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MOLECULAR CHARACTERIZATION OF PH-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS IN UKRAINE

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***Aim*: The aim of this study was to examine the *JAK2* V617F, the G1691A allele of factor V, and the *G20210A* prothrombin gene mutation status, and their predictive value for thrombosis in patients with Ph-negative myeloproliferative neoplasms (MPN) in Ukraine, with special emphasize to patient exposed to ionizing radiation due to the Chernobyl accident. *Materials and Methods*: There were 198 patients with Ph-negative MPN included in the study. Of these, 45 patients had experienced radiation exposure due to the Chernobyl accident. The *JAK2* V617F mutation, the *G1691A* of factor V and the *G20210A* of prothrombin were detected by allele-specific polymerase chain reaction. *Results*: The polycythemia vera and essential thrombocythemia patients in unexposed group and Chernobyl patients were comparable in terms of the *JAK2* V617F mutation prevalence with the frequency of anomaly corresponding well to the published data on unselected cases of these types of Ph-negative MPN. The *JAK2* V617F mutation was less common on the border of statistical significance ($p = 0.08$) in Chernobyl primary myelofibrosis (PMF) patients than in non-exposed patients. *JAK2* V617F positive patients had higher level of leukocytes ($p = 0.03$), hemoglobin ($p = 0.04$) and splenomegaly ($p = 0.04$) than those without mutation. The *JAK2* V617F mutation was strong predictor for thrombosis in essential thrombocytemia patients (relative risk=3.1, 95% CI = 1.7–16.4, $p = 0.03$). In PMF, the association with thrombosis was found for the *G1691A* allele of factor V ($p = 0.03$). The risk of thrombosis associated with the inherited thrombophilia in PMF patients was 7.0-fold (95% CI = 1.41–33.1, $p = 0.03$) higher than in polycythemia vera patients. The inherited thrombophilia increased risk of thrombotic complication 5.4-fold (95% CI = 1.41–18.17, $p = 0.01$) in overall cohort of Ph-negative myeloproliferative neoplasms patients. This trend continued in Chernobyl patients ($p = 0.02$), but not in unexposed cases. *Conclusions*: Our findings confirm previous results of other studies reporting that the *JAK2* V617F mutation significantly and independently influences on a disease phenotype in Ph-negative MPN. The inherited thrombophilia is important risk factors of the thrombosis development in overall cohort primary myelofibrosis patients, and especially in disease developed following radiation exposure.**

***Key Words:* Ph-negative myeloproliferative neoplasms, thrombosis, the *JAK2* V617F mutation, the factor V gene *G1691A*, the prothrombin gene *G20210A*.**

The critical role of the *JAK2* V617F mutation in the pathogenesis of Ph-negative myeloproliferative neoplasms (MPN) has been clearly identified. The mutation frequency is estimated at 95–97% for polycythemia vera (PV) [1, 2] and 50–60% for both essential thrombocythemia (ET) and primary myelofibrosis (PMF) [3, 4]. However, the majority of studies regarding the prevalence of the *JAK2* V617F mutation were conducted in groups of patients with unselected myeloproliferative processes. It is not known whether the *JAK2* V617F mutation is as frequent in patients developed Ph-negative MPN following the radiation exposure as in spontaneous Ph-negative MPN cases. Our previous studies demonstrated that radiation-associated myeloid neoplasms of other types developed following low-dose accidental radiation exposure differ from spontaneous cases of the disease in terms of molecular genetic alterations [5, 6]. In this study, we performed an analysis of the *JAK2* V617F mutation prevalence in Ukrainian Ph-negative MPN patients with special empathizes to patients exposed to ionizing radiation due to the Chernobyl accident aiming to demonstrate its significance in pathogenesis of the radiation-associated disease and its possible correlations with clinical characteristics and laboratory findings. The predictive value of mutational status of the *JAK2* V617F allele for most common complications of Ph-negative MPN such as vascular events, leukemic transformation has not been studied in patients exposed to ionizing radiation before as well. Remarkably, the presence of inherited thrombophilia can further increase the relative risk of the thrombosis development in *JAK2*-positive Ph-negative MPN patients by factor of more than 3 [7]. It remains undetermined how the increased vascular complications risk attributable to Ph-negative MPN developed following the radiation exposure is being modified by carriage of the allelic variant *G1691A* of factor V coagulation and the *G20210A* allele of prothrombin gene. The effect of the combined presence in Ph-negative MPN patients of the *JAK2* V617F mutation and the inherited thrombophilia on the thrombotic risk is also unclear [7–10]. Therefore, we explored how the *JAK2* V617F

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Abbreviations used: CI – confidence interval; DNA – deoxyribonucleic acid; ET – essential thrombocythemia; MPN – myeloproliferative neoplasms; NA – not applicable; PMF – primary myelofibrosis; PV – polycythemia vera.

mutation or coexisting thrombophilic risk factors contribute to thrombotic complications in unselected Ph-negative MPN cases and those developed following the Chernobyl accident.

MATERIALS AND METHODS

We analyzed clinical data (history of thrombosis, hemoglobin and cells counts in peripheral blood, spleen diameter defined by ultrasound scanning) and molecular parameters of 198 patients with Ph-negative MPN diagnosed in different clinics of Ukraine between 2009 and 2013. Of these, there were 45 patients experienced the radiation exposure due to the Chernobyl accident (Chernobyl patients) and 153 non-exposed patients with sporadic Ph-negative MPN (Table 1). The Chernobyl patients group included both clean-up workers, which were involved in recovery operations after the accident, and patients either evacuated from the Chernobyl exclusion zone or domiciled in Ukrainian territories with high contamination from radionuclide fallout. World Health Organization (WHO) criteria 2008 were used for the diagnosis of Ph-negative MPN. The study was carried out in accordance with the principles of the Declaration of Helsinki. Thrombosis was defined as a history of typical and atypical arterial or venous occlusive vascular events. By typical arterial events we meant central nervous system vascular complications, including transient ischemic attacks and a stroke, cardiac vascular complications, including angina pectoris and myocardial infarction, peripheral thrombosis, such as intermittent claudication of the legs. Atypical arterial thrombotic events were intra-abdominal arterial vascular complications. The category of typical venous thrombotic episodes included deep venous thrombosis, pulmonary thromboembolism, whereas atypical venous events were Budd — Chiari syndrome and splenic-portal thrombosis. Microvascular arterial disturbances such as acroparesthesias, tinnitus, dizziness and headache or visual complaints were not considered as thrombotic events in this study.

Genomic DNA was extracted from peripheral blood leukocytes by Quiamp DNA extraction kit (Quiagen, Hilgen, Germany). The *JAK2* V617F mutation was detected according to E.J. Baxter et al. [11]. The *G1691A* allelic variant of factor V gene and the *G20210A* allele of prothrombin gene were determined according to L.V. Chornaya et al. [12].

The association between *JAK2* V617F and clinical and laboratory parameters was tested using the Mann — Whitney U-test. The 2-sided Fisher's exact test for 2×2 tables was used to compare categorical variables between the groups. The value of the *JAK2* V617F mutation, the G1691A allelic variant of factor V gene and the G20210A allele of prothrombin gene in the development of thrombotic events was expressed as relative ratio with the corresponding 95% confidence interval (CI). All calculations were performed with STATISTICA Version 10 (StatSoft, Tulsa, OK, USA). Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The *JAK2* V617F mutation status was assessed in 198 patients (Table 1). There were more males in the group of PV patients experienced radiation exposure due to the Chernobyl accident compared to non-exposed PV patients ($p = 0.03$), which might reflect the gender distribution among Chernobyl accident clean-up workers. Polycythemia vera and ET patients in the non-exposed group and Chernobyl patients were comparable in terms of the *JAK2* V617F mutation prevalence (Table 2) with the frequency of anomaly corresponding well to the published data on unselected cases of these types of Ph-negative MPN [1, 2]. The difference in the presence of *JAK2* V617F mutant allele among radiation exposed and sporadic PMF patients was on the border of statistical significance ($p = 0.08$). Primary myelofibrosis developed following the radiation exposure might be characterized by somewhat different pathogenesis involving other genetic events. Thus, the utility of the *JAK2* V617F mutation screening for diagnosis of PMF in Chernobyl patients needs further research.

In the entire cohort of the PV patients, the presence of the *JAK2* V617F mutation was associated with the leukocytosis above 12×10^9/L and the number of neutrophil above 7×10^9/L. *JAK2* V617F positive PV patients had 3.1-fold (95% CI = 1.7–16.4, p = 0.03) and 3.2-fold (95% CI = 1.1–35.2, p = 0.03) more often leukocytes above 12×10^9/L and neutrophils above 7×10^9/L, accordingly. The 20-year risk of evolution to post-PV myelofibrosis was estimated at 6.8% (7 of 103 patients). In spite the fact that all of post-PV myelofibrosis patients were *JAK2* V617F positive, no significant difference was found between the mutation positive and negative PV patients in terms of post-PV myelofibrosis development. In ET patients the positive *JAK2* V617F mutation status was associated with higher hemoglobin level (p=0.04) and number of leukocytes above 12×10^9/L. The relative risk of the leukocytosis associated with the mutation was 2.77 (95% CI = 1.06–7.19, p = 0.01). *JAK2* V617F positive patients with PMF demonstrated the greater size of the spleen (p = 0.04) and 3.1-fold (95%, CI = 1.04–9.02; p = 0.03) higher probability of having the leukocyte level more than 18×10^9/L than patients with wild type allele of the *JAK2* gene. Three of 36 (8.3%) PMF patients displayed progression to acute leukemia at a median time from diagnosis of 48 months (5–156 months). All of progressed patients were *JAK2* V617F positive, however, because of the limited number of observations, no significant difference for leukemia transformation was found between *JAK2* V617F positive and negative PMF patients (0 of 18 versus 3 of 15 patients, p=0.1). These findings confirm results of other studies [13, 14] reporting that the *JAK2* V617F mutation significantly and independently influences on a disease phenotype and might play a role in progression of Ph-negative MPN. There were no differences in clinical parameters between Chernobyl patients and sporadic Ph-negative MPN patients.

In ET patients, it has been found the association of the allelic variant *JAK2* V617F with a history of thrombosis. The relative risk of thrombosis in whole cohort and in non-exposed *JAK2* V617F positive ET patients accounted 3.5 (95% CI = 1.58–26.6, *p*=0.04) and 3.4 (95% CI = 1.05–11.0, *p*=0.04), respectively. Episodes of atypical thrombotic complications occurred in 7.5% of Ph-negative MPN patients in our study, all of which were *JAK2* V617F positive. It was a trend on the border of statistical significance for the unselected Ph-negative MPN patients with thrombotic complication to have atypical thrombotic episode more often in the *JAK2* V617F positive cases (15 of 46 versus 0 of 7 patients; *p* = 0.08). Recent studies suggested that the *JAK2* V617F mutation is frequent in patients with Budd — Chiari syndrome, or portal vein thrombosis, which might present the Ph-negative MPN manifestation [15, 16].

Four (2%) of 198 Ph-negative MPN patients harbored the G20210A allele of prothrombin gene (Table 2). A larger portion of patients with thrombotic events had this mutation than those without (3 of 53 versus 1 of 145 patients), without reaching statistical significance (p = 0.06). The G1691A allele of factor V gene was detected in 2.5% (5 of 198) of Ph-negative MPN patients, all of them being heterozygote carriers. There was no difference in the mutation prevalence in patients with and without thrombosis (3 of 45 versus 2 of 137 patients, p = 0.1). However, by the separate analysis on PMF patients, the allele frequency of this polymorphism in patients with thrombosis was significantly higher than that in patients without thrombotic episodes in anamnesis (p = 0.03). This trend continued in patients with developed PMF following the Chernobyl accident (p = 0.05), but was not observed in non-exposed patients with PMF. The prevalence of any of the inherited thrombophilia marker in PMF patients with thrombosis was significantly higher than in PMF patients without vascular events (3 of 7 versus 0 of 29 patients, p = 0.04). The presence of thrombophilic allele was equal in PV, ET patients with and without thrombotic complication. It was found that the relative risk of thrombosis associated with the congenital thrombophilia in PMF patients was 7.0-fold (95% CI = 1.41–33.1, p = 0.03) higher than in PV patients. In the overall cohort of Ph-negative MPN, the relative risk of thrombotic complication in patients with the inherited thrombophilia was equal 5.4 (95% CI = 1.41–18.17, p = 0.01). The higher prevalence of the G1691A allele of factor V and/or the G20210A allele of prothrombin gene in patients with thrombosis compared to those without was also confirmed for the Chernobyl patients with Ph-negative MPN (3 of 13 versus 0 of 32 patients, p = 0.02), but not for non-exposed patients (3 of 40 versus 3 of 113 patients, p = 0.18). These data suggest that the inherited thrombophilia impacts more to the thrombosis risk in patients with Ph-negative MPN developed following the radiation exposure than in sporadic myeloproliferative disease.

In the total study population of Ph-negative MPN, the thrombosis prevalence in carriers of both the *JAK2* V617F mutation and any of the inherited thrombophilia genetic marker was higher on the border of statistical significance compared to *JAK2* V617F-positive patients without the inherited thrombophilia (5 of 8 versus 44 of 145 patients, p = 0.06). By separate analysis on PV, ET, and PMF, this tendency retained only for PMF patients (p = 0.06). Furthermore, combined

Table 1. Clinical data in Ph-negative MPN patients included into study

Characteristic	PV (n=103)		ET (n=59)		PMF (n=36)	
	Chernobyl patients (n=28)	Non-exposed patients (n=75)	Chernobyl patients (n=2)	Non-exposed patients (n=57)	Chernobyl patients (n=15)	Non-exposed patients (n=21)
Mean age, year	62.0±13.8	59.0±13.9	72.0±4.24	52.3±15.5	65.2±7.0	58.7±12.5
Male/female, n	20/8	37/38	0/2	25/32	12/3	10/11
Mean follow-up period of disease, months (range)	36.4 (8–264)	37.7 (1–247)	38.5 (35–42)	39.1 (1–173)	48.0 (5–156)	34.3 (7–117)
History of thrombosis, n (%)	9 (32.1)	24 (32.0)	0	13 (22.8)	4 (26.6)	3 (14.2)
Typical arterial thrombosis, n (%)	3 (10.7)	15 (20.0)	0	3 (5.2)	4 (26.6)	0
Acute coronary syndromes, n (%)	2 (7.1)	9 (12.0)	0	2 (3.5)	2 (13.3)	0
Cerebrovascular events, n (%)	1 (3.5)	6 (8.0)	0	1 (1.7)	2 (13.3)	0
Deep venous thrombosis of the limbs and/or pulmonary embolism, n (%)	3 (10.7)	4 (5.3)	0	5 (8.7)	0	1 (4.7)
Atypical arterial thrombosis, n (%)	1 (3.5)	1 (1.3)	0	3 (5.2)	0	0
Atypical venues thrombosis, n (%)	2 (7.1)	4 (5.3)	0	2 (3.5)	0	2 (9.5)

Table 2. The presence of the *JAK2* V617F mutation and the inherited thronmbophilia in Ph-negative MPN patients

	Groups	n	The *JAK2* V617F mutation status assessment				The factor V G1691A mutation status assessment				The prothrombin G20210A mutation status assessment			
			Mutation carriers, n (%)	*JAK2* V617F positive +Thr**, n (%)	*JAK2* V617F negative +Thr**, n (%)	P*	Mutation carriers, n (%)	G1691A positive +Thr**, n (%)	G1691A negative +Thr**, n (%)	P*	Mutation carriers, n (%)	G20210A positive +Thr**, n (%)	G20210A negative +Thr**, n (%)	P*
PV	Unselected	103	99 (96.1)	33 (32.0)	0	*0.2*	3 (2.9)	1 (0.9)	32 (31.0)	*0.69*	1 (1.9)	1 (1.9)	32 (31.0)	*0.32*
	Chernobyl patients	28	27 (96.4)	9 (32.1)	0	*0.6*	0	0	9 (32.1)	*NA****	1 (3.5)	1 (3.5)	8 (28.5)	*0.32*
	Non-exposed patients	75	72 (96.0)	24 (32.0)	0	*0.3*	3 (4.0)	1 (1.3)	23 (30.6)	*0.67*	0	0	24 (32.0)	*NA****
ET	Unselected	59	36 (61.0)	11 (18.6)	2 (3.3)	*0.04*	0	0	13 (22.0)	*NA****	2 (3.4)	1 (1.7)	12 (20.3)	*0.39*
	Chernobyl patients	2	1 (50.0)	0	0	*NA****	0	0	0	*NA****	0	0	0	*NA****
	Non-exposed patients	57	35 (61.4)	11 (19.3)	2 (3.5)	*0.04*	0	0	13 (22.8)	*NA****	2 (3.5)	1 (1.7)	12 (21.0)	*0.41*
PMF	Unselected	36	18 (50.0)	5 (13.9)	2 (5.5)	*0.20*	2 (5.5)	2 (5.5)	5 (13.8)	*0.03*	1 (2.7)	1 (2.7)	6 (16.6)	*0.19*
	Chernobyl patients	15	5 (33.3)	3 (20.0)	1 (13.3)	*0.07*	2 (13.3)	2 (13.3)	2 (13.3)	*0.05*	0	0	3 (20.0)	*NA****
	Non-exposed patients	21	13 (61.9)	2 (9.5)	1 (4.7)	*0.68*	0	0	3 (14.2)	*NA****	1 (4.7)	1 (4.7)	2 (9.5)	*0.14*

Notes: P* – estimated for mutation prevalence between group of patients with and without thrombosis; **+Thr – patients with thrombosis;***not applicable.

carriers of the *JAK2* V617F mutation and the genetic marker of the inherited thrombophilia with PMF more often had thrombosis than *JAK2* V617F-negative PMF patients without the inherited thrombophilia (2 of 2 versus 2 of 18 patients, p = 0.03).

The characteristics of patients with diseases developed following the radiation exposure and non-exposed patients group in this study reflect the current knowledge concerning PV, ET in terms of the thrombosis rate and the *JAK2* V617F mutation prevalence [17]. However, the cumulative rate of thrombosis for PMF patients included into this analysis both unselected and in each study group was significantly higher than expected according to data from the literature [18]. Our findings confirmed the most resent observations on prothrombotic role of the *JAK2* V617F mutation in ET patients [19, 20]. The univariate analysis on the whole cohort and the non-exposed group of ET patients demonstrated an increased risk for thrombosis in individuals with the mutation. The prognostic relevance of the *JAK2* V617F status in terms of the thrombotic risk in PMF has been proposed by T. Barbui et al. [18]. However, neither a meta-analysis [21] nor our study demonstrated the thrombogenic role of the *JAK2* V617F mutation in PMF patients.

The role of the inherited thrombophilia in the thrombosis risk is still unclear in Ph-negative MPN patients. The lack of association between the *G20210A* allele of prothrombin gene or the *G1691A* allele of factor V gene and the increased incidence of thrombotic events in ET and PV in this study is in agreement with published results of a few other studies [8, 9]. At the same time, there are several studies reported the prediction role for thrombosis of the *G1691A* allele of factor V and the *G20210A* allele of prothrombin in Ph-negative MPN patients [7, 10]. Our observations also revealed the significantly increased prevalence of thrombophilic alleles in the overall cohort of PMF with thrombosis. This was probably predetermined by patients with PMF developed following the Chernobyl accident. It was also found the higher prognostic relevance of the inherited thrombophilia determinants in not separated patients developed Ph-negative MPN following the Chernobyl accident. The inherited thrombophilia associated thrombosis risk was higher in PMF than in PV patients. It could be that the higher the thrombosis risk associated to the Ph-negative MPN, the lower the impact of inherited thrombophilia on it. According, to recent studies, the presence of both the *JAK2* V617F mutation and the genetic marker of the inherited thrombophilia increases the risk of thrombosis in Ph-negative MPN patients compared to patients without these anomalies [7, 22], which was also confirmed by our study for PMF cohort.

In summary, there is no difference in *JAK2* V617F prevalence in spontaneous PV and ET and those developed following radiation exposure. However, our findings are suggestive that the *JAK2* V617F mutation is less common in Chernobyl PMF patients than in non-exposed patients. The *JAK2* V617F positive status is predictive of higher leukocytosis and more frequent thrombosis in patients with classic Ph-negative MPN. According to results of the study, testing for the *G1691A* allele of factor V and the *G20210A* allele of prothrombin does not seem to be performed on a routine basis for ET and PV patients. However, the congenital thrombophilia increases the thrombosis risk in PMF, and especially in PMF developed following the radiation exposure. The patients with the inherited thrombophilia are also characterized by the higher risk of thrombosis among Ph-negative MPN patients of the overall cohort. Furthermore, there is the tendency for PMF patients carrying both the *JAK2* V617F mutation and the genetic marker of the inherited thrombophilia to have thrombosis more often than *JAK2* V617F-positive PMF patients without the inherited thrombophilia, which needs additional study to be proved.

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