The Egyptian Journal of Hospital Medicine (April 2022) Vol. 87, Page 2253-2258

Evaluating The Risk of Thrombosis and Its Relation to JAKII Mutation in Egyptian Myeloproliferative Neoplasm Patients

Tarek E. Abouzeid*[1], Shaimaa El-Ashwah[1], Salah Aref[2], Nora A. Farag [2], Rania K. Farag[3]
[1]Clinical Hematology Unit, Mansoura University Oncology Center, [2]Hematology Unit, Clinical Pathology Department, [3] Ophthalmology Center, Mansoura Faculty of Medicine, Mansoura, Egypt
***Corresponding author:** Tarek E. Abouzeid, **Mobile:** 00201223663242, **E-Mail:** drtarekabouzid@yahoo.com

ABSTRACT

Background: Vascular thrombosis represents a common manifestation, and complication in patient with myeloproliferative neoplasm (MPN). It is also associated with higher risk of morbidity and mortality. The acquired point mutation in Janus Associated Kinase II (JAKIIV617F) in these disorders is associated with higher incidence of thrombosis compared to patient devoid of this mutation.

Objective: This study aimed to evaluate the incidence of thrombosis, and its relation to JAK II (V617F) mutation among Egyptian MPN patients

Patients and Method: In this, study 102 patients (49 males and 53 females) at the Outpatient Clinic of Mansoura Oncology Center has been evaluated for the presence and type of thrombosis. In addition, its relation to JAKII mutation.

Results: From the 102-studied patients, 47 (46.15%) had Polycythemia Vera (PV), 27(26.5%) had essential thrombocytosis (ET), 22 (21.6%) had myelofibrosis (MF), and 6 (5.9%) patients had unclassified MPN. Vascular thrombosis has been observed in 34 (33.3%) patients, 28 (27.5%) of them at the time of diagnosis, 6 (5.9%) patients at follow up and 4 (3.9%) were having recurrent thrombosis. According to the pattern of thrombosis, 16 patients had arterial thrombosis, 14 had venous, and 4 patients had combined arterio-venous thrombosis. The association of of JAK II V617F mutation and thrombosis were statistically significant (P = 0.0003).

Conclusion: The presence of JAKII V617F mutation is associated with higher incidence of vascular thrombosis in MPN patients compared to those without this mutation.

Keywords: MPN, JAKII, Thrombosis.

INTRODUCTION

Myeloproliferative neoplasms (MPNs) are a group of disorders, that share the common feature of excessive proliferation of the myeloid stem cells in the bone marrow, and as a result of that, there will be increased number of red blood cells, platelets, and leukocytes in the peripheral blood. Since Dameshek in 1951 first described these disorders, several attempts have been done towards better understanding of their nature, etiology, and differences. With the advances in the field of molecular biology and cytogenetics, and the discovery of several molecular aberration like JAK II (V617F), Philadelphia chromosome, and myeloproliferative leukemia protein mutation (MPL W515L/K), the World Health Organization (WHO) reached the well-established classification of these disorders [(1)]. As they share same pathophysiology, and manifestation, by high tendency towards thrombosis and leukemic transformation, so that the survival is affected, however, they differ in the survival, being better in essential thrombocytosis (ET), while patients with polycythemia vera (PV), or myelofibrosis (MF) their survival is being markedly affected [(2)].

Thrombosis has a wide range of clinical symptoms, from minor microcirculatory abnormalities to more significant problems including arterial and venous thrombosis. Ischemic stroke, acute myocardial infarction, and peripheral artery occlusion are all examples of arterial thrombosis, which contributes for 60 to 70% of MPN-related events. Deep venous thrombosis of the lower legs, pulmonary embolism, intra-abdominal, and cerebral vein thrombosis are all examples of venous system events. Venous thrombosis is quite prevalent in PV, accounting for almost one-third of all occurrences [(2)]. MPNs constitute the most common cause of splanchnic venous thrombosis, accounting for approximately 50% of Budd Chiari syndrome (BCS) cases and 25% of portal vein thrombosis (PVT) [(3)].

Neurological manifestations are also common including transient ischemic attacks, peripheral paresthesia, but hemiparesis is less common. Visual dysfunction may also manifest as transient diplopia and sudden reversible attacks of blurred vision [(4)]. With aggressive cytoreductive– myelosuppressive therapy, such as hydroxyurea, the rate of thrombosis in this patient is markedly reduced, but it carries the risk of therapy-related adverse effect, such as pneumonitis, oral mucositis, and skin hyperpigmentation. Even with blood count control, this condition remains as hypercoagulable states that necessitate continuous medication and close follow up [(5)].

As an acquired thrombophilic state, these patients are prone to vascular complications, the mechanisms ultimately responsible for that remains unclear into a large extent, only several theories have tried to explain it [(6)]. The pathogenesis of the acquired thrombophilic may be multifactorial. However, two main mechanisms are the cause of hypercoagulation in these disorders. The first is the abnormalities of blood cells arising from the clonal proliferation of hematopoietic progenitor cells, which acquire a prothrombotic phenotype. The second arise from the

Received: 02/01/2021
Accepted: 19/2/2021

host inflammatory response to the action of cytokines and other mediators released by the malignant cells [7].

The discovery of the acquired V617F mutation in the tyrosine kinase JAK-2 gene, which represents a gain function mutation, has greatly influenced the diagnostic and therapeutic approach in MPN patients. Several studies have proved the role of the JAK2V617F mutation in the increased thrombotic tendency observed in ET and PV patients. In the analysis by **Ziakas** [8] involving 2905 ET patients, the JAK2V617F mutation was associated with an increased risk of both venous and arterial thrombosis**.**

PATIENTS AND METHODS

This study was carried out on 102 adult patients, attending the Hematology Clinic, at Mansoura Oncology Center. 53 females and 49 males were subjected to thorough history taking, examinations, and full laboratory investigations including the hematological parameters and bone marrow examinations. Real Time polymerase Chain reaction (RT-PCR, Roche diagnostic using JAK II Mutaquant Kit 24, VA Ipsogen, Qiagen (Ref. 673523) was used to examine peripheral blood samples from these patients to detect the presence of JAK II (V617F) mutation.

Accordingly, they were divided into the four different types of MPN based on the World Health Organization (WHO) 2016 classification and diagnostic criteria according to **Arber *et al.*** [9]**,** 47 had polycythemia vera (PV), 27 had essential thrombocytosis (ET), 22 had myelofibrosis (MF), and 6 had unclassified MPN.

Different radiological studies have been done whenever thrombosis is suspected in these patients including angiography for different vessels, fluorescein angiography for retinal vessels

Ethical Approval and consent to participate:

This study was approved by the Institutional Review Board for Clinical Research Committee, Mansoura University (approval number of R.17.11.82). All procedures performed in studies were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki. Written informed consent was obtained from every participant.

Statistical analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test (χ^2) was used to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean ± SD. Independent samples t-test was used to compare between two independent groups of normally distributed variables (parametric data). P value ≤ 0.05 was considered significant.

RESULTS

Table (1) showed that JAKII V617F mutation was detected in 70 (68%) out of 102 MPN patients. The mutation was detected in the different MPN types as follow: in PV 38 (80%), in ET 19 (70%), in MF 13 (59%), while 3 (100%) in unclassified MPN. Incidence of thrombosis was 34 (33.3%) in all MPN patients, and this incidence varies according to the different types as follow: 15 (31.9%) in PV, 9 (33.3%) in ET, in MF 7 (31.8%), while 3 (100%) in unclassified MPN.

Table (1): Patient Demographic data

	Total	**PV**	**ET**	**MF**	**Unclassified**
Age (Mean ± SD)	50.13 ±12.1	52.8 ±10.6	45.7 ±12.8	51.4 ±12.3	44.9±14.3
Sex (M/F)	49 / 53	25 / 22	13 / 14	10 / 12	1 / 5
HTN (present/ Absence)	35 / 67	22 / 25	6 / 21	4 / 18	3 / 3
Bleeding (present/Absence)	15 / 87	3 / 43	3 / 24	5 / 17	3 /3
Thrombosis (present/Absence)	34 / 68	15 /32	9 / 18	7/15	3/3
JAK II V617F mutation (+/-)	70/32	38 / 9	19 / 8	13 / 9	3 / 3
WBC ($X10^3$)	50.13 ± 12.1	15.47 ± 1.38	16.9 ±2.02	14.64 ± 3.22	16.32 ± 2.24
Hb (gm/dl)	14.4 ± 3.2	17.89 ± 2.35	11.9 ± 2.22	9.79 ± 2.51	13.72 ± 2.86
Hematocrit (%)	45.7 ± 3.2	55.69 ± 8.35	38.46 ± 5.91	33.94 ± 3.43	42.48 ± 9.58
Platelet ($X10^3$)	671.7 ± 34.1	539.02 ± 61.88	1200.69±85.87	344.19 ±13.42	543.20 ± 89.82

Table (2) showed that 28 (27.5%) patients had thrombosis at diagnosis, 6 (5.9%) at follow up, and 4 (3.9%) had recurrent thrombosis (at diagnosis (D), and at follow up (FU)).

Table (2): Incidence of thrombosis at Diagnosis and follow up among all patients

Diagnosis	Thrombosis	Thrombosis at D	Thrombosis at FU	Rec. Thrombosis
No	34	28	6	4
%	33.3	27.5	5.9	3.9

Table (3) showed the type of thrombosis in these MPN patients, which ranged from arterial thrombosis in 16 (47.1%), venous thrombosis in 14(41.1%) patients, and arteriovenous thrombosis in 4 (11.76%) patients.

Table (3): Different types of Thrombosis

Vascular System	NO	%
Arterial	16	47.1%
Venous	14	41.1%
Arterio-Venous	4	11.76%

Table (4) showed the relation between the thrombosis and hematologic parameters where were not statistically significant.

Table (4): Correlation between thrombosis and CBC

	Thrombosis (n=34)	No thrombosis (n=68)	Test of significance	P = value
WBC Median (IQR)	12.3 (7.5-18.4)	13 (7-20)	Z=0.226	0.821
Platelet Median (IQR)	545 (241-952)	500 (221-810)	Z=0.430	0.667
Hemoglobin Mean (SD)	14.81 (4.4)	14.17 (4.1)	t =0.707	0.481

Table (5) showed that there was significant relation between the presence of JAKII V617F mutation and thrombosis (P = 0.0003).

Table (5): Correlation between thrombosis and JAKII mutation

JAK II V617F	Thrombosis (n=34)	No thrombosis (n=68)	Test of significance	P = value
(+) ve	30 (88.2%)	40 (58.8%)	$X^2 = 9.1$	0.0003
(-)ve	4 (11.8%)	28 (41.2%)		

IQR: interquartile range, χ^2: Chi square Test, Z: Mann-Whitney test

Table (6) showed that there was significant relation between the presence of JAKII V617F mutation and thrombosis at diagnosis (P = 0.001).

Table (6): The incidence of Thrombosis and JAKII V617F mutation at diagnosis

JAK II V617F	Thrombosis (n=28)	No thrombosis (n=74)	Test of significance	P = value
(+) ve	26 (92.9%)	44 (59.5%)	X 2 =10.5	0.001
(-) ve	2 (7.1%)	30 (40.5%)		

The overall survival was found to be of little significant difference (P= 0.097) between MPN patient with thrombosis and those without thrombosis. While the JAKII V617F mutation positivity did not affect the survival (Figures 1 & 2).

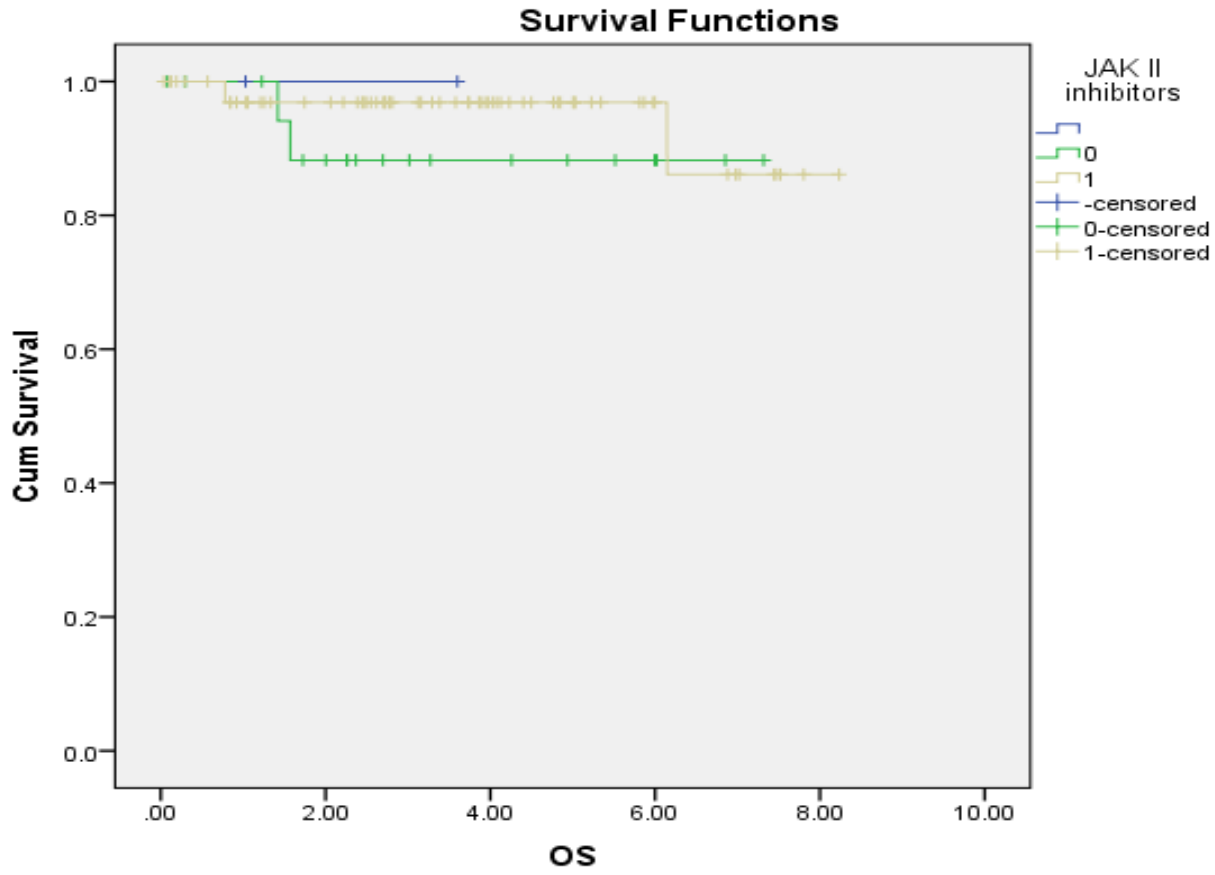

Fig. (1): Test of equality of survival distributions for the different levels of Thrombosis (P = 0.0097).

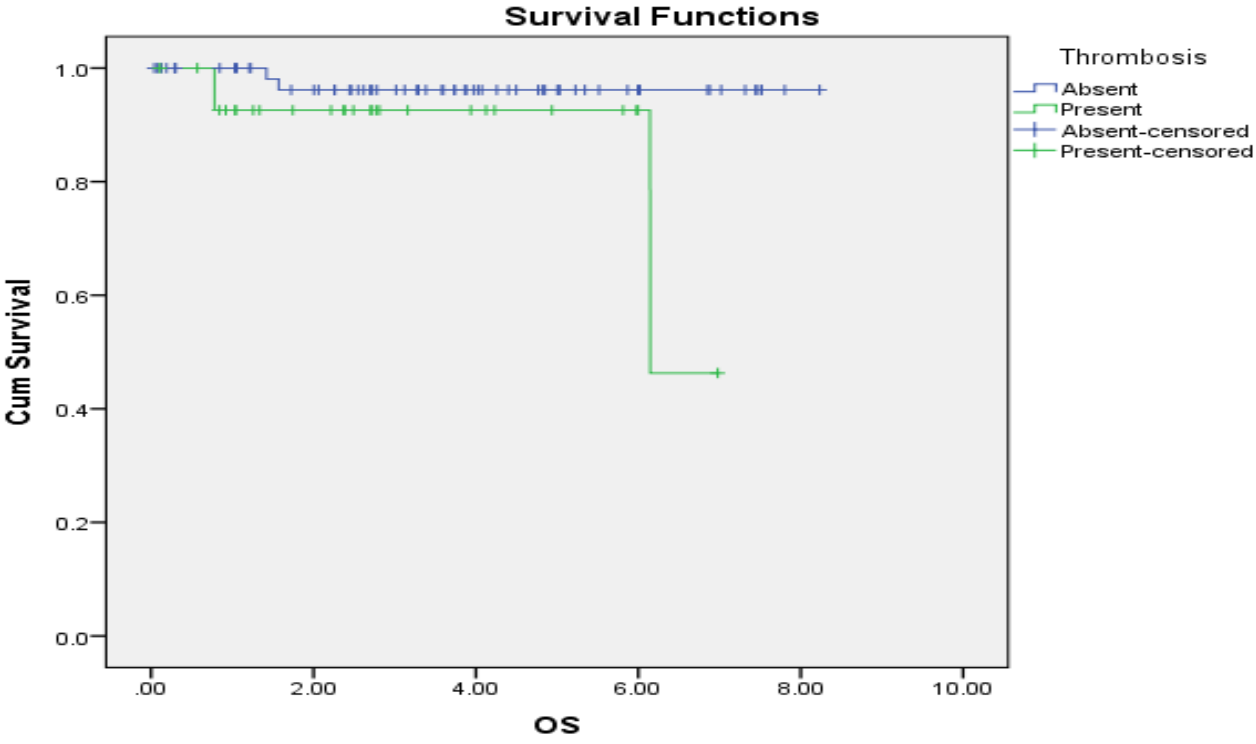

Fig. (2): Test of equality of survival distribution for the different levels of JAK II inhibitors (P = 0.5).

DISCUSSION

Myeloproliferative neoplasms (MPNs) are a group of disorders that share the common feature of excessive proliferation of the myeloid stem cells in the bone marrow, with higher tendency towards arterial and venous thrombosis. **Carobbio *et al.* [10]** reported that in these disorders, due to excessive proliferation, apoptosis inhibition in the bone marrow stem cells, and increase in the hematocrit value, blood viscosity, and blood cell count, will leads to serious complications such as thrombosis. **Park *et al.* [11]** also found that the mortality caused by thrombosis accounted for 35-70% of the total mortality in MPN patients. Therefore, it is a condition generates significant complications for MPN patients.

In this study, thrombosis was found in 34/102 (33.3%) in all MPN patients. The thrombotic incidence in the different types of MPN patient was as follow: 15 (31.9%) in PV, 9 (33.3%) in ET, 7 (31.8%) in MF and 3 (100%) in unclassified MPN. Out of these 34 patients with thrombotic events, 28 (27.5%) had thrombosis at the time of diagnosis, 6 (5.9%) during follow up, and 4 (3.9%) had recurrent thrombosis. These findings agree with that found by **Kreher *et al.* [12].**

The type of thrombosis in these MPN patients were arterial thrombosis in 16 (47.1%), venous thrombosis in 14 (41.1%) patients, and arteriovenous thrombosis were found in 4 (11.7%) patients. Our result differs from that of **Falanga *et al.* [13]**, in which vascular thrombosis, accounted for 60%-70% of events related to MPNs. This might be related to younger age group included in this study, as the risk of thrombosis is increased with advances in the age.

In this study, the most common type of arterial thrombosis was the cerebral, followed by the coronaries, while the venous thrombosis, mostly affected the splenic vein, and followed by deep venous system of the lower limb (DVT). These data are quite different from that obtained by **Kaifie *et al.* [14]** in which DVT was the most common event followed by cardiac events. But the results of this study are in agreement with data reported by **Kiladjian *et al.* [4],** which found that splanchnic venous thrombosis are most commonly caused by MPNs, which account for around half of all Budd Chiari syndrome (BCS) patients and a quarter of all portal vein thrombosis (PVT). Also, these results agree with that of **How *et al.* [15]**, which stated that MPNs are the most common risk factor found in patients with splanchnic vein thrombosis. Clinical risk factors for MPN-associated splenic vein thrombosis include younger age, female sex, and the JAK2 V617F mutation.

In this study, recurrent thrombosis has been seen in 3.9 % of the patients, which is lower than that reported by **De Stefano *et al.* [16],** which was 30%. This might be related to our younger age population, the use of aggressive therapy, such as cytoreductive hydroxyurea, anticoagulant and antiplatelet therapy as early as possible in our patients.

As regards the different incidence of thrombosis related to the different MPN subtypes, no differences have been detected, and the different subtypes were having the same thrombotic incidences, these results are in agreement with that of **Kreher *et al.* [12].**

In this study, by evaluating the relation of JAK II V617F mutation and thrombosis, in addition to other risk factors including age, gender, and hematological parameters, it was found that patients with JAK II V617F mutation, had higher incidence of thrombosis (P = 0.0003), even at diagnosis (P = 0.001), compared to patients without this mutation. These results indicate that the JAK2V617F mutation is an important risk factor for the incidence of thrombosis in MPN patients. These findings agree with that of **Takata *et al.* [17]**. In addition, these results are in agreement with that of **Finazzi *et al.* [18]** who reported that the incidence of thrombosis in patients with the JAK2V617F mutation is higher than that in patients without this mutation**?** This finding may prove the relation between the JAKII V617F mutation and thrombosis, as this mutation is found in about 99% of PV patients, and only in 50% of ET, and MF patients, but the three disorders are associated with the same incidence of thrombosis. Therefore, JAKII-positive ET, and MF patients are having higher thrombotic incidence than those without this mutation, as reported also by **Fleischman and Tyner [19].**

In our study, the overall survival was little affected with the presence of thrombosis (P = 0.097), while the JAKII V617F positivity has no survival affection. However, JAKII V617F may affect survival indirectly as it is associated with higher incidence to thrombosis.

Further studies has to be carried out to compare the thrombotic incidence in JAK II V617F-positive , and negative ET, and MF patients, to show the increased risk of thrombosis with JAKII positivity in these patients. At the end thrombotic events represents a common manifestation and complication of MPN patients, so that MPN patients has to be evaluated for the presence of thrombotic events by clinical examination and if suspected radiological examination has to be performed.

CONCLUSIONS

In conclusion, the presence of JAKII V617F mutation is associated with higher incidence of vascular thrombosis in MPN patients compared to those without this mutation. JAK II V617F mutation is of a strong predictive value for thrombosis in all types of MPN patients.

Abbreviations:

BCS (Budd chiari syndrome), **ET** (essential Thrombosis), **JAKII** (Janus associated Kinase), **MPL** (myeloproliferative leukemia protein), **MPN** (Myeloproliferative Neoplasms), **Ph** (Philadelphia),

PMF (primary myelofibrosis), **PV** (polycythemia vera), and **PVT** (portal vein thrombosis).

Financial support and sponsorship: Nil.
Conflict of interest: Nil.

REFERENCES

1. **Rumi E, Cazzola M (2017):** Diagnosis, risk stratification, and response evaluation in classical myeloproliferative neoplasms. Blood, 129 (6): 680- 692
2. **Reikvam H, Tiu R (2012):** Venous thromboembolism in patients with essential thrombocythemia and polycythemia vera. Leukemia, 26 (4): 563–571.
3. **Kelliher S, Falanga A (2021):** Thrombosis in myeloproliferative neoplasms: A clinical and pathophysiological perspective. Thrombosis Update, 21: 1-5.
4. **Kiladjian J, Cervantes F, Leebeek F *et al.* (2008):** The impact of JAK2 and MPL mutations on diagnosis and prognosis of splanchnic vein thrombosis: a report on 241 cases. Blood, 111 (10): 4922–4929.
5. **Tefferi A (2011):** Annual Clinical Updates in Hematological Malignancies: a continuing medical education series: polycythemia vera and essential thrombocythemia: 2011 update on diagnosis, risk-stratification, and management. Am J Hematol., 86 (3): 292–301.
6. **Falanga A, Marchetti M, Evangelista V *et al.* (2000):** Polymorphonuclear leukocyte activation and hemostasis in patients with essential thrombocythemia and polycythemia vera. Blood, 96 (13): 4261–4266.
7. **Arellano-Rodrigo E, Alvarez-Larrán A, Reverter J *et al.* (2009):** Platelet turnover, coagulation factors, and soluble markers of platelet and endothelial activation in essential thrombocythemia: relationship with thrombosis occurrence and JAK2 V617F allele burden. Am J Hematol., 84 (2): 102–108.
8. **Ziakas P (2008):** Effect of JAK2 V617F on thrombotic risk in patients with essential thrombocythemia: measuring the uncertain. Haematologica, 93 (9): 1412–1414.
9. **Arber d, Orazi A, Hasserjian R *et al.* (2016):** The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood, 127 (20): 2391-2405.
10. **Carobbio A, Finazzi G, Guerini V *et al.* (2007):** Leukocytosis is a risk factor for thrombosis in essential thrombocythemia: interaction with treatment, standard risk factors, and Jak2 mutation status. Blood, 109: 2310-2313.
11. **Park S, Chi H, Cho Y *et al.* (2013):** The allele burden of JAK2 V617F can aid in differential diagnosis of Philadelphia Chromosome-Negative Myeloproliferative Neoplasm. Blood Res., 48: 128-132.
12. **Kreher S, Ochsenreither S, Trappe R *et al.* (2014):** Prophylaxis and management of venous thromboembolism in patients with myeloproliferative neoplasms: consensus statement of the Haemostasis Working Party of the German Society of Hematology and Oncology (DGHO), the Austrian Society of Hematology and Oncology (OGHO) and Society of Thrombosis and Haemostasis Research (GTH e.V.). Annals of Hematology, 93 (12): 1953–63.
13. **Falanga A, Marchetti M (2012):** Thrombotic disease in the myeloproliferative neoplasms. Hematology Am Soc Hematol Educ Program, 12: 571-81.
14. **Kaifie A, Kirschner M, Wolf D *et al.* (2016):** Bleeding, thrombosis, and anticoagulation in myeloproliferative neoplasms (MPN): analysis from the German SAL-MPN-registry. Hemato-Oncol., 9: 18-23.
15. **How J, Zhou A, Stephen T (2017):** Splanchnic vein thrombosis in myeloproliferative neoplasms: pathophysiology and molecular mechanisms of disease. Ther Adv Hematol., 8 (3): 107–118.
16. **De Stefano V, Za T, Rossi E *et al.* (2008):** Recurrent thrombosis in patients with polycythemia vera and essential thrombocythemia: incidence, risk factors, and effect of treatments. Haematologica, 93 (3): 372–80.
17. **Takata Y, Seki R, Kanajii T *et al.* (2014):** Association between thromboembolic events and the JAK2 V617F mutation in myeloproliferative neoplasms. Kurume Med J., 60 (3-4): 89-97.
18. **Finazzi G, Rambaldi A, Guerini V *et al.* (2007):** Risk of thrombosis in patients with essential thrombocythemia and polycythemia vera according to JAK2 V617F mutation status. Haematologica, 92: 135-136.
19. **Fleischman A, Tyner J (2013):** Causal role for JAK2 V617F in thrombosis. Blood, 122 (23): 3705–3706.