101 (93.52%)
Heterozygous for mutant allele	0 (0.0%)	0 (0.00%)	7 (6.48%)
Homozygous for mutant allele	0 (0.0%)	0 (0%)	0 (0.0%)
prothrombin gene mutation (G20210A)			
Homozygous for wild allele	5 (25.00%)	14 (70.00%)	105 (97.22%)
Heterozygous for mutant allele	1 (5.0%)	0 (0.00%)	3 (2.78%)
Homozygous for mutant allele	0 (0.0%)	0 (0.0%)	0 (0.0%)
Mutation in the gene for glycoprotein IIb/IIIa (PLA1/A2)			
Homozygous for wild allele	5 (25.00%)	8 (40.00%)	93 (86.11%)
Heterozygous for mutant allele	1 (5.00%)	6 (30.00%)	14 (12.96%)
Homozygous for mutant allele	0 (0.00%)	0 (0.00%)	1 (0.93%)
Total thrombophilia carriership			
Carriers	2 (10.00%)	6 (30.00%)	25 (23.15%)
Non-carriers	4 (20.00%)	8 (40.00%)	83 (76.85%)
Carriership of JAK2V617F mutation			
Homozygous for wild allele	1 (5.00%)	4 (20.00%)	
Heterozygous for mutant allele	2 (10.00%)	5 (25.00%)	
Homozygous for mutant allele	0 (0.00%)	0 (0.00%)	
Data missing	3 (15.00%)	5 (25.00%)	108 (100%)

When comparing TEs frequency between ET patients, carriers and non-carriers of genetic thrombophilia, carriership was not confirmed as a risk factor in them. Only when comparing thrombotic frequency between ET patients with healthy controls from CG1, risk was

increasing (RR=14.44, 95%CI [0.76-273.31], p=0.08), although the difference was not statistically significant (OR=19.62; 95%CI [0.84-460.59]; p=0.06; t=1.63). This confirmed present literature data, that ET patients were one of the most risky for TEs and it is for them that some local guidelines recommended genetic thrombophilia to be a part of the diagnostic panel. But later on, these recommendations were dropped out.

In ET subgroup no increase in thrombotic risk for G20210A carriership was found, probably because of the small carrier number, no FVL carriers with TEs were present. Based on literature, however, most authors associate these 2 thrombophilia defects carriership in ET with a thrombotic risk increase.

Regardless of the 7 ET patients, who were carriers of PLA1/A2, when comparing TEs frequency between subgroup and healthy volunteers in CG1, no statistically significant difference was found (OR=7.15, 95%CI [0.26-199.69], p=0.25, t=1.08), but the risk was increasing (RR=6.00, 95%CI [0.27-131.35], p=0.26). *This is why carriership of PLA1/A2 was considered a risk factor (although not statistically significant) when ET diagnosis was confirmed as compared to CG1.* Most data available in literature are at the opposite statement.

Based on the literature reference, confirmation of JAK2V617F mutation is found in more than 30% of ET patients, it is associated with higher thrombotic risk and is included in their risk stratification - IPSET (International Prognostic Score for ET). Despite that fact, in the investigated subgroup we did not find any significance when comparing TEs frequency between patients, who were carriers and non-carriers of JAK2V617F – 2 vascular events in 7 carriers and 1 event in 5 non-carriers (OR=1.6, 95%CI [0.10-24.70], p=0.37, t=0.34), and respectively, it was not considered a risk factor (RR=1.43, 95%CI [0.17-11.76], p=0.37). It should be pointed out that results were interpretated, based on a small patient subgroup number.

5.3. Myelofibrosis

In the PG there were 39 patients (28.26%) with MF confirmed diagnosis. In 12 of them TEs were registered – they represented 30.77% of all patients with TEs and 30.77% of all MF patients. *The thrombotic frequency of this subgroup (30.77%)* is higher than reported in the literature (about 10%) and highest of all investigated subgroups (PV, ET and CML) (table 8). In this subgroup 13 patients were diagnosed with secondary MF (TEs were present in 3 of them – 23.08%) and 26 – with primary (TEs were registered in 9 of them – 34.62%). Thrombotic frequency was higher in patients with primary than in secondary MF. Registered events were as follows: 2 MIs, 8 ISs, 1 miscarriage, 1 DVT, 1 splenic infarction.

When comparing TEs between MF patients, carriers of genetic thrombophilia (13 patients with 2 registered TEs) and MF patients, non-carriers (26 patients with 10 registered TEs), the difference in frequency (OR=0.29, 95% CI [0.05-1.59], p=0.08, t=1.42) and risk (RR=0.40, 95%CI [0.10-1.57], p=0.09) were not significant.

Table 8. Frequency of genetic defects in MF subgroup and CG1 according to TEs presence

Genetic defects	**MF subgroup (N=39 patients)**		**CG1, N=108 volunteers, (% of 108)**
	With TEs, N=12, (% of 39)	*Without TEs, N=27, (% of 39)*	
Factor V Leiden (FVL)			
Homozygous for wild allele	12 (30.77%)	27 (69.23%)	101 (93.52%)
Heterozygous for mutant allele	0 (0.00%)	0 (0.00%)	7 (6.48%)
Homozygous for mutant allele	0 (0.00%)	0 (0.00%)	0 (0.0%)
prothrombin gene mutation (G20210A)			
Homozygous for wild allele	11 (28.21%)	23 (58.97%)	105 (97.22%)
Heterozygous for mutant allele	1 (2.56%)	4 (10.26%)	3 (2.78%)
Homozygous for mutant allele	0 (0.0%)	0 (0.0%)	0 (0.0%)
Mutation in the gene for glycoprotein IIb/IIIa (PLA1/A2)			
Homozygous for wild allele	11 (28.21%)	19 (48.72%)	93 (86.11%)
Heterozygous for mutant allele	1 (2.56%)	7 (17.95%)	14 (12.96%)
Homozygous for mutant allele	0 (0.00%)	1 (2.56%)	1 (0.93%)
Total thrombophilia carriership			
Carriers	2 (5.12%)	11 (28.21%)	25 (23.15%)
Non-carriers	10 (25.64%)	16 (41.03%)	83 (76.85%)
Carriership of JAK2V617F mutation			
Homozygous for wild allele	2 (5.12%)	10 (25.64%)	
Heterozygous for mutant allele	5 (12.82%)	4 (10.26%)	
Homozygous for mutant allele	4 (10.26%)	4 (10.26%)	
Data missing	1 (2.56%)	9 (23.08%)	108 (100%)

In this subgroup there were 13 carriers of genetic thrombophilia (33.33%) – 2 of them with TEs. In CG1 there were 25 (23.15%) carriers with no TEs. Difference was not statistically significant (OR=11.09, 95%CI [0.49-249.88], p=0.13, t=1.54), but nevertheless, the factor "carriership" increased the risk for thrombosis (RR=9.29, 95%CI [0.48-180.29], p=0.14). *Carriership of genetic thrombophilia increases 9 times the thrombotic risk in MF patients as compared to healthy volunteers.* Literature data on the topic is not that much and usually includes a small number of patients, rarely attention is paid to the disease itself.

No statistically significant difference was found in thrombotic frequency between MF patients, carriers and non-carriers of G20210A polymorphism (OR=0.52, 95%CI [0.05-5.25], p=0.29, t=0.55), as well as between patients and volunteers from CG1, that were both carriers.

According to our results, no statistically significant difference was found in thrombotic frequency between MF patients, carriers and non-carriers of PLA1/A2 polymorphism (OR=0.20, 95%CI [0.02-1.87], p=0.08, t=1.41), the risk was not changed either (RR=0.29, 95%CI [0.04-1.97], p=0.10). No difference was also found in PLA1/A2 carriership frequency between patients and healthy volunteers of CG1 (OR=5.47, 95%CI [0.20-149.54], p=0.31, t=1.06), *but the risk increased* (RR=4.8, 95%CI [0.22-106.72], p=0.32). Specific data on frequency and TEs association of PLA1/A2 carriership in MPN patients, particularly MF, is missing. This is the reason why presented results for MF subgroup only are a significant scientific contribution.

JAK2V617F mutation has been confirmed in 25.00 to 85.70% of MF patients, as reported in literature. According to some authors no significant relation between carriership and thrombotic risk is confirmed for this patient subgroup. However, according to our results *there was a statistical significance when comparing TEs frequency between MF patients, carriers and non-carriers of JAK2V617F mutation* – 9 events were registered in 17 carriers (thrombotic frequency 52.94%) and 2 events in 12 non-carriers (thrombotic frequency 16.67%) and the difference was significant (OR=5.63, 95%CI [0.94-33.76], **p=0.03**, t=1.89). Data showed a significant 3 times increase in thrombotic risk (RR=3.18; 95%CI [0.83-12.16]; **p=0.05**) for MF carriers. Literature sources confirm age above 60 years and JAK2V617F carriership as important risk factors and especially the combination of the two, that most commonly lead to a vascular event. Our results showed 9 of 12 MF patients with both factors and with TEs – 75% of patients with TEs. Comparing them to the rest of MF patients with both factors but without TEs (7 of 27 without TEs – 27.93%), the difference and risk increase were statistically

significant (OR=8.57, **p=0.004**, RR=2.89, **p=0.0002**). In conclusion, JAK2V617F mutation in MF patients above the age of 60 increases significantly 8 times the risk for TEs.

5.4. Chronic myeloid leukemia

In PG 30 patients (21.74%) with confirmed CML were included. In 7 of them there were 8 TEs registered – these were 17.95% of all patients with TEs and 23.33% of CML patients. The events were as follows: 2 MIs, 3 ISs, 1 miscarriage, 2 DVTs. None of them was predominant in frequency. *Indeed, we found a thrombotic frequency for CML subgroup (23.33%) higher* than reported in the literature 13.00% (1.00–36.00%), but most authors mentioned thrombotic risk in CML mainly in the context of tyrosine kinase inhibitors (TKIs). Results are shown in table 9.

Of all CML patients there were 23 (40.00%) carriers of genetic thrombophilia – 4 of them with a registered TE. In CG1 there were 25 carriers and no TEs. The difference was statistically significant (OR=27.00, 95%CI [1.31-555.02], **p=0.03**, t=2.45). *The thrombotic risk also increased significantly – 18 times in CML patients, who carried genetic thrombophilia* (RR=18.00, 95%CI [1.05-309.61], **p=0.05**), as compared to healthy volunteers of CG1. Thrombotic risk in people, treated with TKIs is well-known. In our PG there were only 3 newly diagnosed patients (10% of all CML patients). We could suggest that therapy for CML was the cause for TEs. But when analysing results, we found that vascular events in CML were registered before antileukemic therapy initiation and it was not possible to define disease phase at that moment.

When comparing genetic thrombophilia carriers (12 patients with 4 TEs) to non-carriers (18 patients with 3TEs), the difference was not statistically significant (OR=2.50, 95%CI [0.45-14.04], p=0.15, t=1.04), *although an increase in thrombotic risk was present* (RR=2.00, 95%CI [0.54-7.39], p=0.15).

Table 9. Frequency of genetic defects in CML subgroup and CG1 according to TEs presence

Genetic defects	**CML subgroup (N=30 patients)**		**CG1, N=108 volunteers, (% of 108)**
	With TEs, N=7, (% of 30)	*Without TEs, N=23, (% of 30)*	
Factor V Leiden (FVL)			
Homozygous for wild allele	7 (23.33%)	21 (70.00%)	101 (93.52%)
Heterozygous for mutant allele	0 (0.0%)	2 (6.67%)	7 (6.48%)
Homozygous for mutant allele	0 (0.0%)	0 (0%)	0 (0.0%)
prothrombin gene mutation (G20210A)			
Homozygous for wild allele	5 (16.67%)	23 (76.66%)	105 (97.22%)
Heterozygous for mutant allele	2 (6.67%)	0 (0.00%)	3 (2.78%)
Homozygous for mutant allele	0 (0.0%)	0 (0.0%)	0 (0.0%)
Mutation in the gene for glycoprotein IIb/IIIa (PLA1/A2)			
Homozygous for wild allele	5 (16.67%)	16 (53.33%)	93 (86.11%)
Heterozygous for mutant allele	2 (6.67%)	7 (23.33%)	14 (12.96%)
Homozygous for mutant allele	0 (0.00%)	0 (0.00%)	1 (0.93%)
Total thrombophilia carriership			
Carriers	4 (13.33%)	8 (26.67%)	25 (23.15%)
Non-carriers	3 (10.00%)	15 (50.00%)	83 (76.85%)

As a conclusion, our results confirmed a higher thrombotic risk in patients, who carry genetic thrombophilia in the context of CML diagnosis. A single Turkish study on this topic is present in literature from 2012 – in a population of children with acute lymphoblastic leukemia, acute myeloid leukemia and CML (6 patients). Probably because of the small CML patients' number, it was not determined whether some of them were carriers and if TEs were present. This led to difficulties in comparing our results to others corresponding.

We found a statistically significant difference in TEs frequency between CML patients, carriers and non-carriers of G20210A polymorphism (OR=21.36, 95% CI [0.89-511.26],

p=0.05, t=11.35) *and the risk also increased significantly for carriers* (RR=5.60, 95%CI [2.53-12.39], **p<0.0001**). No difference was found when comparing thrombotic frequency between CML patients and healthy volunteers from CG1, both carriers of G20210A (OR=35, 95%CI [0.50-2435.88], p=0.10, t=not calculable), but the risk for carriers increased, although not significantly (RR=6.67, 95%CI [0.47-93.59], p=0.16). *In this subgroup the carriership of G20210A itself was associated with a thrombotic risk increase in CML patients as compared to non-carriers.*

We did not find any difference between thrombotic frequency in CML patients and healthy volunteers of CG1, who both carried PLA1/A2 (OR=10.33, 95%CI [0.44-243.34], p=0.15, t=1.60), but although not significantly, *thrombotic risk in PG increased* (RR=8.00, 95%CI [0.43-150.09], p=0.16). No articles were found in literature to investigate carriership of FVL, G20210A and PlA1/A2 in CML patients and to compare their results with ours. This could give a new direction in defining TEs in CML patients outside TKI therapy.

5.5. Summarized data on genetic thrombophilia carriership in patient group

When comparing frequency of vascular events between patients and healthy volunteers of CG1, who were both carriers of genetic thrombophilia, a statistically significant difference was found (OR=17.47, 95%CI [0.99-308.56], **p=0.05**, t=4.00) and *a thrombotic risk increase* (RR=13.27, 95%CI [0.82-215.21], p=0.07). Regarding the whole investigated PG there was no change in thrombotic risk between patients, carriers and non-carriers of genetic thrombophilia but in the context of MPN diagnosis *the risk for patients increased 13 times as compared to healthy volunteers.* We confirmed statistically significant correlation between at least one thrombophilia defect carriership and TEs presence ($\chi 2$=5.16, df=1, p=0.02, Phi=0.15) in MPN patients. Literature data available on the topic is contradictory but does not include CML patients.

6. JAK2V617F carriership – results of task 4 completion

6.1. Frequency

In the whole PG 97 (70.29%) patients were tested for JAK2V617F. Of them 23 (16.67%) were heterozygous and 12 (8.70%) homozygous carriers. Total carriers of JAK2V617F were 35 (25.36%) and 62 (44.92%) were homozygous for the wild allele. Information on carriership was not available for the rest of 41 (29.71%) patients.

6.2. Association between JAK2V617F carriership and TEs presence

Among JAK2V617F carriers there were 17 (48.57%) patients with registered TEs and 7 (11.29%) – in non-carriers. The difference in thrombotic frequency between carriers and non-carriers of the mutation was statistically significant (OR=7.42, 95%CI [2.65-20.76], **p<0.0001**, t=3.82; $\chi 2$=16.526, **p<0.0001**), thrombotic risk increased significantly as well (RR=4.30, 95%CI [1.98-9.35], **p=0.0001**). *Carriership of JAK2V617F mutation in MPN patients significantly 4-times increased thrombotic risk.* These results are in accordance with other studies' conclusions, that positively confirm increased thrombotic risk in patients with Ph-negative MPNs and JAK2V617F presence and also include it in the risk stratification of ET patients.

Thrombotic frequency between hetero- and homozygous carriers was not significantly different (OR=1.09, 95%CI [0.27-4.41], p=0.45, t=0.12; $\chi 2$=0.01, p=0.90). Most literature data confirm the opposite.

In 2009 a thrombotic risk increase was mentioned in JAK2V617F and genetic thrombophilia presence but the topic on cumulative effect of their combination is still debatable.

7. Combination carriership – genetic thrombophilia and V617F mutation in JAK2 (Janus kinase) gene

Combination carriership of genetic thrombophilia defects was found in 3 of our patients – 2 carriers of G20210A and PLA1/A2 (CML and MF diagnoses) and 1 carrier of FVL and PLA1/A2 (CML diagnosis). Only in the CML patient, who was a G20210A and PLA1/A2 carrier, there was a TE presence. The number of patients was too small to draw accurate conclusions.

Combination carriership of all genetic defects, investigated in this study (genetic thrombophilia and JAK2V617F mutation), was found in 14 of our patients – 6 of them with TEs (table 10).

Table 10. Combination carriership of genetic thrombophilia and JAK2V617F mutation in PG

Diagnosis	Number of patients	Number of TEs	Type of genetic defect combinations
PV	6	4	• 5 patients with PLA1/A2 + JAK2V617F • 1 patient with G20210A + JAK2V617F
ET	3	1	3 patients with PLA1/A2 + JAK2V617F
MF	3	0	• 2 patients with PLA1/A2 + JAK2V617F • 1 patient with G20210A + PLA1/A2 + JAK2V617F
CML	2	1	• 1 patient with PLA1/A2 + FVL • 1 patient with PLA1/A2 + G20210A
CG1	2	0	• 2 patients with PLA1/A2 + G20210A

When comparing thrombotic frequency between patients with combination carriership of genetic defects (genetic thrombophilia and JAK2V617F) to patients, carriers of genetic thrombophilia only (14 carriers with 6 TEs to 23 carriers with 6 TEs accordingly), we found a statistically significant difference in thrombotic frequency (OR=3.50, 95%CI [0.88-13.88], **p=0.04**, t=1.78), as well as a thrombotic risk increase (RR=2.43, 95%CI [0.94-6.25], **p=0.03**, t=1.84), i.e. *in the presence of genetic thrombophilia the additional carriership of JAK2V617F mutation significantly increased the risk for thrombosis in MPN patients.* This was also confirmed in literature.

We compared thrombotic frequency of patients with combined carriership of genetic defects to non-carriers (90 non-carriers with 27 TEs). We did not find a significant difference in thrombotic frequency (OR=1.75, 95%CI [0.55-5.53], p=0.17, t=0.95) or in thrombotic risk (RR=1.43, 95%CI [0.72-2.83], p=0.15), but it should be noted that the group of patients, non-carriers of genetic thrombophilia, included some patients with JAK2V617F mutation. Therefore, we compared thrombotic frequency between combined carriers and single JAK2V617F mutation carriers (35 carriers with 17 TEs). Lack of statistical significance in thrombotic frequency (OR=0.79, 95%CI [0.23-2.77], p=0.36, t=0.36) or in risk change (RR=0.89, 95%CI [0.44-1.77], p=0.36) confirmed the conclusion, that *the risk in these patients was mainly increased due to the additional JAK2V617F mutation combined with already present genetic thrombophilia.*

Combination carriership of polymorphism PLA1/A2 and JAK2V617F was most commonly found in the present study – in 10 patients. They presented 71.43% of all patients – combined carriers and half of them (N=5) reported TEs. When comparing this patient population to other patients, carriers of single genetic thrombophilia only (38 carriers with 7 TEs), we found statistically significant difference in TEs (OR=4.43, 95%CI [1.00-19.58], **p=0.02**, t=1.96). *In MPN patients combined carriership of PLA1/A2 and JAK2V617F mutation significantly increased thrombotic risk* (RR=2.71, 95%CI [1.09-6.76], **p=0.02**) as compared to single genetic defect carriers. Similarly to total group of carriers, in combined carriership group of patients the presence of JAK2V617F was an additional risk thrombotic factor for MPN patients with genetic thrombophilia presence.

In PV subgroup highest frequency of patients with combined carriership was found. The difference in thrombotic frequency with the rest of the patients (ET, MF, CML), who were also combined carriers, was not statistically significant (OR=6.00, 95%CI [0.58-61.84], p=0.07, t=1.51), but *thrombotic risk increased in PV diagnosis and combined carriership* (RR=2.67, 95%CI [0.71-10.05], p=0.07) although not significantly. Probably significance was not present due to the small patient number in some subgroup analyses.

8. Impact of FBC parameters on the thrombotic risk in MPN patients – results of task 5 completion

8.1. Patient results according to the levels of leukocytes, hemoglobin and platelets

Patient results according to the values of main FBC parameters were presented graphically. They were divided into 3 groups – patients with low, normal and increased levels of leukocytes, hemoglobin and platelets (table 11). The references for normal ranges of the parameters are shown in annex 5.

Table 11. Patient groups according to some FBC parameters' changes

FBC parameters	Changes	Women, N=63 (% of 63)		Men, N=75 (% of 75)		Total, N=138 (% of 100)	
Leukocytes	Leukocytosis	CML, N= 2 PV, N=8 ET, N=5 MF, N=8	Total N=23 (36.51%)	CML, N= 1 PV, N=6 ET, N=5 MF, N=10	Total N=22 (29.33%)	CML, N=3 PV, N=14 ET, N=10 MF, N=18	Total N=45 (32.61%)
	Normal	Total N=37 (58.73%)		Total N=49 (65.33%)		Total N=86 (62.32%)	
	Leukope-nia	ET, N=1 MF, N=2	Total N=3 (4.76%)	MF, N=4	Total N=4 (5.33%)	ET, N=1 MF0, N=6	Total N=7 (5.07%)
Hemoglobin	High	PV, N=9 ET, N=4 MF, N=3	Total N=16 (25.4%)	PV, N=22 MF, N=1	Total N=23 (30.67%)	PV, N=31 ET, N=4 MF, N=4	Total N=39 (28.26%)
	Normal	Total N=30 (47.62%)		Total N=25 (33.33%)		Total N=55 (39.86%)	
	Low	CML, N= 2 PV, N=1 ET, N=7 MF, N=7	Total N=17 (26.98%)	CML, N= 1 PV, N=1 ET, N=4 MF, N=21	Total N=27 (36.00%)	CML, N= 3 PV, N=2 ET, N=11 MF, N=28	Total N=44 (31.88%)
Platelets	Thrombocyto-sis	CML, N= 1 PV, N=7 ET, N=15 MF, N=5	Total N=28 (44.44%)	CML, N=1 PV, N=2 ET, N=3 MF, N=7	Total N=13 (17.33%)	ХМЛ, N=2 ПВ, N=9 ET, N=18 МФ, N=12	Total N=41 (29.71)
	Normal	Total N=30 (47.62%)		Total N=54 (72.00%)		Total N=84 (60.87%)	
	Thrombo cyto-penia	MF, N=5	Total N=5 (7.94%)	PV, N=1 ET, N=1 MF, N=6	Total N=8 (10.67%)	PV, N=1 ET, N=1 MF, N=11	Total N=13 (9.42%)

8.2. Impact of leukocytosis for TEs development

Patients of each subgroup according to MPN type were divided into 2 cohorts – patients with increased leukocytes and low/normal leukocytes (normal/leukopenia) (table 12).

Table 12. Patient distribution of different MPNs according to the level of leukocytes and TEs presence

	Total number (N)	*With TEs (N)*	*Without TEs (N)*	**OR**	**p**	**RR**	**p**
CML with leukocytosis	3	2	1	**8.80**	**0.05**	**3.60**	**0.01**
CML with normal/leukopenia	27	5	22				
PV with leukocytosis	14	6	1	2.53	0.08	1.88	0.08
PV with normal/leukopenia	35	6	1				
ET with leukocytosis	10	3	7	1	0.5	1	0.5
ET with normal/leukopenia	10	3	7				
MF with leukocytosis	18	5	13	0.77	0.35	0.83	0.35
MF with normal/leukopenia	21	5	13				

As a conclusion to our analysis, leukocytosis was a risk thrombotic factor in CML and PV patients but a significance was only confirmed for CML. Still leukocytosis in MPN patients is not a confirmed risk factor, important for therapeutic decisions and our results confirmed this. Other studies with higher patients' number, however, define it as an independent prognostic marker for TEs development and in PV it determines higher arterial events frequency, especially in the context of JAK2V617F carriership.

8.3. Impact of increased hemoglobin level for TEs development

Patients of each subgroup depending on the type of MPN disease were divided into 2 cohorts – patients with increased and low/normal level of hemoglobin (normal/anemia) (table 13).

Table 13. Patient distribution according to different MPNs, hemoglobin level and TEs presence

	Total number (N)	*With thrombosis (N)*	*Without thrombosis (N)*	**OR**	**p**	**RR**	**p**
CML with increased hemoglobin	0	0	0	3.13	0.58	2.07	0.49
CML with normal/anemia	30	7	23				
PV with increased hemoglobin	31	7	24	0.46	0.11	0.58	0.11
PV with normal/anemia	18	7	11				
ET with increased hemoglobin	4	2	2	3	0.17	2	0.15
ET with normal/anemia	16	4	12				
MF with increased hemoglobin	4	1	3	0.73	0.4	0.80	0.4
MF with normal/anemia	35	11	24				

No statistically significant differences between patients with increased hemoglobin level and patients with normal/low hemoglobin level were found, regarding TEs in different MPNs. The expected and logical thrombotic risk increase in patients with high blood viscosity is not always confirmed in studies, as well as by our results.

8.4. Impact of thrombocytosis for the TEs development

Patients of each MPN subgroup were divided into two cohorts – patients with increased platelet count and with low/normal platelet count (normal/thrombocytopenia) (table 14).

Table 14. Patient distribution according to MPN subtypes, platelet level and TEs presence

	Total number (N)	*With TEs (N)*	*Without TEs (N)*	**OR**	**p**	**RR**	**p**
CML with thrombocytosis	2	2	0	**21.36**	**0.05**	**5.60**	**<0.0001**
CML with normal/thrombocytopenia	28	5	23				
PV with thrombocytosis	9	2	7	0.67	0.32	0.74	0.33
PV with normal/thrombocytopenia	40	12	28				
ET with thrombocytosis	18	5	13	0.38	0.26	0.56	0.23
ET with normal/thrombocytopenia	2	1	1				
MF with thrombocytosis	12	3	9	0.67	0.30	0.75	0.31
MF with normal/thrombocytopenia	27	9	18				

In our investigated PG thrombocytosis appeared to be a risk factor in CML patients and it increased thrombotic risk significantly (**p=0.05**). This is in accordance with available literature data for a significant correlation between thrombocytosis (>1000/1500x10^9 /l) and hemorrhagic but not thrombotic complications.

9. CD11b/CD18 expression on neutrophils' surface – results of task 6 completion

For surface CD11b/CD18 expression on neutrophils 113 patients were tested. Of them 32 had TEs and 81 – did not. CG2 consisted of 46 healthy volunteers with no TEs.

9.1. Expression of CD11b/CD18 on neutrophils' surface in patient group and control group 2

In table 15 average number of patient neutrophils, that expressed CD11b/CD18 on their surface, are shown. Results for patient subgroups are presented depending on diagnosis and for CG2 as well.

Table 15. Surface CD11b/CD18 expression on neutrophils in patients (diagnosis subgroups inclusive) and in healthy volunteers of CG2 – Mean, SD, minimum/maximum value (Min/max), Median, Interquartile range (IQR).

Diagnosis	**Number (N)**	**Mean**	**SD**	**Min/max**	**Median**	**IQR**
CML	28	6555.50	±1161.73	4202/8310	6847	1847
PV	35	7180.91	±1428.63	3310/8900	7610	1988
ET	15	6921.93	±2241.64	734/9000	7726	1913
MF	35	6846.57	±1749.71	2532/9310	7168	2796
CG2	46	1437.46	±2421.71	88/8286	306	912

No statistically significant difference in median number of neutrophils, that expressed CD11/CD18 on their surface, was found between patient subgroups (CML, PV, ET and MF) (H=6.06, df=3, N=113, p=0.11), but an important point was that *lowest value was found in CML subgroup and highest – in PV subgroup.*

To compare PG (each subgroup according to diagnosis inclusive) with CG2 Independent-Samples Kruskal-Wallis H Test and Mann-Whitney U Test were used (table 16).

Table 16. Statistical significance between PG (diagnosis subgroups as well) and CG2 according to tests used

	Mean rank score	**Statistical significance compared to CG2**	**Z score**	**U value**	**Statistical significance compared to CG2**
CML (N=28)	68.54	**P<0.001**	5.86	118	**P<0.0001**
PV (N=35)	107.11	**P<0.001**	6.75	97	**P<0.0001**
ET (N=15)	106.13	**P<0.001**	4.92	51	**P<0.0001**
MF (N=35)	99.83	**P<0.001**	6.7	115	**P<0.0001**
PG (N=113)	99.63	**P<0.001**	8.42	381	**P<0.0001**
CG2 (N=46)	31.78			4817	

The results' significance was also confirmed with Post-hoc Dunn's test (Bonferroni corrected alpha of 0.005). In PG significantly higher number of patients' neutrophils expressed CD11b/CD18 on their surface than in healthy volunteers of CG2 (**p<0.0001**) and this was also

confirmed when patient subgroups, depending on diagnosis, were compared to CG2. This correlated with *neutrophil hyperactivity of patients with 4 MPN entities.* Increasing number of studies in literature focus on blood cell activity in MPNs but almost all of them separate CML because of different pathogenesis and Ph-chromosome presence. However, we did not find any articles, that investigate complex thrombogenesis of all MPNs in the cell activity aspect. Probably an important point is the right moment to perform these tests – they should be followed-up appropriately.

9.2. Expression of CD11b/CD18 on neutrophils' surface and relation to TEs presence

Results for PG (diagnosis subgroups inclusive) depending on TEs presence are shown in table 17.

Table 17. Mean, SD and statistical significance of neutrophil number, expressing CD11b/CD18, between patients with TEs and without TEs – for the PG total and in subgroups, depending on diagnosis

PG	**PG N=113 (100%)**			
	With TEs, N=32 (28.32% of 113)		*Without TEs, N=81 (71.68% of 113)*	
	Mean±SD	**Total number N (% of 113)**	**Mean±SD**	**Total number N (% of 113)**
Total PG (N=113)	7412.81±1320.30	32 (100%)	6680.68±1658.74	81 (100%)
P=0.008, t=2.46				
CML (N=28)	6590.80±1210.01	5 (5.63%)	6547.83±1178.89	23 (28.40%)
P=0.47, t=0.07				
PV (N=35)	7846.979	10 (31.24%)	6914.88±1507.96	25 (30.86%)
P=0.02, t=2.15				
ET (N=15)	8110.80±825.66	5 (15.63%)	6327.50±2517.09	10 (12.34%)
P=0.03, t=2.03				
MF (N=35)	7103.50±1595.18	12 (37.50%)	6712.52±1845.07	23 (28.40%)
P=0.26, t=0.65				

Significantly higher number of neutrophils expressed CD11b/CD18 on their surface in patients, who experienced TEs, than in patients without TEs (**p=0.008**) (figure 5), which is in accordance with literature data. *Significant differences were also found in PV and ET subgroups.* If validated in bigger patient cohorts, these average values may be used as predictive for TEs, especially when dynamically followed-up. Our results may contribute to define the missing "neutrophilic threshold", critical for TEs appearance in MPN patients – generally increased neutrophils or above a concrete value.

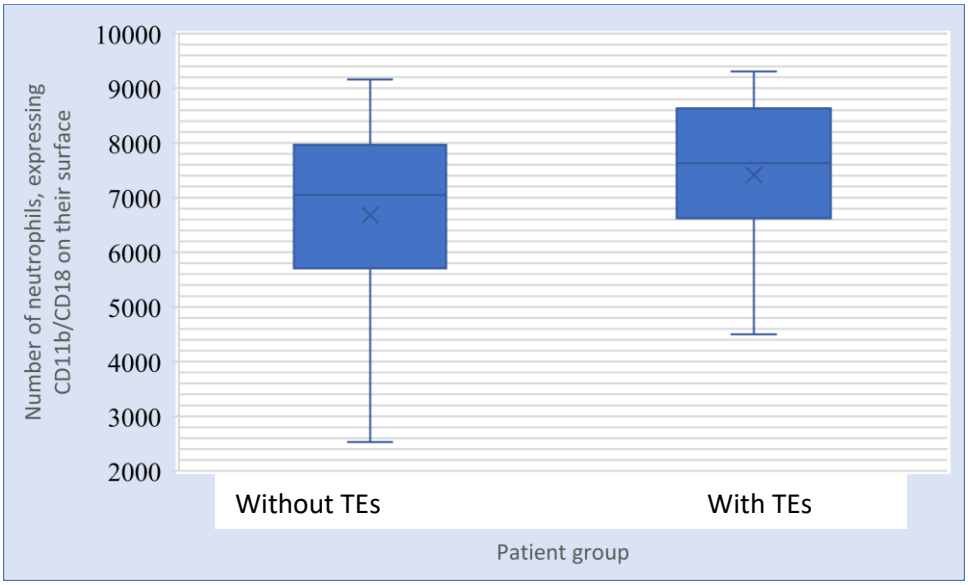


Figure 5. Comparison of CD11b/CD18 neutrophil expression between patients with and without TEs

9.3. Comparison of neutrophils' CD11b/CD18 surface expression between patient group and control group 2

We found a statistically significant difference in neutrophils, that expressed CD11b/CD18 on their surface, between PG and CG2 (U=381.000, N=159, z= -8.425, **p<0.0001**, t=14.07). Mann-Whitney U Test as used. These data have been confirmed in other articles, investigating neutrophil activity in PV and ET patients but no information is available on the whole MPN group.

9.4. Logistic regression

To investigate the effect of neutrophil cells, expressing CD11b/CD18 on their surface, on TEs a logistic regression was performed. Binary logistic regression was constructed to evaluate whether the independent variable "number of neutrophils, that expressed CD11b/CD18

on their surface" significantly predicted probability for TEs occurrence. The regression model was statistically significant ($\chi 2$=22.58, df=1, **p<0.001**). This model explained between 13.20% (Cox & Snell) and 21.10% (Nagelkerke R^2) of dispersion in TEs and classified 80.50% of observations correctly. Wald criterion showed that the independent variable "number of neutrophils, that expressed CD11b/CD18 on their surface" *(Wald=50.18, df=1,* ***p<0.0001)*** *influenced significantly the probability for TEs occurrence.* The value of regression coefficient was 0.045 and of regression constant -4.316. The exponent of regression coefficient Exp(B) showed that *every 1 percent increase in the number of neutrophils, expressing CD11b/CD18 on their surface, led to 1.046 increase in the chance that a person will experience a TE.* It was CD11b itself that some authors investigated to define the level of neutrophil "stickiness" to platelets for leuko-thrombocytic aggregates formation.

9.5. Surface CD11b/CD18 neutrophils' expression in MPN patients and leukocytosis

In 35 patients, tested for CD11b/CD18 expression, we found leukocytosis and they had average number of expressing neutrophils 7549.14±1559.37. Of them 13 had TEs with average number of 8157.08±1240.16 and 22 had no TEs with average number of 7189.91±1641.36 (table 18). The difference was statistically significant (**p=0.03**), i.e. *patients with leukocytosis and TEs had significantly higher number of expressing neutrophils than patients with leukocytosis but without TEs.*

In the absence of leukocytosis TEs were not found to be associated with neutrophilic CD11b/CD18 expression.

Table 18. Number of neutrophils, expressing CD11b/CD18 on their surface, in patients with changes in the leukocytic count, depending on TEs presence

Patient subgroup according to leukocytes (N=113)	**CD11b/CD18 expression on neutrophils – Mean**		
	With TEs (N=32)	*Without TEs (N=81)*	*Mean group value*
Patients with leukocytosis	8157.08±1240.16 (N=13)	7189.91±1641.36 (N=22)	7549.14±1559.37 (N=35)
Patients with normal/low leukocytes	6903.58±1141.51 (N=19)	6490.80±1638.47 (N=59)	6591.35±1535.76 (N=78)

When dividing patients with TEs into patients with leukocytosis (total of 13 patients with average expressing neutrophils 8157.08±1240.16) and the rest – with normal/low leukocytes (total of 19 patients with average expressing neutrophils 6903.58±1141.51), the difference in average number of neutrophils, expressing CD11b/CD18, was statistically significant (**p=0.001**).

Difference in average number of neutrophils, expressing investigated markers, between patients with leukocytosis (N=35) and patients with normal/low leukocytes (N=78), 7549.14±1559.37 and 6591.35±1535.76 accordingly, was significant as well (**p=0.002**).

In conclusion to our data, *leukocytosis is an important parameter for TE development and a specific value of CD11b/CD18 expressing neutrophils may be validated as predictive for vascular event occurrence.* Most authors support our data and some of them even point out leukocytosis as a more significant factor than thrombocytosis. However, there is still ambiguity on specific values for leukocytosis, although some authors mention relatively low ones. Opposite statements are also present, probably because of unclear threshold.

9.6. Surface CD11b/CD18 neutrophils' expression in MPN patients and thrombocytosis

In the CD11b/CD18 tested PG there were 34 patients with thrombocytosis and average cell count, expressing markers (Mean), of 7125.12±1899.99. Of them 10 reported TEs – average value of 7574.40±1228.60, and 24 reported no TEs – average value 6937.92±2112.94 (table 19). The difference was not statistically significant (p=0.14). When comparing this expression between patients with and without TEs with normal/low platelets, the difference was statistically significant (**p=0.01**). In our study *thrombocytosis was not found to be a risk factor.*

Table 19. Number of neutrophils, expressing CD11b/CD18 in their surface, in patients with platelet count changes, depending on TEs presence

Patient subgroup according to platelets (N=113)	**CD11b/CD18 expression on neutrophils – Mean**		
	With TEs (N=32)	*Without TEs (N=81)*	*Mean group value*
Patients with thrombocytosis	7574.40±1228.60 (N=10)	6937.92±2112.94 (N=24)	7125.12±1899.99 (N=34)
Patients with normal/low platelets	7339.36±1381.41 (N=22)	6572.37±1434.11 (N=57)	6785.96±1452.61 (N=79)

As a conclusion, *we did not find an association between platelet count (increased one inclusive), neutrophil hyperactivity and TEs presence.* This is in accordance with other authors findings as well but our data confirmed it for the whole MPN group.

9.7. Surface CD11b/CD18 neutrophils' expression in MPN patients and genetic defects

Data on average number of neutrophils, expressing CD11b/CD18, in the investigated PG and different genetic defects according to TEs presence are shown in table 20.

Table 20. Values of neutrophils, expressing CD11b/CD18 on their surface according to the genetic defect.

Genetic defects/ subgroups	**Neutrophils with CD11b/CD18 expression (Mean)**	*With TEs*		*Without TEs*		**Statistical significance between patients with and without TEs**
		Number	*Neutrophils with CD11b/CD18 expression (Mean)*	*Number*	*Neutrophils with CD11b/CD18 expression (Mean)*	
FVL	7347±26.87	0	0	2	7347±26.87	-
G20210A carriers	6029.33±2109.17	3	6647.33±2066.14	6	5720.33±2250.88	P=0.3
G20210A non-carriers	6962.32±1538.03	29	7492±1247.30	75	6757.51±1597.32	**P=0.007**
PLA1/A2 carriers	6953.87±1422.56	7	7368±1260.55	23	6827.83±1470.73	P=0.17
PLA1/A2 non-carriers	6864.20±1655.96	25	7425.36±1361.48	58	6622.33±1736.26	**P=0.01**
JAK2V617F carriers	7304.97±1503.34	15	7684.53±1364.68	16	6949.13±1582	P=0.08
JAK2V617F non-carriers	6676.36±1558.13	9	6886.67±1507.24	47	6636.09±1580.31	P=0.33

Statistically significant differences were found in the number of neutrophils, expressing CD11b/CD18, when comparing patients with and without TEs of two subgroups – G20210A non-carriers (**p=0.007**) and PLA1/A2 non-carriers (**p=0.01**). No association was found for FVL, G20210A, PLA1/A2 and JAK2V617F mutation carriers with or without TEs.

Statistically significant results were found when neutrophil count with CD11b/CD18 expression in MF patients with TEs and JAK2V617 mutation was compared to patients without this mutation - 7380.22±1658.03 compared to 5582±328.10 – **p=0.01**, t=2.98. This significance was only found in MF subgroup and confirmed the importance of mutation carriership in these patients.

As a conclusion of our results, *we did not confirm that genetic defects carriership was associated with number of neutrophils, expressing CD11b/CD18, the thrombotic risk was not different as well.* Most literature data state the opposite and associate JAK2V617F mutated cells with hypercoagulability state. Our data confirmed this statement for MF patients only.

In our literature reference we did not find any articles to investigate leukocytic activity through CD11b/CD18 expression in MPN patients, carriers of genetic thrombophilia.

10. Comorbidities and thrombotic risk in MPN patients – results of task 7 completion

10.1. PG data

Information about comorbidity/risk factors, that could influence thrombotic risk, was gathered for PG. Patients were divided into 6 groups depending on the number of factors they have (no comorbidity/risk factors, 1 factor, 2 factors, 3 factors, 4 factors, 5 factors) and in 2 subgroups depending on TEs presence – with or without TEs. Data are shown in table 21.

A significant increase was noted in the number of patients (and their percentage) with 2 comorbidity/risk factors, who experienced TEs. Comparing thrombotic frequency between groups of patients without comorbidity/risk factors and 1 factor to groups of patients with 2 and more factors, we found a statistically significant difference (OR=0.28, 95%CI [0.13-0.61], **p=0.0007**). It was confirmed that presence of less factors decreased thrombotic risk (RR=0.41; 95%CI [0.23-0.71]; **p=0.0008**).

Table 21. Patient distribution according to the number of comorbidity/risk factors.

	With TEs (N=39)	Total number (% of patient subgroup)	*Without TEs (N=99)*	Total number (% of patient subgroup)
Patients without comorbidity/risk factors (N=32)	• 2 CML • 3 PV • 1 MF	6 (18.75%)	• 12 CML • 6 PV • 4 ET • 4 MF	26 (81.25%)
Patients with 1 comorbidity/risk factor (N=48)	• 2 CML • 2 PV • 1 ET • 3 MF	8 (16.67%)	• 9 CML • 14 PV • 4 ET • 13 MF	40 (83.33%)
Patients with 2 comorbidity/risk factors (N=38)	• 1 CML • 4 PV • 4 ET • 5 MF	14 (36.84%)	• 2 CML • 10 PV • 6 ET • 6 MF	24 (63.16%)
Patients with 3 comorbidity/risk factors (N=15)	• 2 CML • 4 PV • 1 ET • 1 MF	8 (53.33%)	• 4 PV • 3 MF	7 (46.66%)
Patients with 4 comorbidity/risk factors (N=4)	• 1 PV • 1 MF	2 (50.00%)	• 1 PV • 1 MF	2 (50.00%)
Patients with 5 comorbidity/risk factors (N=1)	• 1 MF	1 (100.00%)	-	0 (0.00%)

Presence of ≤1 comorbidity/risk factor in MPN patients was associated with lower thrombotic risk as compared to ≥2 factors. A significantly higher frequency for IHD, AH and HF in patients with TEs was noted (table 22). Most articles present in literature do not find a difference in thrombotic frequency between MPN patients and different comorbidity/risk

factors but rather report their higher frequency with a follow-up recommendation. Their data do not include the whole MPN group but mostly Ph-negative diseases. The frequency of AH, obesity, DM and hypercholesterolemia we found was higher than reported in literature, though some of them did not reach a significance in thrombotic risk increase.

Table 22. Significance of IHD, AH and HF as the most common comorbidity/risk factors in MPN patients with TEs presence.

Comorbi-dity/risk factor	Presence/ lack of factors (yes/no)	TEs			OR	p	RR	p
		With TEs, N=39 (% of 39)	*Without TEs, N=99 (% of 99)*	Total number, N=138 (% of 138)				
IHD	Yes	12 (30.77%)	6 (6.06%)	18 (13.04%)	6.89	**0.0002**	2.96	**0.000002**
	No	27 (69.23%)	93 (93.94%)	120 (86.96%)				
AH	Yes	28 (71.79%)	50 (50.51%)	78 (56.52%)	2.49	**0.01**	1.96	**0.002**
	No	11 (28.21%)	49 (49.49%)	60 (43.48%)				
HF	Yes	6 (15.38%)	5 (5.05%)	11 (7.97%)	3.42	**0.03**	2.10	**0.009**
	No	33 (84.62%)	94 (94.95%)	127 (92.03%)				

10.2. Comorbidity according to patient subgroups

- CML

When comparing TEs frequency between patients with ≤1 comorbidity/risk factor with >1, the difference was statistically significant (OR=0.12, 95%CI [0.02-1.02], **p=0.03**), *presence of >1 factor increased thrombotic risk in CML patients* (RR=0.27; 95%CI [0.09-0.84]; **p=0.01**).

Because most TEs arose early in CML patients' lives, a special attention to potential comorbidity/risk factors is recommended. Our data also confirmed that in this patient subgroup TEs appeared before TKIs initiation.

Patients with TEs were 7 of total 30. *A significance was confirmed for smoking as a risk thrombotic factor* (**p=0.05**; RR=6.57). The connection between smoking and TEs risk has already been positively confirmed which is in accordance with our data and is an important recommendation to everyday living of CML patients.

- PV

When comparing TEs frequency between patients with ≤1 comorbidity/risk factor to >1, the difference in this subgroup was not significant (OR=0.42, 95%CI [0.12-1.50], p=0.09), the risk was also not changed (RR=0.53, 95%CI [0.21-1.36], p=0.09). There was a significance (OR=0.30, 95%CI [0.07-1.27], **p=0.05**), when comparing groups of patients with ≤2 factors to >2 (RR=0.46, 95%CI [0.20-1.07], **p=0.04**). *Higher number of comorbidity/risk factors was needed to increase thrombotic risk in PV patients.* In literature more than 70% of PV patients have comorbidity/risk factors – in our patient population 81.63%.

In our PV subgroup there was no significant increase in thrombotic risk for patients with a specific comorbidity/risk factor. Opposite to our results, most investigators report a significantly higher frequency and increased risk for patients with AH, DM, dislipidemia and smokers.

- ET

When comparing TEs frequency between patients with ≤1 comorbidity/risk factor to >1, the difference was not statistically significant (OR=0.15, 95%CI [0.01-1.64], p=0.06), presence of ≤1 factor was not enough to decrease thrombotic risk in ET patients (RR=0.24, 95%CI [0.04-1.73], p=0.08). A higher number of patients is necessary to make significant conclusions.

No significant increase in thrombotic risk for patients with a specific comorbidity/risk factor was noted. Opposite to our results, most authors report higher frequency and increased risk in patients with AH, DM, dislipidemia and smokers.

- MF

When comparing TEs frequency between patients with ≤1 comorbidity/risk factor to >1, the difference was statistically significant (OR=0.29, 95%CI [0.07-1.232], **p=0.05**), *presence*

of >1 factor increased the risk for vascular events in MF patients (RR=0.43, 95%CI [0.15-1.19], **p=0.05**). At least 1 factor was found in 87.18% of this patient population.

In our MF subgroup we found *statistically significant increase in thrombotic risk for patients with AH and especially IHD.* Similar to our data, there are reports about AH, dislipidemia, DM and smoking.

11. Complex thromboetiopathogenesis in MPN

Summarizing all data available on our patients, we confirmed a complex, multifactorial etiopathogenesis of thrombotic events in MPN patients.

Of all 138 investigated MPN patients **39** (28.26%) **reported TEs,** 99 (71.74%) patients did not. Of patients with TEs (total of 39) 12 (30.77%) were confirmed to carry genetic thrombophilia, which was a potential reason for TEs presence but in the context of additional risk factors. Even in the presence of a confirmed risk thrombotic factor (some genetic thrombophilia defects, for example), patients with TEs have more than one factor and probably the combination was the reason for thrombotic complication occurrence.

Of 39 patients with TEs 27 (69.23%) did not carry genetic thrombophilia but 12 (30.77%) of them were JAK2V617F carriers, which was also a risk thrombotic factor. In this patient subgroup carriership of mutation was a leading, probably even only cause for TEs. Nevertheless, there were patients with risk factor combination.

For 15 of 39 patients with TEs (33.33%) a different number of comorbidity/risk factors was present. In this subgroup there were patients with no obvious reason for TEs presence. But our investigations did not include other data such as procoagulant proteins and factors levels, inflammatory cytokines, coagulation status, natural fibrinolytics, endothelial dysfunction, microparticles, platelet receptors, etc. This once again confirmed the multifactorial thrombogenesis of MPN patients, necessity for complex approach to evaluate thrombotic risk, comprehensive risk scores development, including different criteria and their burden. To validate them, investigations of many patients are needed.

As a confirmation to our conclusion a pathogenesis of thrombosis in MPN patients is shown in figure 6, recently published by Falanga et al.

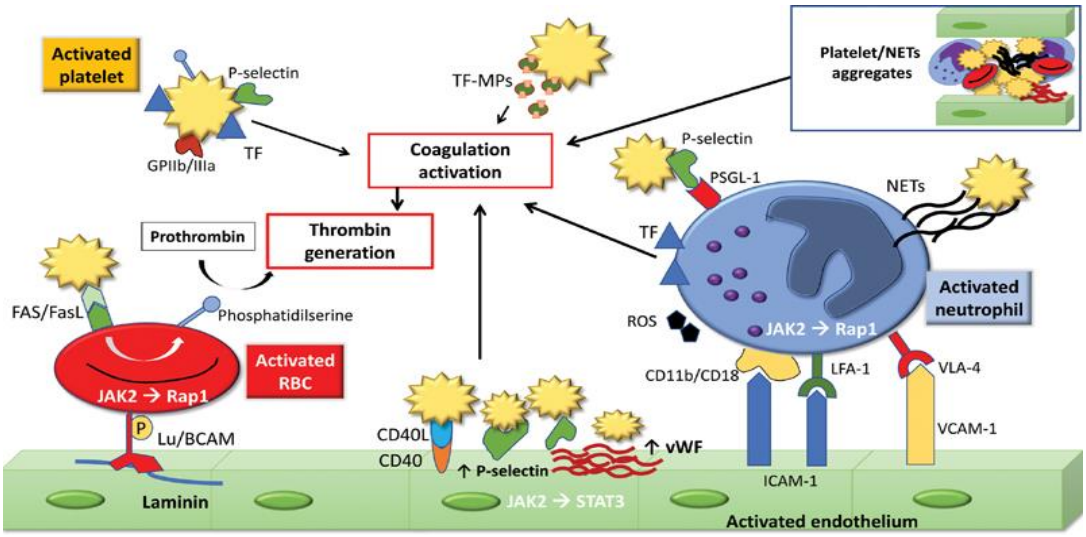


Figure 6. Pathogenesis of thrombosis in MPN patients

(License Number 5724270659565, License date – Feb 08, 2024, Publisher – Georg Thieme Verlag KG, Publication – Hämostaseologie, Title - Prevention and Management of Thrombosis in BCR/ABL-Negative Myeloproliferative Neoplasms, Author - Anna Falanga, Marina Marchetti, Francesca Schieppati, Date – Feb 15, 2021, vol. 41, Issue 01)

12. Risk factors in patients and recommended follow-up in routine practice

According to our data, we could summarize different factors, that were important to thrombotic risk in patients of PG and of different subgroups depending on the disease.

<u>Risk factors for the whole MPN group of patients (Ph-negative and CML):</u>

- Carriership of a thrombophilia defect – increased 13 times thrombotic risk as compared to healthy volunteers;
- Carriership of PLA1/A2 – more common among patients and increased 8 times thrombotic risk as compared to healthy volunteers;
- Carriership of G20210A and FVL – increased slightly thrombotic risk;
- Carriership of JAK2V617F mutation – increased 4.3 times thrombotic risk as compared to non-carriers;
- Combined carriership of investigated genetic defects – increased 2 times thrombotic risk as compared to carriership of thrombophilia defects only and 2 times as compared to healthy volunteers;
- Neutrophilic CD11b/CD18 expression – significantly higher in PG than in CG2 (**$p<0.0001$**);

- Neutrophilic CD11b/CD18 expression in patients with TEs – significantly higher as compared to patients without TEs (**p=0.008**), patients with leukocytosis and normal/low platelets inclusive;
- An increase in neutrophilic CD11b/CD18 expression of patients with 1% increased the chance for TEs with 1.046;
- Presence of at least 2 comorbidity/risk factors significantly increase thrombotic risk as compared to patients with ≤1 factor (p=0.02);
- Comorbidities, such as AH, IHD and HF increased thrombotic risk 2, 3 and 2 times accordingly.

Risk factors for CML patients:

- Leukocytosis and thrombocytosis – increased thrombotic risk 4 and 11 times accordingly;
- Carriership of genetic thrombophilia – increased 18 times risk as compared to healthy volunteers and 2 times as compared to CML patients, who were non-carriers;
- Carriership of G20210A – increased 7 times risk as compared to healthy volunteers and 5.6 times as compared to non-carriers;
- Carriership of PLA1/A2 – increased 8 times the risk as compared to healthy volunteers;
- Smoking – increased 6.57 times the risk;
- Presence of 2 and more comorbidity/risk factors – increased significantly thrombotic risk (**p=0.01**).

Risk factors for PV patients:

- Leukocytosis – increased 2 times the risk;
- Carriership of genetic thrombophilia – increased 18 times thrombotic risk as compared to healthy volunteers;
- Carriership of PLA1/A2 – increased 14 times the risk as compared to healthy volunteers and 2 times as compared to non-carriers;
- Carriership of JAK2V617F mutation – increased the risk 7 times as compared to PV patients, who were not carriers;
- Combined carriership – increased the risk 2.67 times as compared to other subgroups combined carriership;

- Neutrophil CD11b/CD18 expression in patients with TEs – significantly higher as compared to PV patients without TEs (**p=0.02**);
- Presence of at least 3 comorbidity/risk factors – significantly increased the risk (**p=0.05**).

Risk factors in ET patients:

- Carriership of genetic thrombophilia – increased the risk 14 times as compared to healthy volunteers;
- Carriership of PLA1/A2 – increased the risk 6 times as compared to healthy volunteers;
- Neutrophilic expression of CD11b/CD18 in patients with TEs – significantly higher than ET patients without TEs (**p=0.03**);
- Age above 60 years – increased the risk 10 times.

Risk factors in MF patients:

- Carriership of genetic thrombophilia – increased the risk 9 times as compared to healthy volunteers;
- Carriership of G20210A – increased the risk 2 times as compared to healthy volunteers;
- Carriership of PLA1/A2 - increased the risk 5 times as compared to healthy volunteers;
- Carriership of JAK2V617F mutation – increased the risk 3 times as compared to non-carriers;
- Carriership of JAK2V617F mutation and age above 60 years were both found in 75% of MF patients with TEs and in only 26% of ET patients without TEs;
- Neutrophil expression of CD11b/CD18 in patients with JAK2V617F mutation and TEs – significantly higher as compared to MF patients with TEs without JAK2V617F mutation (**p=0.01**);
- Presence of at least 2 comorbidity/risk factors – significantly increased risk (**p=0.05**);
- Presence of AH and IHD – increased the risk 3 times.

As a conclusion to our results, we suggest a practical algorithm to evaluate thrombotic risk in MPN patients step by step (figure 7).

The algorithm allows risk factors evaluation in MPN patients with TEs, using a multicomponent score. It may be used as an element in a complex approach to precise and individualize thrombotic risk in this patient population. That would serve as a base for new therapies, directed to a specific patient according to their genetic and environmental characteristics and would improve therapy to be more effective in the context of personalized medicine.

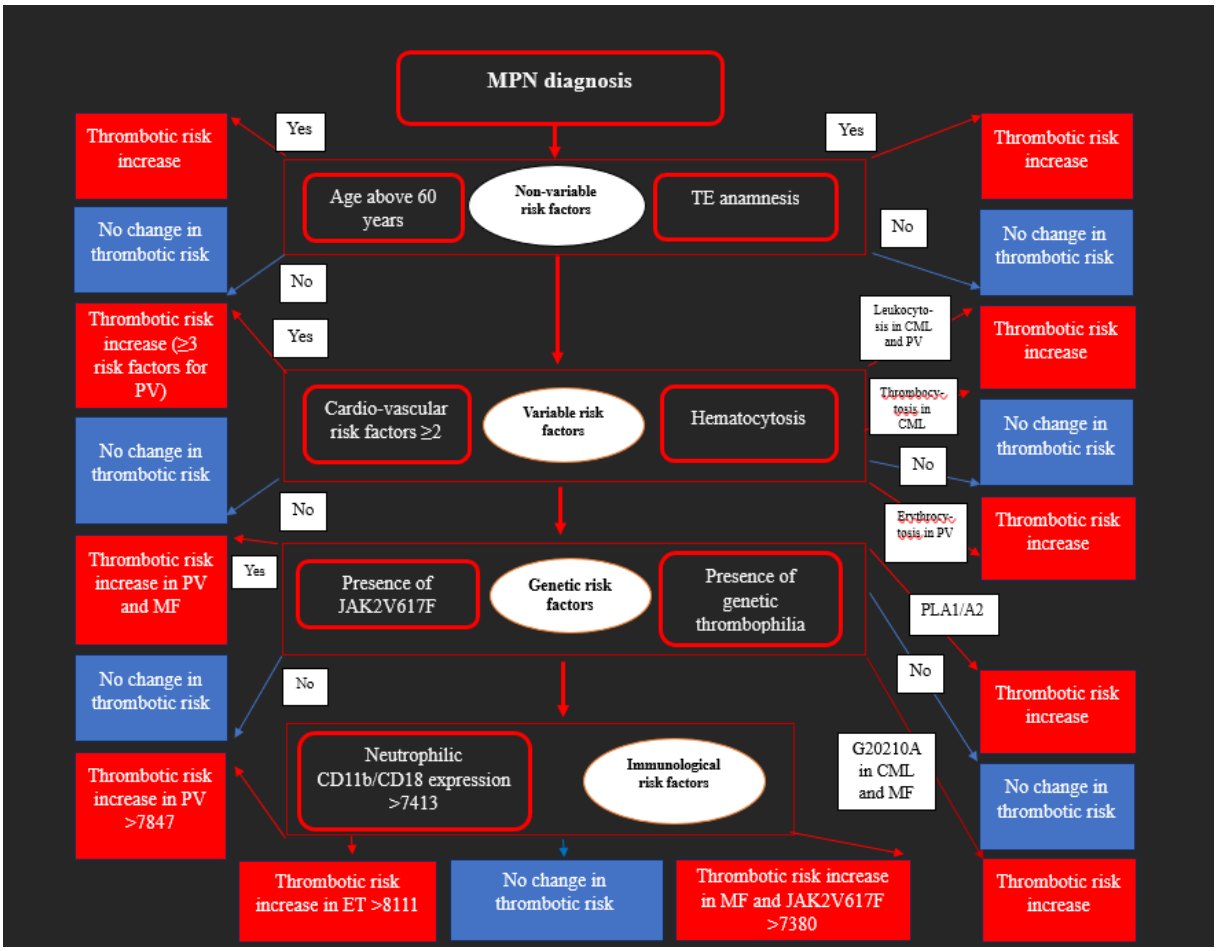


Figure 7. Practical algorithm to evaluate thrombotic risk in MPN patients

Therapy in MPN patients follow the principles of cytoreduction with different medications, target molecules, antiplatelet prophylaxis, anticoagulation and symptomatic agents, appropriate TKI choice, risk factor correction and allogeneic stem cell transplantation sometimes.

VI. CONCLUSIONS

1. Carriership of genetic defects for thrombophilia was significantly more common in the investigated patient group than in healthy volunteers (OR=1.77). The most significant difference in defect frequency between the two groups was found for PLA1/A2 (OR=1.93), especially for PV patients (OR=22.73).
2. Regardless of the fact that in the total MPN patient group thrombophilia defects generally did not show an association with thrombotic risk, carriers of thrombophilia defects had a significant 13 times increase in thrombotic risk as compared to healthy volunteers.
3. Carriership of PLA1/A2 was significantly more frequent in PV patients (OR=22.73), but it was also associated with thrombotic risk in less studied groups, like MF and CML (OR=5.47 and OR=10.33 accordingly). The CML subgroup carriership of genetic thrombophilia increased thrombotic risk 2 times and G20210A in particular – 5.6 times.
4. JAK2V617F carriership was associated with increased thrombotic risk in PV and MF patients (7 and 3 times accordingly). Combined carriership of genetic mutations (thrombophilia and JAK2V617F) was found 6 times more frequent in PV patients as compared to other subgroups. Double carriers for genetic thrombophilia and JAK2V617F mutation had significantly 2 times higher risk for TEs than carriers of genetic thrombophilia only (RR=2.43, p=0.03).
5. Leukocytosis was a confirmed significant risk thrombotic factor in CML and PV patients (RR=3.60 and RR=1.88 accordingly) and thrombocytosis – only in CML patients (RR=5.60).
6. Neutrophilic CD11b/CD18 expression was significantly higher in all MPN patients as well as in separate subgroups as compared to healthy volunteers ($p<0.001$). This was also confirmed for patients with TEs in comparison with patients without thrombosis (p=0.008), especially in PV and ET ($p<0.05$). In the total MPN group carriership of genetic mutations (thrombophilia and JAK2V617F) was not associated with significantly different number of expressing neutrophils when comparing patients with TEs and without TEs.
7. Presence of ≥2 comorbidity/risk factors significantly increased thrombotic risk in MPN patients (p=0.0007). Factors such as AH, IHD and HF were significantly more frequent in patients with TEs than in patients without thrombosis ($p<0.005$).
8. The algorithm for risk thrombotic factors evaluation in MPN patients may serve as an element in a complex approach to precise and individualize risk in this patient population.

VII. CONTRIBUTIONS

Contributions with scientific and original character:

1. This study is the first complex investigation of some risk factors for thrombogenesis in patients with CML and Philadelphia-negative MPNs.
2. Scientific data is added to the frequency and risk change when some genetic factors for thrombophilia are present in CML and MF patients, they have not been studied in this context yet, especially for the PLA1/A2 carriership.
3. Neutrophilic expression of CD11b/CD18 has been studied as a marker for leukocytic activity in classical MPNs (and in separate diagnostic subgroups) and its significance is determined for TEs as well as in the context of blood counts.
4. A comprehensive information is collected and the significance of different comorbidity/risk factors is reported for the thrombogenesis in all patients.
5. An attempt is made to present the complex view of complicate and multifactorial etiopathogenesis of TEs and define the role of each factor when assessing the risk for thrombotic complications in all MPN patients.

Contributions of practical character

1. Thrombotic risk in different MPN entities is precisely reported on the basis of genetic thrombophilia mutations.
2. An association between change in MPN patient neutrophils, expressing CD11b/CD18, and thrombotic risk increase is confirmedd.
3. When validated in large patient populations these levels would serve as “cut-off” or predictive of thrombotic event in different MPN subgroups.
4. Precise comorbidity/risk factors are established, associated with thrombogenesis in patients.
5. An algorithm is developed to evaluate the risk factors for thrombotic events in MPN, based on the complex approach to precise and individualize thrombotic risk in these patient population.

VIII. PUBLICATIONS AND PARTICIPATIONS IN SCIENTIFIC FORUMS IN CONJUCTION WITH THE DISSERTATION WORK

A STUDY ON THE ROLE OF THROMBOPHILIC GENETIC DISORDERS AS A RISK FACTOR FOR THROMBOTIC COMPLICATIONS IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS. Doroteya K. Todorieva-Todorova, Katya S. Kovacheva, Nikolay T. Tzvetkov, Stefan V. Trifonov, Galya Ts. Stavreva, Tihomir R. Rashev, Alexander A. Todorov, Petar D. Ivanov. JBCR, Vol.12 Number 1, 2019, p 19-26.

GRANULOCYTIC EXPRESSION OF CD11B/CD18 AND THROMBOTIC RISK IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS. Doroteya K. Todorieva-Todorova, Katya S. Kovacheva, Nikolay T. Tzvetkov, Svetla O. Blazheva, Tzvetan H. Lukanov. JBCR Vol. 14, No. 1, 2021, p. 47-53.

ROLE OF NEUTROPHIL CD11B/CD18 EXPRESSION IN THROMBOGENESIS OF PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS, D. Todorieva-Todorova, L. Gercheva, K. Kovacheva, E. Mineva-Dimitrova, p. 47-53 “HEMATOLOGY” issue 1-2/2023, Vol. LX, ISSN 2367-7864

Dineva, Dobrinka & Paskaleva, Ivanka & Todorieva-Todorova, D. (2019). Evaluation of platelet reactivity on dual antiplatelet therapy in patient with essential thrombocythemia and acute coronary syndrome. Clinica Chimica Acta. 493. S405. 10.1016/j.cca.2019.03.863.

Velkova A., Tzvetkov N., Kovacheva K., Todorieva D., Ivanov P., Todorov A., Penkova R., Golemanov G., Panayotova S., Popova V. A STUDY ON THE ROLE OF GENETIC THROMBOPHILIC DISORDERS AS A RISK FACTOR FOR THROMBOTIC COMPLICATIONS IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS. Oral presentation at XII Anniversary International Medical Scientific Conference for Students and Young Doctors, Pleven, Bulgaria. 8-11 Oct, 2013

Тодориева Д., Ковачева К., Цветков Н., Тодоров А., Иванов П., Пенкова Р. , Големанов Г., Панайотова Ст., Попова В., Велкова А. ГЕНЕТИЧНИ ДЕФЕКТИ ЗА ТРОМБОФИЛИЯ КАТО РИСКОВИ ФАКТОРИ ЗА ТРОМБОТИЧНИ УСЛОЖНЕНИЯ ПРИ ПАЦИЕНТИ С МИЕЛОПРОЛИФЕРАТИВНИ ЗАБОЛЯВАНИЯ. Орална презентация, Втори национален симпозиум „Млад хематолог“, Сливен, България. 9-10 Ноември, 2013

Цветков Н.*, Ковачева К.**, Тодориева Д.*, Тодоров А.***, Панайотова Ст.*, Попова В.*, Иванов П. ****, Комса-Пенкова Р. ****, Велкова А.*****. ПРОУЧВАНЕ ВЪРХУ РОЛЯТА НА ГЕНЕТИЧНИТЕ ДЕФЕКТИ ЗА ТРОМБОФИЛИЯ КАТО РИСКОВИ ФАКТОРИ ЗА ТРОМБОТИЧНИ УСЛОЖНЕНИЯ ПРИ ПАЦИЕНТИ С МИЕЛОПРОЛИФЕРАТИВНИ ЗАБОЛЯВАНИЯ. Постер, X Национален конгрес по хематология, Правец, България, 22-25.10.2015 г.

ANNEXES

Annex 1. An information list and an informed consent form for patients

Информационен лист за пациента

Заглавие на проекта:

ПРОУЧВАНЕ НА АДИТИВНИЯ ЕФЕКТ ОТ НЯКОИ ГЕНЕТИЧНИ, ИМУНОЛОГИЧНИ И КОМОРБИДНИ ФАКТОРИ ВЪРХУ ТРОМБОГЕНЕЗАТА И ТРОМБОГЕННИЯ РИСК ПРИ ПАЦИЕНТИ С МИЕЛОПРОЛИФЕРАТИВНИ ЗАБОЛЯВАНИЯ

Обяснения и информация за пациента относно същността на проекта:

Уважаеми Господине/Госпожо/Госпожице,

Бихме искали да Ви помолим да участвате в изследователски проект с горепосоченото заглавие, тъй като при вас е диагностицирано заболяване от групата на Миелопролиферативните неоплазии. Участието е изцяло доброволно и ако не желаете, не трябва да се включвате в този проект.

Важно е да прочетете внимателно тази информация, преди да решите дали ще се включите в проекта. След като се запознаете с информацията имате право да зададете въпроси и ако получите удовлетворяващи Ви отговори, моля, попълнете формуляра, като с това ще потвърдите доброволното си желание за участие в проекта.

Ако решите да откажете своето участие или да се оттеглите от проучването, което имате право да направите по всяко време, без да давате обяснения, лечението Ви няма да бъде повлияно от това Ваше решение. То няма да се отрази и на отношението на медицинския персонал към Вас, както и на цялостните грижи за Вашето здраве. Ако се откажете от участие в проекта, Ви молим да уведомите изследователския екип.

Проектът ще се осъществява от лекари от Клиника по хематология. По всички интересуващи Ви въпроси можете да се отнасяте към водещия изследовател проф. Катя Ковачева и д-р Доротея Тодориева.

Бихме искали да Ви помолим да участвате в проекта, за да получим отговор на въпроса каква роля има генетичното предразположение за развитие на тромбози, които са тежките усложнения на Миелопролиферативните заболявания. С изпълнението на този проект ще получим нови знания за генезата на тромбообразуването при тези състояния. Това би определило по-точно от сега съществуващите методи за диагностика и индивидуализиране на терапевтичния подход за всеки пациент.

Проектът ще продължи 1 година.

При започването му, лекар-член на изследователския екип ще Ви зададе въпроси относно заболяванията, от които страдате сега или сте страдали в миналото, както и някои лични данни – рождена дата, адрес и телефони на Вас , за да може да се осъществява контакт с Вас и след като бъдете изписан(а) от

болницата. Данните ще се нанасят в Карта на пациента – документ, до който ще имат достъп само членовете на изследователския екип.

Освен обичайните изследвания, задължително изисквани при болни с Миелопролиферативни заболявания, ще Ви бъдат взети допълнително 2 епруветки 5-7 мл. венозна кръв, за изследване на 3 ДНК маркера – изменения в гените на фактор V (FVL), на протромбина и един полиморфизъм -PLA2 алелът на GPIIa, MTHFR, както и за имунологично изследване за CD11b/CD18. Вземането на венозна кръв за това изследване не надвишава обичайните рискове, свързани с тази манипулация. Този риск ще бъде минимизиран поради това, че кръвната проба ще се взема от обучен персонал и при задължително спазване на утвърдените изисквания за стерилност при вземане на кръвни проби.

Ще бъдете помолен да посетите болницата УМБАЛ „Д-р Георги Странски" по-късно, за да получите резултатите си.

Не са предвидени средства в проекта за обезпечаване на Вашите транспортни разходи, свързани с осъществяване на контролните прегледи.

Ако решите да участвате, цялата информация за Вас ще остане поверителна. Определени упълномощени лица (д-р Доротея Тодориева) ще имат достъп до Вашата медицинска карта, но при строга поверителност. Не са предвидени компенсации в случай на претърпени вреди от участие в изследването и допълнителните грижи, тъй като от това изследване не произтичат допълнителни рискове или те са минимални, при спазване на условията за безопасност и при извършването им от обучен персонал, както е предвидено.

Формуляр за информирано съгласие

Заглавие на проекта:

ПРОУЧВАНЕ НА АДИТИВНИЯ ЕФЕКТ ОТ НЯКОИ ГЕНЕТИЧНИ, ИМУНОЛОГИЧНИ И КОМОРБИДНИ ФАКТОРИ ВЪРХУ ТРОМБОГЕНЕЗАТА И ТРОМБОГЕННИЯ РИСК ПРИ ПАЦИЕНТИ С МИЕЛОПРОЛИФЕРАТИВНИ ЗАБОЛЯВАНИЯ

Моля, подчертайте **Да** или **Не** за всички посочени по-долу твърдения (**подчертава се вярното твърдение**).

Бях помолен да се съглася сам	Да	Не
Прочетох Информационния лист на пациента	Да	Не
Дадена ми бе възможност да задам всички важни за мен въпроси и да обсъдя този проект	Да	Не
Получих удовлетворяващи ме отговори на всички мои въпроси	Да	Не
Получих достатъчна информация относно проекта	Да	Не

Проектът ми беше обяснен, зададох въпросите си и получих отговори на тях от Д-р Доротея Тодориева.

(име на изследователя)

……

(Подпис на изследователя)

Разбирам, че съм свободен да се откажа от участие в проекта по всяко време, без да давам обяснения за отказа си и без това да повлияе на полагащите ми се в бъдеще медицински грижи.

Дата:……

Име, презиме, фамилия на пациента:

……

Подпис на пациента:……

Annex 2. An information list and an informed consent form for healthy volunteers

Информационен лист за доброволеца

Заглавие на проекта:

ПРОУЧВАНЕ НА АДИТИВНИЯ ЕФЕКТ ОТ НЯКОИ ГЕНЕТИЧНИ, ИМУНОЛОГИЧНИ И КОМОРБИДНИ ФАКТОРИ ВЪРХУ ТРОМБОГЕНЕЗАТА И ТРОМБОГЕННИЯ РИСК ПРИ ПАЦИЕНТИ С МИЕЛОПРОЛИФЕРАТИВНИ ЗАБОЛЯВАНИЯ

Обяснения и информация за доброволци относно същността на проекта:

Уважаеми Господине/Госпожо/Госпожице,

Бихме искали да Ви помолим да участвате като доброволци в изследователски проект с горепосоченото заглавие. Участието е изцяло доброволно и ако не желаете, не трябва да се включвате в този проект.

Проектът се осъществява от лекари от Хематологична клиника. По всички интересуващи Ви въпроси можете да се отнасяте към водещия изследовател проф. д-р Катя Ковачева и към д-р Доротея Тодориева.

Участието Ви в проекта, ще даде възможност на изследователите да получат отговор на въпроса, каква роля има генетичното предразположение и някои имунологични фактори за развитие на тромбози, които са тежките усложнения на заболявания от групата на Миелопролиферативните болести. Това би имало значение за избора на най-подходящ лечебен подход за всеки пациент, съобразен с неговите наследствени особености.

Участието Ви в проекта се състои във вземането от Вас на 5-7 мл. венозна кръв, за изследване на имунологични маркери върху гранулоцитната мембрана – CD11b/CD18. Вземането на венозна кръв за това изследване не надвишава обичайните рискове, свързани с тази манипулация. Този риск ще бъде минимизиран поради това, че кръвната проба ще се взема от обучен персонал и при задължително спазване на утвърдените изисквания за стерилност при вземане на кръвни проби.

КОМИСИЯ ПО ЕКИПА НА НАУЧНО-ИЗСЛЕДОВАТЕЛСКАТА ДЕЙНОСТ ПРИ МЕДИЦИНСКИ УНИВЕРСИТЕТ – ПЛЕВЕН ОДОБРЯВА ГОРНОТО ИЗЛОЖЕНИЕ ЗА ИНФОРМАЦИОНЕН ЛИСТ НА ДОБРОВОЛЕЦА:

Подпис:.....
(Председател на Комисия по етика на научно-изследователската дейност)

Дата:.....

Формуляр за информирано съгласие

Заглавие на проекта:

ПРОУЧВАНЕ НА АДИТИВНИЯ ЕФЕКТ ОТ НЯКОИ ГЕНЕТИЧНИ, ИМУНОЛОГИЧНИ И КОМОРБИДНИ ФАКТОРИ ВЪРХУ ТРОМБОГЕНЕЗАТА И ТРОМБОГЕННИЯ РИСК ПРИ ПАЦИЕНТИ С МИЕЛОПРОЛИФЕРАТИВНИ ЗАБОЛЯВАНИЯ

Моля, подчертайте **Да** или **Не** за всички посочени по-долу твърдения (**подчертава се вярното твърдение**).

Бях помолен да се съглася сам	Да	Не
Прочетох Информационния лист на доброволеца	Да	Не
Дадена ми бе възможност да задам всички важни за мен въпроси и да обсъдя този проект	Да	Не
Получих удовлетворяващи ме отговори на всички мои въпроси	Да	Не
Получих достатъчна информация относно проекта	Да	Не

Проектът ми беше обяснен, зададох въпросите си и получих отговори на тях от……..

(име на изследователя)

……

(Подпис на изследователя)

Разбирам, че съм свободен да се откажа от участие в проекта по всяко време, без да давам обяснения за отказа си.

Дата:……

Име, презиме, фамилия на доброволеца:

……

Подпис на доброволеца:…….

Annex 3. Questionnaire

АНКЕТНА КАРТА №

за изследване на генетични дефекти за тромбофилия при пациенти с Миелопролиферативни заболявания

1. Име..... възраст .....пол.....
2. Адрес, телефон.....
3. Клинична диагноза .....

- дата на поставяне на диагнозата.....
- клинични данни/ анамнеза .....
- първоначални патологични промени
  - лабораторни.....

4. Минали и придружаващи заболявания и състояния:
   - инфаркт на миокарда, ИБС, хипертония, сърдечна недостатъчност, или др.ССЗ .....
     □ □ □ □ □
   - диабет, затлъстяване, хиперлипидемия, неоплазия, чернодробно страдание
     □ □ □ □ □
   - предишни прояви на тромботични инциденти преди хематологичното заболяване
     - венозна тромбоза (ВТ) и възраст ..... □ да □ не
     - венозен тромбоемболизъм (ВТЕ) и възраст.....□ да □ не
   - *информация касаеща само жени, относно изходи на бременности:*
     - спонтанни аборти брой (.....); срок на бременността (.....)
     - усложнения на бременността
       - прееклампсия □ да □ не
       - abruption placentae □ да □ не
       - интраутеринно изоставане в развитието □ да □ не
       - мъртвораждане брой (.....); срок на бременноста (.....)
       - перорални контрацептиви □ да □ не
5. Провокиращи фактори за тромботични събития □ да □ не
   - ако " да " – кой от следните :
     - оперативна интервенция □ да □ не
     - травма □ да □ не
     - продължителна имобилизация □ да □ не
     - злокачествени заболявания □ да □ не
     - хормонозаместителна терапия □ да □ не
6. Допънителни провокиращи фактори – тютюнопушене □ да □ не
7. Усложнение на основното заболяване/ в хода на заболяването прояви на тромбози или хеморагии - локализация:
   - тромбози (инсулт, инфаркт, НАП, ДВТ, БТЕ, портална/далачна тромбоза) □ да □ не
     - вени на долен крайник – дълбоки / повърхностни
     - други локализации:.....
   - хеморагии □ да □ не локализация.....
8. Лечение:
   - провеждано в момента лечение на хематологичното заболяване.....
   - прием на Хидрея □ да □ не
   - провеждано в момента лечение на придружаващите заболявания.....
   - данни за оперативно лечение.....
9. Фамилна история за тромбози □ да □ не
   Ако " да " – какви родственици .....
10. Доказано носителство на тромбофилична мутация в родственик от I степен.... □ да □ не
11. Клиника.....
12. Лекуващ лекар.....
13. Дата на вземане на материала .....

Материал за изследване – 5-9 мл венозна кръв се взема в 2 специални пластмасови епруветки, съд. EDTA маркирана с лилава капачка и ***се съхранява в хладилник на 4 °C*** *не повече от* ***24 часа*** *(****да не се замразява****)*
Телефони за контакт: 064/ 886 – 392
Лице за контакт д-р Д. Тодориева 0892 21 25 20

Annex 4. Permissions by the ethics committee of scientific and research activity in Medical university - Pleven

МЕДИЦИНСКИ УНИВЕРСИТЕТ
гр. ПЛЕВЕН
КЕНИД
Изх. №349-КЕНИД/24.06.2015

КОМИСИЯ ПО ЕТИКА НА НАУЧНО-ИЗСЛЕДОВАТЕЛСКАТА ДЕЙНОСТ
МЕДИЦИНСКИ УНИВЕРСИТЕТ - ПЛЕВЕН
ул. "Климент Охридски" № 1
Телефон: 884 196 / 884 197

РЕШЕНИЕ НА КОМИСИЯТА ПО ЕТИКА НА НАУЧНО-ИЗСЛЕДОВАТЕЛСКАТА ДЕЙНОСТ ПРИ МЕДИЦИНСКИ УНИВЕРСИТЕТ – ПЛЕВЕН

Спонсор	Медицински Университет-Плевен, ул. „Климент Охридски"1
Главен изследовател	Доц. Д-р Николай Цветков, дм
Изследователски център(ове)	✓ Сектор „Генетика", катедра „Микробиология, вирусология и медицинска генетика," МУ – Плевен ✓ Клиника по Хематология, УМБАЛ „Д-р Г. Странски" Плевен ✓ Клиника по Имунология, УМБАЛ „Д-р Г. Странски" Плевен
Заглавие на проекта	***„Проучване върху генетични и имунологични фактори, определящи тромбогенезата при пациенти с миелопролиферативни заболявания"***
Протокол № 37	
Получени, разгледани и одобрени от КЕНИД документи	✓ Форма-заявление за разглеждане и даване на решение за извършване на научни изследвания върху човешки същества ✓ План-проект на научното изследване ✓ Информационен лист и формуляр за информирано съгласие на пациента ✓ Информационен лист и формуляр за информирано съгласие на доброволеца ✓ Анкетна карта за изследване на генетични дефекти за тромбофилия при пациенти с Миелопролифераитвни заболявания ✓ Творчески автобиографии на изследователя

Комисията по етика на научно-изследователската дейност при Медицински Университет - Плевен **реши да разреши провеждането на научно изследване на тема**: *„Проучване върху генетични и имунологични фактори, определящи тромбогенезата при пациенти с миелопролиферативни заболявания"* с главен изследовател доц. д-р Николай Цветков, д.м. и определя срок за **периодичен надзор** съгласно изискванията на КЕНИД (*приложение 2*) на 12-тия месец от началото на проучването.

24.06.2015г.
гр. Плевен

Председател на КЕНИД:
(доц. д-р С. Александрова-Янкуловска, д.м.н)

КОМИСИЯ ПО ЕТИКА НА НАУЧНО-ИЗСЛЕДОВАТЕЛСКАТА ДЕЙНОСТ
МЕДИЦИНСКИ УНИВЕРСИТЕТ - ПЛЕВЕН
Ул. "Климент Охридски" № 1
Телефон: 884 196 / 884 197

МЕДИЦИНСКИ УНИВЕРСИТЕТ
ГР. ПЛЕВЕН
КЕНИД
Изх. № 464-КЕИД/21.06.2017

РЕШЕНИЕ НА КОМИСИЯТА ПО ЕТИКА НА НАУЧНО-ИЗСЛЕДОВАТЕЛСКАТА ДЕЙНОСТ ПРИ МЕДИЦИНСКИ УНИВЕРСИТЕТ – ПЛЕВЕН

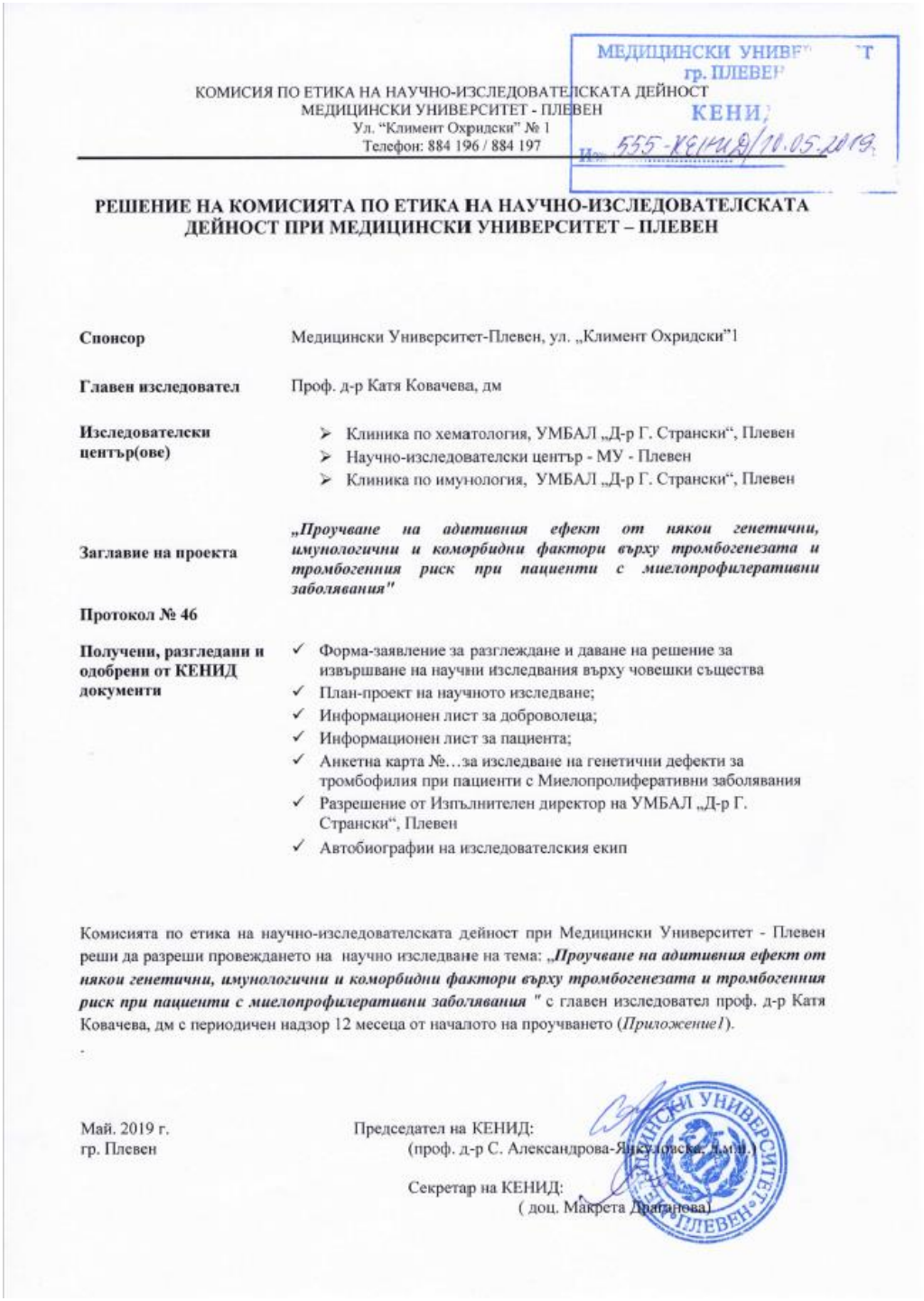
Спонсор	Медицински Университет-Плевен, ул. „Климент Охридски"1
Главен изследовател	Доц. Д-р Николай Цветков, дм
Изследователски център(ове)	➢ Клиника по хематология, УМБАЛ „Д-р Г. Странски" ЕАД; ➢ Клиника по имунология, УМБАЛ „Д-р Г. Странски" ЕАД ➢ Научно-изследователски център – МУ - Плевен
Заглавие на проекта	***„ Проучване върху генетични и имунологични тромбофилични фактори, определящи тромбогенезата при пациенти с миелопролиферативни заболявания"***
Протокол № 43	
Получени, разгледани и одобрени от КЕНИД документи	✓ Форма-заявление за разглеждане и даване на решение за извършване на научни изследвания върху човешки същества ✓ План-проект на научното изследване ✓ Информационен лист за пациента ✓ Анкетна карта за изследване на генетични дефекти за тромбофилия при пациенти с Миелопролиферативни заболявания ✓ Заявление за разрешение за провеждане на проучване от Изпълнителен директор на УМБАЛ – Плевен ✓ Автобиографии на изследователския екип

Комисията по етика на научно-изследователската дейност при Медицински Университет - Плевен реши да разреши провеждането на научно изследване на тема: ***„„Проучване върху генетични и имунологични тромбофилични фактори, определящи тромбогенезата при пациенти с миелопролиферативни заболявания"*** с главен изследовател доц. д-р Николай Цветков, дм с периодичен надзор 12 месеца от началото на проучването *(Приложение 1)*.

21. 06. 2017 г..
гр. Плевен

Председател на КЕНИД:
(проф. д-р С. Александрова-Янкуловска, д.м.н.)

Секретар на КЕНИД:
(доц. Маргрета Драганова)



МЕДИЦИНСКИ УНИВЕР... гр. ПЛЕВЕН КЕНИД

КОМИСИЯ ПО ЕТИКА НА НАУЧНО-ИЗСЛЕДОВАТЕЛСКАТА ДЕЙНОСТ
МЕДИЦИНСКИ УНИВЕРСИТЕТ - ПЛЕВЕН
Ул. "Климент Охридски" № 1
Телефон: 884 196 / 884 197

№ 555-КЕИД/10.05.2019

РЕШЕНИЕ НА КОМИСИЯТА ПО ЕТИКА НА НАУЧНО-ИЗСЛЕДОВАТЕЛСКАТА ДЕЙНОСТ ПРИ МЕДИЦИНСКИ УНИВЕРСИТЕТ – ПЛЕВЕН

Спонсор	Медицински Университет-Плевен, ул. „Климент Охридски"1
Главен изследовател	Проф. д-р Катя Ковачева, дм
Изследователски център(ове)	➢ Клиника по хематология, УМБАЛ „Д-р Г. Странски", Плевен ➢ Научно-изследователски център - МУ - Плевен ➢ Клиника по имунология, УМБАЛ „Д-р Г. Странски", Плевен
Заглавие на проекта	***„Проучване на адитивния ефект от някои генетични, имунологични и коморбидни фактори върху тромбогенезата и тромбогенния риск при пациенти с миелопрофилеративни заболявания"***
Протокол № 46	
Получени, разгледани и одобрени от КЕНИД документи	✓ Форма-заявление за разглеждане и даване на решение за извършване на научни изследвания върху човешки същества ✓ План-проект на научното изследване; ✓ Информационен лист за доброволеца; ✓ Информационен лист за пациента; ✓ Анкетна карта №…за изследване на генетични дефекти за тромбофилия при пациенти с Миелопролиферативни заболявания ✓ Разрешение от Изпълнителен директор на УМБАЛ „Д-р Г. Странски", Плевен ✓ Автобиографии на изследователския екип

Комисията по етика на научно-изследователската дейност при Медицински Университет - Плевен реши да разреши провеждането на научно изследване на тема: ***„Проучване на адитивния ефект от някои генетични, имунологични и коморбидни фактори върху тромбогенезата и тромбогенния риск при пациенти с миелопрофилеративни заболявания*** " с главен изследовател проф. д-р Катя Ковачева, дм с периодичен надзор 12 месеца от началото на проучването (*Приложение1*).

Май. 2019 г.
гр. Плевен

Председател на КЕНИД:
(проф. д-р С. Александрова-Янкулоска, д.м.н.)

Секретар на КЕНИД:
(доц. Макрета Драганова)

МЕДИЦИНСКИ УНИВЕРСИТЕТ ПЛЕВЕН

Annex 5. References of FBC parameters

	Leukocytes	**Hemoglobin**	**Platelets**
Reference	3.5-10.5x10^9 /l	120-150 g/l for women, 130-170 g/l for men	130-420x10^9 /l
Low	Leukopenia	Low	Thrombocytopenia
High	Leukocytosis	High	Thrombocytosis