Predictive factors of thrombosis for patients with essential thrombocythaemia: A single center study*

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Abstract

Background. Thrombotic complications are the leading cause of mortality in essential thrombocythemia (ET), but the definition of thrombotic risk remains far from clear.

Objectives. The aim of this study was to evaluate the prognostic markers for thrombosis to identify ET patients at risk.

Material and methods. Forty-five consecutive patients with ET were studied. This group was divided into two subgroups ET patients with (A) and without (B) history of thrombosis. Each patient has been tested for complete blood count, fibrinogen, factor VIII, D-dimer, protein C, APCr, TAT and F1+2. JAK2 mutation was assessed by RT-PCR. Factor V Leiden and prothrombin genes mutations were screened by DNA sequencing.

Results. The median age of ET patients was 62.0 years. JAK2 mutation was found in 24 patients, 21 of them had a history of thrombotic events, and 17/21 were JAK2 positive. Compared to controls, ET patients had a significantly higher WBC and PLT counts, and higher mean platelet volume (MPV), but not Hgb level or RBC count. In ET subgroup A, apart from changes seen in the whole ET, the Hgb level, RBC count, and Hct were also significantly elevated. Interestingly, the MPV was significantly larger in subgroup A, but not in B. Fibrinogen and D-dimers levels were significantly higher in ET group than in controls, but not F1+F2 or TAT. The results of hemostatic tests did not markedly differ between subgroups A and B. APCr was found in 5/45 patients with ET, and 2 out of 5 had a factor V Leiden heterozygous mutation. No prothrombin gene mutation was observed.

Conclusions. Our results suggest that MPV can serve as a simple test for assessing the hypercoagulable state in ET patients. It has been confirmed that JAK2 mutation and leukocytosis are independent predictors for thrombotic events in ET patients.

Key words: essential thrombocythemia, thrombosis, JAK2 mutation, platelets, leukocytes

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Introduction

Essential thrombocythemia (ET) is a stem cell disorder whose distinguishing characteristic is a markedly elevated platelet count caused by excessive megakaryocyte proliferation. The activating *JAK2V617F* mutation is seen in nearly 60% of ET patients¹, *CALR* mutation in about 20–25%, *c-MPL* gene mutation in a few percent, and about 10% to 25% are defined as “triple negative”^{2,3}. An annual incidence rate in the United States is approximately 0.5 to 1.5 cases per 100,000 persons per year. The median age at diagnosis is approximately in the mid-seventh decade of life, but it can occur cross in all age groups, and there is a 1.5 to 2-fold female predominance, especially in those diagnosed in the third to fifth decade of life.

Thrombotic complications are present at diagnosis in about 9–22% of patients. Thromboembolic risk has been associated with age (> 60 years), prior thrombosis, leukocytosis, and possibly also with *JAK2V617F* mutation.⁴

Morbidity and mortality from ET in the first decade of the disease are predominantly related to thrombotic, vasomotor, and less commonly, to hemorrhagic complications. Long standing ET can progress to myelofibrosis or acute myeloblastic leukemia.⁵

Material and methods

This study included 45 consecutive patients with essential thrombocythemia (ET) attending the out-patient clinic at the Department of Hematology, Blood Neoplasms, and Bone Marrow Transplantation in Wrocław, Poland. The diagnosis of ET was made on the basis of the 2008 World Health Organization criteria.⁶ Among the 45 ET patients, there were 14 men and 31 women; the age range was between 25–89 years. Neither of the ET patients had a new thrombotic complication at the time of entering the study. A group of 45 healthy subjects (18 men, 27 women; age range, 22–78 years) was recruited from healthy adults without history of thrombotic events to serve as a control group.

All investigations were approved by the Bioethics Commission of Wrocław Medical University, Poland. The study was carried out in accordance with the Declaration of Helsinki of 1975, as revised in 2000. Written informed consent was obtained from all patients and controls before entering the study.

The characteristics of the patients and the control subjects were summarized in Table 1. Of 45 ET patients, 12 asymptomatic patients did not receive any treatment for ET, 22 were treated with anagrelide (thromboreductin), 8 with hydroxyurea (HU), and 3 with aspirin (ASA). Twenty-one patients had thrombotic events in the past. Fifteen patients had arterial thrombosis: 6 patients had myocardial infarction (MI), 7 patients had transient ischemic attacks (TIA), 4 patients had peripheral arterial thrombosis (PAT) of the

Table 1. Characteristic of patients with essential thrombocythemia (ET)

Data/Group	Essential thrombocythemia (n = 45)	Controls (n = 45)
Age: median ± SD (range)	62.0 (range 25–89)	58.6 (range 22–78)
Gender (male/female)	14/31	18/27
<i>JAK2</i> positive (male/female)	24 (10/14)	0
Thromboembolic events (male/female)	21 (6/15)	0

ET – essential thrombocythemia; *JAK2* mutation – Janus kinase (*JAK2*) V517F mutation.

legs, and 6 patients had deep venous thrombosis (DVT). Microvascular disturbances and superficial thrombophlebitis of the legs were not considered in this analysis.

Methods

Each patient has been tested by automated methods for blood morphology (Sysmex). Plasma fibrinogen level, D-dimer level, factor VIII activity, protein C level, activated protein C resistance (APCR) were measured in coagulometer Ceverone alpha (Technoclone). Plasma concentrations of thrombin-anti-thrombin complexes (TAT) and prothrombin fragment F₁₊₂ (F1+2) were determined by ELISA, using commercially available kits (Enzygnost TAT and Enzygnost F1+2, respectively; Siemens).

Testing for the factor V Leiden (G11691A) gene and prothrombin gene (FII G20210A) hereditary mutations were screened by DNA sequencing.

JAK2 mutation was screened by the real-time RT-PCR assay.

The applied dispersed computer system was used for the accumulation and safety storage of patients' data and the results of the laboratory tests.

Statistical analysis

All calculations were performed with the R Project for Statistical Computing, with the threshold of statistical significance set at a p-value of 0.05 or lower.

Patients' characteristics were compared with the use of the two-tailed Fisher's exact test for categorical variables, and the Mann-Whitney U test for continuous variables.

Results

The study population consisted of 45 subjects with ET, 14 males and 31 females, with the median age of 62.0. Their general characteristics are presented in Table 1. Among the 45 ET patients, 24 tested positive for the *JAK2* heterozygous mutation, predominantly among fe-

males 14/24. Thromboembolic events were reported in 21 out of 45 ET patients, in 6 males and in 15 females.

The comparison of blood picture parameters and hemostatic tests in ET and the control group are shown in Table 2.

Leukocyte ($p < 10^{-6}$) and platelet ($p < 10^{-6}$) counts were significantly higher, and MPV markedly bigger ($p = 0.0001$) in ET patients than in healthy subjects, but not hemoglobin level or erythrocyte count.

Fibrinogen concentration ($p = 0.0015$) and D-dimers ($p < 10^{-6}$) were significantly higher in the ET group than in controls, but not F1 + F2, TAT, factor VIII activity, PC activity and activated protein C resistance.

APCR was found in 5/45 ET patients with ET and in 2/45 controls. None of ET patients were carriers of the FV Leiden homozygous mutation, but two ET patients had the FV Leiden heterozygous mutation and 2 controls. Prothrombin gene mutation (G20210A) was not present in either group.

Compared to the control groups, ET patients with thrombosis (A) had significantly elevated leukocyte ($p < 10^{-6}$) and platelets ($p < 10^{-11}$) counts, MPV ($p < 10^{-8}$), D-dimers level (0.0001).

Table 4 shows that in ET patients without thrombosis (B) the hemoglobin level ($p = 0.003$), leukocyte count ($p < 10^{-10}$), and platelet count ($p < 10^{-10}$) were significantly higher than in controls, but not MPV ($p = 0.771$). In the ET group without thrombosis (subgroup B) the fibrinogen ($p = 0.004$) and D-dimers ($p < 10^{-6}$) levels were also significantly elevated by comparison with the control groups.

As shown in Table 5, the blood count differs significantly between ET patients with (A) and without (B) thrombosis. The hemoglobin level ($p = 0.0002$), erythrocyte count ($p = 0.01$), hematocrit ($p = 0.012$), leukocyte count ($p = 0.0033$) and platelet count ($p = 0.046$) were significantly increased in ET patients with thrombosis (A). The mean platelet volume was significantly larger in ET subgroup with (A) than that in the subgroup (B) without thrombosis ($p < 10^{-8}$).

The coagulation tests did not show any statistically significant differences concerning fibrinogen, D-dimers, factor VIII activity, protein C activity, APCR, F1+F2 and TAT levels between two ET subgroups with (A) and without (B) thrombosis.

Discussion

We have confirmed that the occurrence of *JAK2* mutation can be a strong predictor for thromboembolic events in ET patients. Seventeen out of 21 ET patients with thrombotic complications were *JAK2* (+). Since its discovery in 2005, the *JAK2* mutation has been considered as an important participant in the development of thrombotic complications in Ph-myeloproliferative neoplasms, but

the agreement was not universal.¹ Carobbio et al. in 657 studied ET patients did not find a significant association for *JAK2* mutation and the frequency of thromboembolic events.⁷ De Stefano et al. confirmed that in *JAK2* (+) ET patients aged < 60 years the risk of thrombosis increased 3.8 fold in the presence of homozygous and 2.2 fold in the case of a heterozygous type of FV Leiden mutation.⁸ The systematic review and meta-analysis of 2,436 patients with ET shows that *JAK2* positivity correlated with a clear increase in the odds of thrombosis (OR = 1.83)⁹, and this was also observed by others.^{10–12} Our findings are in line with the results of Schwartz et al.,¹³ who have proven, on the basis of an analyzed cohort of 1,179 thrombocytemic Ph-myeloproliferations, that *JAK2* mutation affected significantly the incidence of thrombosis (both arterial and venous). The *JAK2* mutation has been included recently into the major criteria for ET^{14,15}, and is also considered as a major prothrombotic risk factor in the International Prognostic Score in Essential Thrombocythemia (IPSET) risk factor study in WHO-defined ET.¹⁶

It is not easy to analyze laboratory results obtained in our patients with ET, as it is necessary to consider that some results might have been conflated not only by the disease itself, but also by the treatment effects of anagrelide, hydroxyurea or ASA. Many other investigators faced similar problems.

In ET patients, leukocyte and platelet counts were found to be significantly higher, and the mean platelet volume (MPV) markedly bigger than in healthy subjects, but not hemoglobin level or erythrocyte count.

However, in the subgroup of ET patients with thrombotic events (A), apart from significantly elevated platelet and leukocyte counts and higher MPV, the hemoglobin level, hematocrit, and erythrocyte counts were also increased significantly compared with that in ET patients without thrombosis (B). Belluci and Michiels suggest that heterozygous *JAK2* mutation can be responsible for the increased hemoglobin and hematocrit in some ET patients.¹⁷ Rotunno G et al. found that *JAK2*(+) ET patients have a higher Hb level and are more prone to thrombotic events than *CALR* (+) ET patients.³

Further analysis of our results revealed that between the ET subgroups with (A) and without thrombosis (B) the statistical change in the leukocyte count was much stronger ($p = 0.0033$) than in the platelet count ($p = 0.046$). Leukocyte count > 11 g/L is an established risk factor for thrombosis in ET. It shall be stressed that the median platelet count in subgroup A was 676.0 g/L (ranged from 544.0 g/L to 837.0 g/L), but in subgroup B though, the median platelet count was 535.0 g/L. This subgroup was much more heterogeneous as platelet counts ranged from 300.0 g/L to 2022.0 g/L.

Interestingly, the presence of bigger, younger and more hemostatically active platelets caused a significant increase in the mean platelet volume in all ET patients and in the subgroup of ET with thrombosis, but not in ET pa-

Table 2. Blood picture parameters and hemostatic tests in patients with essential thrombocythaemia

Parameter	ET (n = 45)				SD	Controls (n = 45)					p-value
	median	minimum	maximum	mean		median	minimum	maximum	mean	SD	
Hemoglobin (g/dL)	14.5	9.3	17.3	14.19	1.96	14.6	11.8	17.0	14.60	1.09	0.654
Erythrocyte (T/L)	482	3.15	5.91	4.87	0.66	4.94	3.91	5.97	4.89	0.50	0.827
Hematocrit (%)	43.0	28.6	54.8	42.2	6.61	44.3	33.7	50.1	43.5	4.07	0.434
Leukocyte (G/L)	10.17	6.0	24.2	10.73	4.17	6.3	4.0	9.7	6.37	1.51	p < 10−6
Platelet G/L	625.0	300.0	2022.0	651.88	254.18	255.0	183.0	421.0	263.13	54.7	p < 10−6
MPV (fl)	10.0	7.3	19.0	10.63	2.57	8.6	7.3	10.2	8.63	0.69	0.0001
Fibrinogen (g/L)	2.92	1.06	5.41	3.06	0.91	2.41	1.48	4.6	2.53	0.72	0.0015
F1 + F2 (pmol/L)	222.79	23.39	1440.85	335.52	313.37	196.1	73.36	1907.25	320.52	388.69	0.619
TAT (µg/L)	3.05	0.45	9.5	3.29	1.93	2.73	0.1	103.6	4.9	15.28	0.076
D-dimer (ng/mL)	186.0	0	1463.2	262.22	303.33	18.5	0	245.5	41.08	59.43	p < 10−6
PC (%)	99.0	36.1	153.8	98.14	23.66	103.9	73.8	157.8	102.8	20.85	0.563
APCR	2.5	1.44	3.65	2.54	0.48	2.61	1.5	4.48	2.67	0.55	0.237
FVIII (%)	91.2	38.5	200.6	98.69	32.02	97.7	66.1	185.1	103.12	28.62	0.47

MPV – mean platelet volume; F1 + F2 – prothrombin fragments F1 + F2; TAT – thrombin-antithrombin complex; D – dimer; PC – protein C; APCR – activated protein C resistance; FVIII – factor VIII.

Table 3. Blood picture parameters and hemostatic tests in patients with essential thrombocythaemia with thrombosis (A) and in controls

Parameter	ET patients with thrombosis (A), n = 21				SD	Controls (n = 45)				p-value
	median	minimum	maximum	mean		median	minimum	maximum	mean	SD
Hemoglobin (g/dL)	15.6	12.4	17.3	15.32	1.12	14.6	11.8	17.0	14.60	1.09
Erythrocyte (T/L)	5.41	3.76	5.91	5.12	0.64	4.94	3.91	5.97	4.89	0.50
Hematocrit (%)	46.0	29.0	54.8	44.8	5.40	44.3	33.7	50.1	43.5	4.07
Leukocyte (G/L)	11.5	6.09	17.4	11.53	2.39	6.3	4.0	9.7	6.37	1.51
Platelet G/L	676.0	544.0	837.0	671.4	81.49	255.0	183.0	421.0	263.13	54.7
MPV (fl)	12.7	9.0	19.0	12.75	2.11	8.6	7.3	10.2	8.63	0.69
Fibrinogen (g/L)	2.85	1.06	5.41	3.05	1.03	2.41	1.48	4.6	2.53	0.72
F1 + F2 (pmol/L)	236.0	23.39	865.28	352.85	257.03	196.1	73.36	1907.25	320.52	388.69
TAT (µg/L)	3.06	0.95	9.26	3.24	1.83	2.73	0.1	103.6	4.9	15.28
D-dimer (ng/mL)	163.3	0	1144.5	245.04	276.37	18.5	0	245.5	41.08	59.43
PC (%)	96.5	36.1	140.2	91.5	25.46	103.9	73.8	157.8	102.8	20.85
APCR	2.33	1.87	3.03	2.45	0.38	2.61	1.5	4.48	2.67	0.55
FVIII (%)	90.1	38.5	150.8	91.4	26.57	97.7	66.1	185.1	103.12	28.62

MPV – mean platelet volume; F1 + F2 – prothrombin fragments F1 + F2; TAT – thrombin-antithrombin complex; D – dimer; PC – protein C; APCR – activated protein C resistance; FVIII – factor VIII.

Table 4. Blood picture parameters and hemostatic tests in patients with essential thrombocythaemia without thrombosis (B) and in controls

Parameter	ET patients without thrombosis (B), n = 24					Controls (n = 45)					p-value
	median	minimum	maximum	mean	SD	median	minimum	maximum	mean	SD	
Hemoglobin (g/dL)	13.6	9.3	16.3	13.2	2.02	14.6	11.8	17.0	14.60	1.09	0.003
Erythrocyte (T/L)	4.72	3.15	5.62	4.65	0.62	4.94	3.91	5.97	4.89	0.50	0.175
Hematocrit (%)	41.1	28.6	51.6	39.9	6.84	44.3	33.7	50.1	43.5	4.07	0.013
Leukocyte (G/L)	8.24	6.0	24.21	10.0	5.21	6.3	4.0	9.7	6.37	1.51	< 10 ⁻¹⁰
Platelet G/L	535.0	300.0	2022	634.7	342.3	255.0	183.0	421.0	263.13	54.7	< 10 ⁻¹⁰
MPV (fl)	8.65	7.3	11.0	8.77	1.03	8.6	7.3	10.2	8.63	0.69	0.771
Fibrinogen (g/L)	3.16	1.46	4.62	3.07	0.81	2.41	1.48	4.6	2.53	0.72	0.004
F1 + F2 (pmol/L)	212.11	35.33	1440.85	320.36	360.4	196.1	73.36	1907.25	320.52	388.69	0.915
TAT (μg/L)	2.89	0.45	9.5	3.34	2.06	2.73	0.1	103.6	4.9	15.28	0.127
D-dimer (ng/mL)	200.0	0	1463.2	277.2	330.0	18.5	0	245.5	41.08	59.43	< 10 ⁻⁸
PC (%)	100.3	72.6	153.8	103.9	20.78	103.9	73.8	157.8	102.8	20.85	0.801
APCR	2.55	1.44	3.65	2.62	0.54	2.61	1.5	4.48	2.67	0.55	0.865
FVIII (%)	95.05	72.2	200.6	105.1	35.44	97.7	66.1	185.1	103.12	28.62	1

MPV – mean platelet volume; F1 + F2 – prothrombin fragments F1 + F2; TAT – thrombin-antithrombin complex; D – dimer; PC – protein C; APCR – activated protein C resistance; FVIII – factor VIII.

Table 5. Blood picture parameters and hemostatic tests in patients with essential thrombocythaemia with (A) and without thrombosis (B)

Parameter	ET patients with thrombosis (A), n = 21					ET patients without thrombosis (B), n = 24					p-value
	median	minimum	maximum	mean	SD	median	minimum	maximum	mean	SD	
Hemoglobin (g/dL)	15.6	12.4	17.3	15.32	1.12	13.6	9.3	16.3	13.2	2.02	0.0002
Erythrocyte (T/L)	5.41	3.76	5.91	5.12	0.64	4.72	3.15	5.62	4.65	0.62	0.01
Hematocrit (%)	46.0	29.0	54.8	44.8	5.40	41.1	28.6	51.6	39.9	6.84	0.012
Leukocyte (G/L)	11.5	6.09	17.4	11.53	2.39	8.24	6.0	24.21	10.0	5.21	0.0033
Platelet G/L	676.0	544.0	837.0	671.4	81.49	535.0	300.0	2022	634.7	342.3	0.046
MPV (fl)	12.7	9.0	19.0	12.75	2.11	8.65	7.3	11.0	8.77	1.03	< 10 ⁻⁸
Fibrinogen (g/L)	2.85	1.06	5.41	3.05	1.03	3.16	1.46	4.62	3.07	0.81	0.8288
F1 + F2 (pmol/L)	236.0	23.39	865.28	352.85	257.03	212.11	35.33	1440.85	320.36	360.4	0.4362
TAT (μg/L)	3.06	0.95	9.26	3.24	1.83	2.89	0.45	9.5	3.34	2.06	0.9546
D-dimer (ng/mL)	163.3	0	1144.5	245.04	276.37	200.0	0	1463.2	277.2	330.0	0.7935
PC (%)	96.5	36.1	140.2	91.5	25.46	100.3	72.6	153.8	103.9	20.78	0.1583
APCR	2.33	1.87	3.03	2.45	0.38	2.55	1.44	3.65	2.62	0.54	0.2366
FVIII (%)	90.1	38.5	150.8	91.4	26.57	95.05	72.2	200.6	105.1	35.44	0.2748

MPV – mean platelet volume; F1 + F2 – prothrombin fragments F1 + F2; TAT – thrombin-antithrombin complex; D – dimer; PC – protein C; APCR – activated protein C resistance; FVIII – factor VIII.

tients without thrombosis. If confirmed in a larger group of ET patients, MPV can possibly be useful in predicting a prothrombotic state. Arellano-Rodrigo et al.¹⁸ observed that ET patients had higher values of MPV, especially those with *JAK2* mutation.¹⁸ At a multivariate study it has been confirmed that ET patients with thrombosis had a significantly higher percentage of reticulated platelets. Increased neutrophil and platelet activation play an important role in the pathogenesis of thrombotic complications in ET patients.^{19–22}

We have observed that both fibrinogen ($p = 0.0015$) and D-dimer ($p < 10^{-6}$) levels were significantly higher in our ET patients than in controls, but they did not differ between ET subgroups with and without thrombosis. Blann et al.²³ observed some elevation in the fibrinogen level in treated and untreated patients with ET, but it remained within normal ranges.

Some authors described elevated D-dimer levels in patients with ET at diagnosis or treated with ASA or hydroxyurea and ASA^{23,24}, but others have shown its reduction after cytoreductive therapy.²⁵ However, the increased D-dimer level has not been proven as a clinically useful predictor for thrombosis, when compared with some other confirmed predictors such as age and history of thrombosis or leukocytosis.^{26,27} In patients with ET we did not find an increase in factor VIII activity, trombin-anti-thrombin complexes and prothrombin fragments F1 + F2 levels or protein C activity.

Our observations are not in keeping with the results of some other investigators who observed elevated F1 + F2 fragments¹⁹ in ET patients.

No statistically significant difference has been found in the frequency of factor V Leiden or prothrombin gene mutation compared to the control group. It is in agreement with the results of other authors.^{11,13} Out of five ET patients presenting APCR the factor V Leiden heterozygous mutation has been confirmed in 2, and the remaining three had acquired APCR. Both ET patients with FVL mutation experienced thrombotic events. The knowledge of carriership of FVLeiden can be helpful in the individual thrombosis risk assessment.

However, hereditary thrombophilia with essential thrombocythaemia increases the risk of thrombosis 2.7 fold in carriers and 5 fold in *JAK2* (+) ET patients.¹⁰ Schwartz et al. have shown that in ET the specific thrombophilic markers, e.g. FVLeiden mutation and protein C deficiency, were connected with thrombotic venous events.¹³

In conclusion, our results strongly suggest that changes of mean platelet volume can serve as an additional and simple test in prothrombotic state assessment in ET patients, but this needs to be confirmed in a larger population. The presence of *JAK2* mutation and leukocytosis has been proven to be a strong predictor for thromboembolic events in ET patients.

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