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Original Article

Thrombotic Incidents in Patients with Myelofibrosis Suggest to be Independent of *JAK2* V617F Mutational Status

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Abstract

Introduction: Myelofibrosis (MF) belongs to a group of conditions known as Philadelphia-negative myeloproliferative neoplasms (MPN). Bleeding or various vascular complications could be the main causes of morbidity and mortality in patients with MF. MPN-related thrombosis is a multifactorial process and in the case of myelofibrosis, little is known. The risk factors for thrombotic complications in MF have been rarely assessed.

Aim: The purpose of this study was to investigate the incidence of thrombotic events in MF and the role of *JAK2* V617F mutation as a risk factor for thrombotic incidents in patients with MF.

Materials and methods: In our study of 37 patients, 35% had thrombotic events in the past. All patients were admitted to the Clinic of Hematology, St Ivan Rilski University Hospital, Sofia, Bulgaria between 2016 and 2019 and diagnosed based on the WHO criteria of 2016.

Results: The majority of patients (23, 62%) proved positive for *JAK2* (Janus kinase) V617F mutation carrying one (16, 70%) or two (7, 30%) mutated alleles. Thirteen of the patients (35%) had a thrombotic event in the past and 9 of them (69%) were carriers of *JAK2* V617F mutation. Fourteen patients of those without thrombotic history (24, 58%) were also carriers of *JAK2* V617F mutation.

Conclusions: As a whole, we did not find a statistically significant difference between *JAK2* V617F mutation and the frequency of thrombotic events. Rendering an account to the possible life-threatening complications, treatment decisions should be undertaken upon possible antithrombotic prevention in MF.

Keywords

JAK2 mutation, myelofibrosis (MF), thrombotic incidents, risk factor

INTRODUCTION

Myelofibrosis is a myeloproliferative neoplasm (MPN) characterized by proliferation of clonal hematopoietic stem cells and presence of extra fibrous tissue in the bone marrow of the patient.[1] *JAK2* (V617F) is one of the three well-known driver mutations for the development of MF, together with point mutation in MPL and ins/deletion in CALR genes. It has been proven in several studies that *JAK2* mutation prevails in patients with MF. According to

different sources, this percentage is between 50% and 60% of the patients.[2]

Generally, the prognosis of MF is poor – between five and six years – and increases if treatment with Janus kinase (JAK) inhibitors is initiated or appropriate usage of bone marrow transplantation. Thrombotic events (venous and arterial thrombosis) in MPN could additionally decrease the lifetime of patients. That is why it is important to investigate whether patients with MF are prone to develop thrombotic episodes and what the factors increasing this risk are.

Venous or arterial thromboses are the main complications of BCR-ABL negative myeloproliferative neoplasms, including polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF).[3] *JAK*2 V617F mutational status has been commented as an independent prognostic factor for fatal and nonfatal cardiovascular complications in patients with myelofibrosis (MF).[4] The link between the risk for the occurrence of thrombotic events in patients with MF and the carriership of *JAK2* V617F mutation is still under investigation.

Our hypothesis was that *JAK2* V617F mutation could be a risk factor for thrombotic events in patients with myelofibrosis. What is known at present is that the persistence of this mutation is usually low in ET (with incidence rates of 23%–57%), higher in PV (frequently over 50%, usually 65-97%), and 35-57% in idiopathic MF.[5]

AIM

The aim of this study was to investigate the incidence of thrombotic events in patients with MF and to clarify whether *JAK2* V617F mutation could be considered as a single risk factor for thrombotic incidents in patients with MF.

MATERIALS AND METHODS

Patients and ethical consent

Thirty-seven patients were diagnosed with myelofibrosis (MF) between May, 2015 and December, 2019 at the Clinic of Hematology, St Ivan Rilski University Hospital, Sofia, Bulgaria based on the criteria of WHO 2016.[6] Written informed consent was obtained from the patients for their anonymized personal information to be published in this article.

Detection of *JAK2* V617F by RFLP analysis

All patients were subjected to targeted DNA analysis for the detection of *JAK2* V617F mutation by RFLP procedure which has been previously described.[2] The presence of fragments of three different sizes (241, 189 and 30 bp) on 2% agarose gel certifies the presence of the wild type allele, while the mutant allele remains undigested.

History of thrombotic events and statistical analysis

The history of thrombotic events after setting the diagnosis was collected and summarized from the medical documents of the patients (thrombosis of the arteries/veins). The data were compared to the mutational status of the patients and the results were statistically analyzed by chi-square test. *P*-value less than 0.05 was accepted as a statistically significant difference between the analyzed groups.

RESULTS

Thirty-four of 37 patients were classified with primary MF (PMF), stage I (17 out of 34, 21%), stage II (17 out of 34, 50%), stage III (10 out of 34, 29%), respectively. Three patients had secondary MF developed after the initial diagnosis of essential thrombocythemia (post-ET).

The history of thrombotic events was recorded for all patients **(Table 1)**. The thrombotic events observed in our patients included mainly portal vein thrombosis (PVT) and brain or splenic infarction. Interestingly, post-ET myelofibrotic patients did not show any thrombotic complications **(Table 1)**. Around 35% (13 out of 37) of the myelofibrotic patients had confirmed positive thrombotic history. They were distributed approximately evenly by their sex – seven were males (53%) and six (47%) were females. **Table 1** presents some clinical characteristics of the patients as age, sex, *JAK2* mutational status, the presence of thrombotic incidents, and treatment of the specific patient, if any.

The mean values of the clinical parameters of thrombocytes (Thr), hemoglobin (Hg) and hematocrit (Hct) for patients with or without thrombotic events are shown in **Table 2**. The levels of thrombocytes, Hg and Hct are slightly higher in patients with thrombotic incidents compared to non-thrombotic patients. In our study, we did not find a statistical significance between the levels of Hg and Hct and the presence of *JAK2* V617F mutation in homozygous or heterozygous state (p=0.173 and p=0.695, respectively); however, it was correlated with the level of Thr (p=0.098).

JAK2 V617F status was analyzed for all patients with MF: 23 patients (62%) were positive for the mutation having one or both alleles mutated (homozygous by the mutation, MM or heterozygous MN), while only 14 were *JAK2* negative (34%) **(Fig. 1)**. Among patients with thrombotic history (n=13), *JAK2* V617F positive were nine (69%), the rest (4, 31%) were negative for the mutations. Among non-thrombotic patients (n=24), *JAK2* V617F positive were 14 (58%), *JAK2* V617F negative – 10 (42%) **(Fig. 1)**.

DISCUSSION

The three major types of myeloproliferative neoplasms (myelofibrosis, essential thrombocythemia and polycythemia vera) have differences in terms of bleeding and throm-

Table 1. Characteristics of the patients with MF

Year	Sex	Age	*JAK2* status	Diagnosis and stage	Thr ($\times 10^9$/L)	Hg (g/L)	Hct (%)	Thrombotic event	Management
2017	Male	51	NN	PMF, stage I	293	45	0.144 (14.4)	-	Blood transfusion
2015	Male	65	**MN**	PMF, stage III	84	48	0.134 (13.4)	-	Observation
2017	Male	73	**MN**	Secondary MF, stage III, post-ET	267	60	0.231 (23.1)	-	Blood transfusion, targeted therapy by JAKAVI
2017	Male	51	NN	PMF, stage I	830	69	0.233 (23.3)	Brain infarction	Observation
2018	Male	73	NN	PMF, stage II	36	69	0.217 (21.7)	-	Blood transfusion
2017	Male	94	**MM**	PMF, stage III	254	71	0.22 (22)	-	Hydrea
2019	Male	73	**MN**	PMF, stage III	533	72	0.21 (21)	-	Targeted therapy by JAKAVI
2017	Female	86	NN	PMF, stage II	97	76	0.215 (21.5)	-	Hydrea, anticoagulant therapy
2019	Female	83	NN	Secondary MF, post -ET	209	76	0.237 (23.7)	-	Observation
2016	Female	76	**MN**	PMF, stage III	42	80	0.24 (24)	-	Transfusion of erythrocyte concentration, targeted therapy by JAKAVI
2019	Male	66	**MN**	PMF, stage III	66	80	0.254 (25.4)	-	Blood transfusion
2018	Male	65	**MN**	PMF, stage III	37	85	0.289 (28.9)	Splenic infarction	Observation
2018	Female	79	NN	PMF, stage II	926	88	0.278 (27.8)	-	Hydrea
2019	Male	69	**MM**	PMF, stage I	199	88	0.302 (30.2)	-	Hydrea
2019	Female	55	**MN**	PMF, stage III	104	91	0.314 (31.4)	Splenic infarction	Targeted therapy by JAKAVI
2016	Male	60	NN	PMF, stage II	135	94	0.281 (28.1)	-	Observation
2018	Female	80	**MN**	Secondary MF, stage II, post-ET	550	98	0.303 (30.3)	-	Observation
2019	Male	72	**MN**	PMF, stage II	159	104	0.34 (34)	-	Observation
2018	Male	63	**MN**	PMF, stage II	335	105	0.338 (33.8)	-	Hydrea
2017	Male	46	NN	PMF, stage II	81	107	0.329 (32.9)	Portal vein thrombosis	Observation
2016	Female	29	**MN**	PMF, stage II	304	109	0.316 (31.6)	Portal vein thrombosis	Hydrea
2018	Female	47	NN	PMF, stage II	657	112	0.334 (33.4)	Portal vein thrombosis	Hydrea, anticoagulant therapy
2015	Female	58	NN	PMF, stage I	177	115	0.32 (32)	-	Observation
2018	Female	80	**MM**	PMF, stage II	699	121	0.409 (40.9)	Ischemic stroke of the brain	Anticoagulant therapy
2018	Female	57	NN	PMF, stage III	262	125	0.406 (40.6)	-	Observation
2017	Female	45	**MM**	PMF, stage II	281	126	0.395 (39.5)	Brain disease, unclassified	Anticoagulant therapy
2019	Male	38	NN	PMF, stage I	234	133	0.433 (43.3)	Splenic infarction	Hydrea
2017	Male	54	**MN**	PMF, stage II	507	135	0.514 (51.4)	Mesenteric venous thrombosis	Hydrea, anticoagulant therapy
2018	Female	76	**MM**	PMF, stage III	145	136	0.41 (41)	-	Hydrea, anticoagulant therapy
2019	Male	65	NN	PMF, stage III	248	137	0.427 (42.7)	-	Observation
2017	Female	65	**MM**	PMF, stage II	1052	141	0.414 (41.4)	-	Hydrea, anticoagulant therapy
2016	Male	59	**MN**	PMF, stage II	674	145	0.424 (42.4)	-	Anticoagulant therapy, targeted therapy by JAKAVI

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Year	Sex	Age	*JAK2* status	Diagnosis and stage	Thr ($\times 10^9$/L)	Hg (g/L)	Hct (%)	Thrombotic event	Management
2017	Male	59	**MN**	PMF, stage I	292	148	0.384 (38.4)	Microthrom-boses	Hydrea, anticoagulant therapy
2018	Female	56	**MN**	PMF, stage II	811	148	0.462 (46.2)	Throm-boangiitis obliterans	Hydrea
2019	Male	69	**MM**	PMF, stage II, post-PV	524	152	0.557 (55.7)	Brain infarc-tion	Targeted therapy by JAKAVI
2018	Male	71	**MN**	PMF, stage II	173	156	0.557 (55.7)	-	Hydrea
2016	Male	57	NN	PMF, stage I, II PV	169	197	0.515 (51.5)	-	Bloodletting, anticoagulant therapy

Hct: hematocrit; Hg: hemoglobin; Thr: thrombocytes; PMF: primary myelofibrosis; post-EV: post erythremia vera; post-ET: post essential thrombocythemia; NN: individuals homozygous for the wild type *JAK2 (*lack of mutation); MN: individuals heterozygous for the *JAK2* V617F mutation (mutation in one allele); MM: individuals homozygous for the *JAK2* V617F mutation (mutation in both alleles)

Table 2. Mean values of thrombocytes, hemoglobin, and hematocrit in patients with or without thrombotic events

	Thr (×109/L)	Hg (g/L)	Hct (%)
Patients with thrombotic incident	412.4±276.6	118.2±25.8	0.380±0.1 (38%±1%)
Patients without thrombotic incident	295.2±266.9	100.3±37.3	0.309±0.1 (31%±1%)

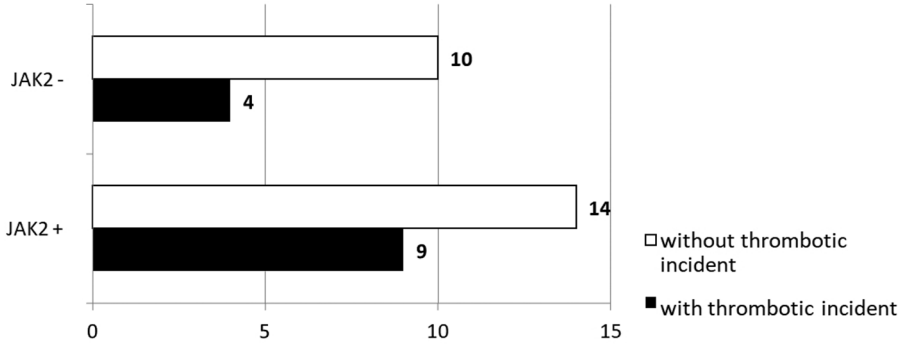

Figure 1. Incidence of thrombotic events and *JAK2* mutational status of the patients.

bosis, although they also share common features.[7-10] Bleeding and thrombotic events are accepted as common complications of myelofibrosis and contribute significantly to the morbidity and mortality of the condition. A few studies only have evaluated the frequency of these events, their characteristics, and their prognostic impact. According to Devendra et al.[11], thrombotic events in MF are about as common as in essential thrombocythemia but less common than in polycythemia vera, while bleeding events are relatively more common in MF than in ET or PV. In a study of 155 patients, 11.6% had thrombotic events during a median follow-up of 4.2 years.[7] In a study of 707 patients with PMF, thromboses were diagnosed in 7.2% patients. A subsequently larger number of patients (35%) in our study showed positive history of thrombotic/vascular events, independently from the sex and the *JAK2* mutational status.

The pathogenesis of vascular events in MF requires further investigation. Some authors state that age >60 and prefibrotic PMF are consistently associated with higher risk of thrombosis while thrombocytosis and *JAK2* positivity are not associated with risk of bleeding.[11] The link between MF with other hereditary or acquired thrombophilic states is virtually unknown. The pro-thrombotic role of *JAK2* V617F mutation in PV or ET is well established and is included in the thrombotic risk model for ET[12], but remains to be clarified in patients with MF. More recently, *JAK2* V617F mutation has been found to carry an increased risk of thrombotic complications.[13] The majority of patients in our study with MF have one or both alleles mutated for *JAK2* V617F mutation (23/37 or 62%). 69% of patients with *JAK2* mutation have previous thrombotic event, while 58% of *JAK2* negative patients are non-thrombotic. Even if there is an observed difference between the groups, a statistical significance has not been found. Those results need to be further confirmed by including more patients and increasing the statistical power of the analysis.

The role of *JAK2* V617F mutation in thrombosis in patients with MPN has been recognized. A mutated *JAK2*

may not only increase the platelet number but also alter the platelet function, thereby playing a role in thrombogenesis. *JAK2* V617F mutation has been reported to cause changes in the process of platelet formation from megakaryocytes in a knock-in mouse model of ET.[14] The platelets were found to be prothrombotic and demonstrated enhanced reactivity to different agonists.[14] The levels of thrombocytes, Hg and Ht are slightly higher in patients with thrombotic incidents compared to non-thrombotic patients. However, we have found no statistical significance among the groups.

The pathogenesis of thrombosis in MPN patients is complex. Disease related factors, such as an increase in blood cell counts (i.e., leukocytosis, erythrocytosis, and thrombocytosis), and more importantly, presence of *JAK2* mutation, can interact with non-disease patient related factors such as age, previous history of thrombotic events, obesity, hypertension, hyperlipidemia, and presence of thrombophilic defects.[15] In our study, we did not find statistical significance also between the levels of Hg and Ht and the presence of *JAK2* V617F mutation in homozygous or heterozygous states (p=0.173 and p=0.695, respectively); however, it was correlated with the Thr level (p=0.098). The idea that patients with MPN-related cerebral venous thrombosis (CVT) should be treated with long-term anticoagulation with VKAs (vit. K antagonists) is presented by some authors.[15] Based on our results, we think that patients with MF, irrespectively of their *JAK2* mutational status, are more prone to develop thrombotic complications and have to be treated with caution, including some anticoagulants in the regular therapeutic regimes.

CONCLUSIONS

Patients with myelofibrosis are prone to developing thrombotic complications independently of their *JAK2* mutational status. Even in a cohort of small size (myelofibrosis is a rare event with an incidence of approximately 1.5:100 000 in USA) (https://rarediseases.org/rare-diseases/primary-myelofibrosis), the frequency of a thrombotic incidence is not significantly related to *JAK2* V617F mutation. Rendering an account to the possible life-threatening complications of such events, treatment decisions should be undertaken upon possible antithrombotic prevention in MF. The thrombotic risk assessment should be based on a combination of clinical risk factors (patient, disease-related, and treatment-related) and a panel of biological markers extending beyond the full blood count.

Author contributions

D.N. wrote the manuscript, A.R. revised it and supplied patients' data

Conflict of Interest

The author reports no conflicts of interest. The authors are responsible for the content and writing of the paper.

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Тромботические инциденты у пациентов с миелофиброзом предполагают, что они не зависят от мутационного статуса *JAK2* V617F

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Резюме

Введение: Миелофиброз (МФ) относится к группе состояний, известных как филадельфийско-негативные миелопролиферативные новообразования (МПН). Кровотечения или различные сосудистые осложнения могут быть основными причинами заболеваемости и смертности больных МФ. Тромбоз, связанный с МПН, представляет собой многофакторный процесс, и в случае миелофиброза мало что известно. Факторы риска тромботических осложнений при МФ оценивались редко.

Цель: Целью этого исследования было изучение частоты тромботических событий при МФ и роли мутации *JAK2* V617F как фактора риска тромботических событий у пациентов с МФ.

Материалы и методы: В нашем исследовании было обследовано 37 пациентов у 35% которых в прошлом были тромботические явления. Все пациенты были госпитализированы в гематологическую клинику Университетской больницы „Свети Иван Рилски", София, Болгария, в период с 2016 по 2019 год, и им был поставлен диагноз на основании критериев ВОЗ 2016 года.

Результаты: У большинства пациентов (23, 62%) обнаружена мутация *JAK2* (янус-киназа) V617F, несущая один (16, 70%) или два (7, 30%) мутантных аллеля. У тринадцати пациентов (35%) в прошлом были тромботические явления, а 9 из них (69%) являются носителями мутации *JAK2* V617F. Четырнадцать пациентов без тромботического анамнеза (24, 58%) также были носителями мутации *JAK2* V617F.

Заключение: В целом мы не обнаружили статистически значимой разницы между мутацией *JAK2* V617F и частотой тромботических событий. С учетом возможных жизнеугрожающих осложнений решение о лечении следует принимать на фоне возможной антитромботической профилактики при МФ.

Ключевые слова

мутация *JAK2*, миелофиброз (МФ), тромботические явления, фактор риска