VEGF, D-dimer and Coagulation Activation Markers in Indonesian Patients with Polycythemia Vera and Essential Thrombocythemia and Their Relation with Recurrence of Thrombosis

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SrySuryani Widjaja¹,
Karmel L Tambunan²,
Yahwardiah Siregar¹,
Rahajuningsih Dharma³,
Stephen CL Koh⁴

¹ Biochemistry department, Medical Faculty, University Sumatera Utara, Medan, Indonesia

² Hemato-Oncology Department, Medical Faculty, University Indonesia, Jakarta, Indonesia

³ Clinical Pathology Department, Medical faculty, University Indonesia, Jakarta, Indonesia

⁴ Clinical Pathology Department, Faculty of Medicine, University Sumatera Utara, Medan, Indonesia

Abstract

Background: PV and ET have high predisposition to thrombosis and recurrence of thrombosis. We determined VEGF, D-dimer and coagulation activation markers in clinically stable patients and recurrence of thrombosis.

Methods: Thirty-five Indonesian patients diagnosed with PV and ET and under treatment for the disease were recruited. The following assays were performed: VEGF, D-dimer, fibrinogen, TAT-complex, vWF, β -TG and JAK2 V617F mutation. Data between patients who were clinically stable (n=20) and those with recurrent thrombosis (n=15) at the time of study was analysed.

Results: The mean age for PV/ET was 51.7 ± 14.9 years. Thrombosis episode was recorded for 94.3% (33/35) patients. Twenty (57.1%) clinically stable and 15 (42.9%) patients had recurrence of thrombosis. D-dimer ($P < 0.001$), fibrinogen ($P = 0.005$) were statistically significant and VEGF ($P = 0.06$) were seen in recurrence of thrombosis compared to clinically stable patients who had normal D-dimer. Elevated D-dimer seen in recurrence thrombosis was significantly correlated with VEGF ($P = 0.002$) levels. Elevated VEGF were seen in 45% of clinically stable patients and 73.3% in recurrence of thrombosis.

Contact information:

Dr Widjaja Sry Suryani

✉ srysuryani@gmail.com

Keywords

PV/ET, VEGF, D-dimer, recurrence of thrombosis

Conclusions: Elevated D-dimer and fibrinogen with higher mean VEGF levels were seen in recurrence of thrombosis. VEGF and D-dimer measurements have clinical use in determining the risk of patients with vascular complications.

Introduction

Polycythemia vera (PV) and essential thrombocythemia (ET) are Philadelphia negative myeloproliferative neoplasms (MPNs) with cardinal features of an increased red cell mass in PV and high platelet count in ET (1, 2). They have high predisposition to thrombosis with a rate of major thrombosis as high as about 50% (3, 4). It is associated with JAK2V617F somatic mutation which carries an enhanced risk of thrombosis (5) but their role in thrombotic complications is not entirely clear. Clinical factors (age, previous history of thrombotic events, JAK2 V617F somatic mutation (6) as well as leukocytosis, erythrocytosis and thrombocytosis is associated with enhanced risk of thrombosis (7). Blood hyperviscosity causes high shear stress of vessel wall and accounts for chronic endothelial dysfunction, platelet and leukocyte activation (8). Blood cell activation effects on the endothelium and plasma clotting factors are most likely the cause of thrombin formation in MPN have been reported (9). The mechanisms leading to thrombosis remain largely speculative, but are likely to be complex and multifactorial (10). Vascular endothelial growth factor (VEGF) a potent regulator of vascular permeability and angiogenesis is also an indirect procoagulant released by platelets (11, 12). Increased angiogenic activity has been reported in PV and ET (13, 14) and platelets are the major physiological transporter of VEGF in blood (15). VEGF induces platelet activation and elevated levels stimulate endothelial activation, enhance tissue factor

synthesis and induce thrombin formation (16,17). Increased levels might be responsible for endothelium activation and for occurrence of a thrombotic event (18). The role of D-dimer assays in the diagnosis of patients with suspected deep vein thrombosis (DVT) has been extensively studied (19). It is a very useful diagnostic tool in the management of patients with suspected DVT and pulmonary embolism (PE) (20) and has been shown to have a high sensitivity and a high negative predictive value for DVT exclusion (21). It is a marker for hypercoagulability and links with venous as well as arterial thrombotic events and has been used to determine the hypercoagulable state leading to thrombosis in PV and ET (22, 23). The main strategy of management in PV and ET is to prevent thrombosis besides treating the disease (24).

The aim of the study is to determine VEGF, D-dimer and coagulation activation markers in Indonesian patients with polycythemia vera and essential thrombocythemia and their relation to recurrence of thrombosis.

Methods

Subjects

The study received ethical approval from the Health Research Ethical Committee (197/KOMET/FK/USU2010), Medical School, University of North Sumatra, Indonesia. Only patients diagnosed with PV and ET as defined by the WHO Protocol 2008

(25) and who gave signed informed consent were admitted to the study. Patients were recruited between August 2010 and January 2011 from the hospitals in Medan, Indonesia: Adam Malik Hospital, Dr Pirngadi Hospital, Herna Hospital, Imelda and Elizabeth Hospital. Recruitment of patients with either PV/ET on their visit to various hospitals and clinics was referred to the study. This include fourteen patients who were diagnosed with recurrence

of thrombosis at admission to the study and one patient who developed recurrence of thrombosis three days later.

A total of 45 patients were recruited and only 35 patients (PV n=24 females n=3; ET n=11 females n=4) who fulfilled the inclusion criteria was admitted into the study. Their age ranged between 14 years and 79 years (mean 52.0 ± 15.1 years). Their characteristics are shown in **Tables 1 and 2**

Table 1. Characteristics of patients with PV and ET

	PV	ET	PV/ET
N	24	11	35
Age mean (SD) years	54.6 (12.9)	45.3 (17.4)	51.7 (14.9)
Sex: Male/Female	21/3	7/4	28/7
<i>History of thrombosis</i>			
Arterial	7	2	9
Venous	5	3	8
Arterial + venous	2	0	2
Microvascular	10	4	14
No history	0	2	2
<i>At time of Study</i>			
Clinically stable	12	8	20
Clinical symptoms	12	3	15

Table 2. Duration of diagnosis, JAK2 V617F mutation and history of thrombosis in clinically stable patients (A) and those who developed recurrent thrombosis (B).

	A		B	
	PV	ET	PV	ET
Diagnosis (years ago)	n			
<1year	0	1	0	0
1-5 years	6	3	10	1
6-10 years	2	4	1	1
>10 years	4	0	1	1
JAK2 V617F mutation	6	4	7	2
History of thrombosis (years ago) n				
1year ago	3	2	2	1
2 years ago	3	0	5	2
3 years ago	6	4	4	0
4-5 years ago	0	0	1	0
No history of thrombosis n	0	2	0	0

Blood collection

Blood sampling was performed from a clean venepuncture using the Vacutainer system (Beckton Dickinson, New Jersey, USA). About 8 mL of blood was collected and 3 mL gently mixed into citrate tubes (sodium citrate 0.109M) and the remainder blood into plain tubes for serum collection. The citrated blood samples were double spun for 15 min each time at 2000g within an hour of blood collection. Serum and plasma samples were aliquoted and kept at -70° C until assayed. Blood sampling was performed only at admission to the study; in recurrence of thrombosis blood sampling was performed post-diagnosis at between 1 to 5 days (n=8), 10-20 days (n=5), and 30 days (n=1) except for one blood sampling was performed 3 days before diagnosis. Most of the patients were on aspirin treatment regime.

Laboratory assays

The following assays were performed: JAK2 V617F mutation (IPSOGEN JAK2 MutantQuant Kit) was performed at the Molecular Diagnostic Centre, National University Health System (NUHS), Yong Loo Lin School of Medicine, National University of Singapore. Serum VEGF Immunoassay (R&D systems, Minneapolis, USA) and beta Thromboglobulin (βTG) (Asserachrome Kit, Stago, France). Von Willebrand Factor (VWF), in-house modified Elisa method using International Standard from National Biological Standards and Control (NIBSC), Potters Bar, Hertfordshire, UK, Fibrinogen (Clauss method) using international standard from NIBSC, plasma D-dimer (Zymu D-dimer Kit, France). The above tests were performed at the Coagulation Laboratory, Department of Obstetrics & Gynaecology, NUHS.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS17; SPSS Inc, Chicago, IL, USA) was used to perform the statistical analysis. Non-parametric Mann Whitney U test was performed together with independent

t-test to evaluate patients under treatment who were clinically stable and those who developed clinical symptoms of recurrence of thrombosis at the time of admission to the study. Pearsons correlation analysis was used to determine for correlation between elevated D-dimer against VEGF seen in recurrence of thrombosis. A *P* value of less than 0.05 was considered statistically significant.

Results

In total, 35 patients who fulfilled the WHO defined criteria for PV/ET was studied. Their characteristics with history of thrombotic episodes are shown in **Tables 1 and 2**, VEGF and haemostatic parameters in **Table 3**. The statistics of evaluating PV/ET patients who were clinically stable and those who developed clinical symptoms of recurrence of thrombosis are shown in **Table 4**. The scatter plots of VEGF and D-dimer levels between clinically stable and recurrent thrombosis patients are shown in **Figure 1** and the correlation between elevated D-dimer and VEGF levels in patients who developed recurrence of thrombosis in **Figure 2**.

Characteristics of patients studied

The mean age of the patients was 51.7 ± 14.9 years and ranged between 14 and 79 years. The ages between PV and ET with their history of thrombotic episodes are shown in **Table 1**. All patients were diagnosed with PV/ET between six months and ten years (n=29) and greater than ten years (n=6) (**Table 2**). Past history of thrombosis was recorded between one to seventeen years post diagnosis of PV/ET, one to five years ago in PV (100%, 24/24) and ET (81.8%, 9/11), of patients except for 2 patients (ET) who were clinically stable and diagnosed two and six years ago. Twenty patients were considered clinically stable (PV n=12; ET n=8) and 15 developed clinical symptoms of recurrence of thrombosis (PV n=12, ET n=3) at the time of study: microvascular

Table 3. VEGF and haemostatic parameters in PV/ET patients (n=35)

	Mean (SD)	Range	Normal Reference
VEGF pg/mL	202.4(164.1)	24.4 – 529.8	<115.0
D-dimer ng/mL	295.6 (179.4)	49.0 - 825.0	<400.0
TAT-complex µg/L	2.80 (1.74)	1.01 - 9.13	<2.0
vWF IU/mL	1.20 (0.43)	0.49 – 2.41	<1.5
Fibrinogen g/L	3.47 (1.71)	1.00 – 8.00	2.00 – 4.00
β-TG IU/mL	799.3 (883.8)	105.2 – 3987.0	<40.0

Table 4. Statistics of parameters studied in PV/ET patients who are clinically stable (A) compared to those who developed recurrent thrombosis (B).

n	A	B	P
	20	15	
Age mean (SD) years	47.7 (17.7)	57.1 (7.5)	0.07
VEGF mean (SD) µg/mL	150.7 (134.0)	271.3 (179.2)	0.06
D-dimer mean (SD) ng/mL	199.4 (78.2)	423.9 (197.4)	<0.001
TAT-complex mean (SD) µg/L	2.6 (2.1)	3.1 (1,2)	0.07
VWF mean (SD) IU/mL	1.10 (0.45)	1.34 (0.37)	0.05
Fibrinogen mean (SD) g/L	2.78 (0.89)	4.41 (2.11)	0.005
β-TG IU/mL	630.5 (531.5)	1024.0 (1191.0)	0.83

(B): MVD 5; DVT 3; CHD 2; stroke 4; PE 1

disease (MVD) 5, Deep Vein thrombosis (DVT) 3, Coronary heart disease (CHD) 2, stroke 4 and Pulmonary Embolism (PE) 1. In clinically stable patients 16 received aspirin 100 mg and one Clopidogrel 75mg with phlebotomy treatment in 11 patients and 15 patients received both phlebotomy and aspirin at the time of blood sampling. In patients who developed recurrence of thrombosis (n=15) seven received aspirin and one Clopidogrel while eleven

had phlebotomy treatment. In 3 patients diagnosed with DVT (PV n=2, ET n=1) low molecular weight heparin was given in addition to aspirin (n=2) and clopidogrel (n=1).

VEGF and haemostatic parameters

No statistically significant differences in the parameters studied between PV and ET were seen and therefore their data were combined for anal-

Figure 1: D-dimer and VEGF levels in recurrence of thrombosis (n=15) and clinically stable states (n=20) in patients with PV/ET.

Figure 2: Correlation of elevated D-dimer against VEGF in recurrence of thrombosis.

ysis. Elevated levels of VEGF (mean 202.4 ± 164.1 pg/mL), TAT-complex (mean 2.80 ± 1.74 ug/mL) and β -TG (mean 799.3 ± 883.8 IU/mL) were evident in PV/ET patients compared to normal reference. Although D-dimer, VWF and fibrinogen mean levels were within normal reference range their ranges were widely variable (**Table 3**). Elevated VEGF levels were seen in 20/35 (57.1%) patients, D-dimer 10/35 (28.6%), Fibrinogen 9/35 (25.7%) and VWF 8/35 (23.6%) patients. The age of patients at 65 years old and above was 20% (7/35). The reference ranges quoted was from the manufacturer's normal reference and therefore, no age-matched to the study population was done (**Table 3**).

Statistics of parameters studied in PV/ET patients who were clinically stable ($n=20$) were compared to those who had developed clinical symptoms of recurrence of thrombosis ($n=15$) at admission to the study (**Table 4**). Recurrence of thrombosis occurred in 13.3% (2/15) of patients at 65 years old and above. The mean age was higher for patients who experienced a recurrence of thrombosis (mean 57.1 ± 7.5 years vs mean 47.7 ± 17.7 years) relative to those that were clinically stable but this observation did not reach statistical significance ($P=0.07$). Statistically significant elevated D-dimer ($P < 0.001$) and fibrinogen ($P=0.005$) were seen in patients with recurrence of thrombosis compared to patients who were clinically stable. Higher mean trends were seen for TAT-complex ($P=0.07$) and VWF ($P=0.05$) in recurrence of thrombosis but they did not reach statistical significance. No significant differences were also seen for VEGF and Beta-TG levels even though the levels were elevated above the normal reference in both groups (**Table 4**). Elevated VEGF levels were seen in 45.0% (9/20) of clinically stable patients and 73.3% (11/15) in patients with recurrence of thrombosis. Clinically stable patients had no elevated D-dimer levels whilst in the recurrence of thrombosis group 66.7% (10/15) patients had elevated levels (>400 ng/mL). The scatter plot for VEGF and D-dimer in both groups is shown in Fig. 1.

Correlation between VEGF and D-dimer levels

Statistically significant correlation between elevated D-dimer (10/15) and VEGF levels in recurrence of thrombosis group were seen ($r=0.8540$, $P=0.002$) (Fig. 2). D-dimer was not elevated in clinically stable patients even though 45% (9/20) had elevated VEGF levels (>115 pg/mL).

JAK2 V617F mutation

JAK2 V617F mutation was confirmed in 54.2% (13/24) patients with PV and in 54.5% (6/11) patients with ET. The overall mutation seen in PV/ET was 54.3%. No statistically significant differences in the parameters studied were seen between JAK2 V617F positive and negative patients except for VWF ($P=0.04$) in negative patients (mean 1.32 ± 0.35 IU/mL vs mean 1.10 ± 0.47 IU/mL) in JAK2 V617F positive mutation.

Discussion

The thrombotic risk in PV and ET is more pronounced in those over 60 years old and with a history of previous thrombosis as has been previously reported (3, 4). PV and ET have high predisposition to thrombosis with a rate of major thrombosis as high as about 50% (3, 4). Incidence of recurrent thrombosis was 5% in those below 65 years old and 10.9% in those above 65 years old (26) whilst another retrospective study the rate was 15% (27). Overall incidence of recurrence is 5.9% for those below 60 years old and risk factor of 1.7 fold increase in those greater than 60 years old (28). In our study recurrence occurred in 42.9% (15/35) of which 14.7% (2/15) were over 65 years of age. VEGF measurement have been suggested to assess high and low risk PV patients (18). In this retrospective study of 35 patients diagnosed with PV/ET, 100% patients with PV had history of thrombosis between year one to seventeen while in ET it was 81.8%

between year one to twelve from diagnosis, the incidence for PV/ET was 94.3% which was much higher than reported (3, 4). At admission to the study, 15 patients (42.9%), PV n=12, ET n=3 were reported to have developed recurrence of thrombosis between one and five years from last thrombotic episode whilst the remaining 20 patients were clinically stable including two (ET) patients with no history of thrombosis. This recurrence of thrombosis was higher than reported (26, 8). A hypercoagulable state for this population was evident from elevated D dimer and fibrinogen levels seen in patients with recurrence of thrombosis, which is in agreement with the study of Wautier and co-workers (9). In support of this observation, D-dimer levels were not elevated in clinically stable patients. VEGF levels were elevated in 45.0% of patients who were clinically stable and 73.3% in patients who experienced recurrence of thrombosis. The elevated levels are of concern as they are known as a pro-coagulant, inducing endothelial activation and thrombin formation (16, 17). Markedly increased D-dimer was found in PV/ET and no differences of coagulation activation markers was found in relation to JAK2-V617F mutational status (29). JAK2 V617F mutation was confirmed in 54.2% PV and 54.5% ET patients. We did not find any significant differences in the plasma levels of D-dimer and coagulation activation markers between JAK2 V617F positive and negative patients even though JAK2 V617F mutation associated with thrombosis (16, 17). Considerable variation of JAK2 V617F mutation frequency have been reported with PV of between 65- 97% and ET 23-57% (30) and PV 95%, ET 50-60% (31). The frequency of the mutation in PV patients (54.2%) in our study is rather low, whilst it was comparable to the published literature in ET patients (54.5%). The published reports for JAK2 V617F frequency are for Caucasian population and the frequency in Asians especially with PV need to be further investigated. Possible variations in mutation discrepancies

observed in PV include assay sensitivity, diagnostic accuracy and treatment effect (32) have been suggested.

Conclusion

In conclusion, plasma VEGF levels were elevated (57.1%) in PV/ET patients relative to controls, although the difference was not significant. Similarly, plasma D-dimer and fibrinogen levels were significantly higher in patients with recurrence of thrombosis relative to clinically stable patients, whereas plasma VEGF levels were non-significantly elevated. Elevated plasma D-dimer seen in patients with recurrence of thrombosis was found to correlate significantly with elevated plasma VEGF levels. VEGF and D-dimer measurements have been used in clinical practice to determine the risk of PV/ET patients with vascular complications.

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Conflict of interest

The authors state they have no conflict of interest.

References

1. Vannucchi MA, Guglielmelli P, Tefferi A. Advances in understanding and management of myeloproliferative neoplasms. *A Cancer J for Clinicians* 2009; 59:171-91.
2. Campbell PJ, Green AR. Mechanisms of diseases: the myeloproliferative disorders. *N Engl J Med* 2006; 355:2452-66.
3. Elliot MA, Tefferi A. Thrombosis and haemorrhage in polycythemia vera and essential thrombocythemia. *Br J Haematol* 2005; 128:275-90.
4. Cortelazzo S, Viero P, Finazzi G, D'Emilio A, Rodeghiero F, Barbui T. Incidence and risk factors for thrombotic complications in a historical cohort of 100 patients with essential thrombocythemia. *J Clin Oncol* 1990; 8:556-62.
5. Tefferi A, Vainchecker W. Myeloproliferative neoplasms in molecular pathophysiology, essential clinical understanding and treatment strategies. *J Clin Oncol* 2011; 29:573-82.
6. Robertson B, Urquhart C, Ford I, Townend J, Watson GH, Vickers MA et al. Platelet and coagulation markers in myeloproliferative diseases: relationships with JAK2V617F status, clonality, and antiphospholipid antibodies. *Journal of Thrombosis and Haemostasis* 2007; 5:1679-85.
7. Vianello F, Battisti A, Cella G, Marchetti M, Falanga A. Defining the thrombotic risks in patients with myeloproliferative neoplasms. *The Scientific World J* 2011; 11:1131-37.
8. Landolfi R, Di Gennaro L. Pathophysiology of thrombosis in myeloproliferative neoplasms. *Haematologica* 2011; 96(2):183-6.
9. Wautier MP, El Nemer W, Gane P, Rain JD, Cartron JP, Colin Y, Le Van Kim C, Wautier JL. Increased adhesion to endothelial cells of erythrocytes from patients with polycythemia vera is mediated by laminin alpha5 chain and Lu/BCAM. *Blood* 2007; 110:894-901.
10. Casini A, Fontana P, Lecompte TP. Thrombotic complications of myeloproliferative neoplasms: risk assessment and risk-guided management. *J Thromb Haemost* 2013; 11:1215-27.
11. Connolly DT. Vascular permeability factor: a unique regulator of blood vessel function. *J Cell Biochem* 1991; 47:219-23.
12. Mohle R, Green D, Moore MAS, Nachman RL, Rafii S. Constitutive production of thrombin-induced release of vascular endothelial growth factor by human megakaryocytes and platelets. *Proc Natl Acad Sci* 1997; 94:663-8.
13. Raimondo DF, Giuseppe PA, Stefano M, Rosario G. Angiogenesis in chronic myeloproliferative diseases. *Acta Haematologica* 2001; 106:177-83.
14. Stamatia T, Timoleon V, Sofia V, Vassilios P, Konstantinos T, Athanassians V et al. Elevated levels of serum vascular endothelial growth factor in patients with polycythemia vera. *Acta Haematologica* 2003; 110:16-9.
15. Verheul HM, Hoekman K, Luyckx-de Bakker S et al. Platelet transporter of vascular endothelial growth factor. *Clinical Cancer Research* 1997; 3:2187-90.
16. Dvorak HF, Senger RD, Dvorak AM, Harvey VS, McDonagh J. Regulation of extravascular coagulation by microvascular permeability. *Science (Washington DC)* 1985; 227:1059-61.
17. Zucker S, Mirza H, Conner CE. Vascular endothelial growth factor induces tissue factor and matrix metalloproteinase production in endothelial cells: conversion of prothrombin to thrombin results in progelatinase-A activation and cell proliferation. *Int J Cancer* 1998; 75:780-6.
18. Cacciola RR, Di Francesco E, Glustolisi R, Cacciola E. Elevated serum vascular endothelial growth factor levels in patients with polycythemia vera and thrombotic complications. *Haematologica* 2002; 87:774-5.
19. Goodacre S, Sampson FC, Sutton AJ, Mason S, Morris F. Variation in the diagnostic performance of D-dimer for suspected deep vein thrombosis. *Q J Med* 2005; 98:513-27.
20. Kovacs MJ, MacKinnon C, Ginsberg J, Wells PS. A comparison of three rapid D-dimer methods for the diagnosis of venous thromboembolism. *Br J Haematol* 2001; 115:140-4.
21. Prisco D, Grifori E. The role of D-dimer testing in patients with suspected venous thromboembolism. *Semin Thromb Hemost* 2009; 35:50-9.
22. Kleinegris MC, Ten Cate H, Ten Cate HAJ. 2013. D-dimer as a marker for cardiovascular and arterial thrombotic events in patients with peripheral arterial disease. A systematic review. *Thromb Haemost* 2013; 110(2):233-43.
23. Gomez K, Tuddenham EGD, McVey JH. 2011. Normal Haemostasis. *Post Graduate Haematology* 2011; 6th ed:747-71. Wiley Blackwell.
24. Landolfi R and Marchioli R et al. Efficacy and safety of low dose aspirin in polycythemia vera. *The New England Journal of Medicine* 2004; 350:114-24.
25. Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point of care diagnostic algorithms. *Leukemia* 2008; 22:14-22.
26. Marchioli R, Finazzi G, Landolfi R, Kutti J, Gisslinger H, Patrono C et al. Vascular and neoplastic risk in a large cohort of patients with polycythemia vera. *J Clin Oncol* 2005; 23:2224-32.
27. Ruggeri M, Gisslinger H, Tosetto A, Rintelen C, Mannhalter C, Pabinger I, et al. Factor Leiden mutation carriership and venous thromboembolism in polycythemia vera and essential thrombocythemia. *Am J Hematol* 2002; 71:1-6.

28. De Stefano V, Tommaso Z, Rossi E, Vannucchi AM, Ruggeri M, Elli E, et al. Recurrent thrombosis in patients with polycythemia vera and essential thrombocythemia: incidence, risk factors, and effects of treatments. *Haematologica* 2008; 93:372-80.
29. Trelinski J, Wierzbowska A, Krawczynska A, Sakowicz A, Pietrucha T, Smolewski P, Robak T, Chojnowski K. Circulating endothelial cells in essential thrombocythemia and polycythemia vera: correlation with JAK2-V617F mutational status, angiogenic factors and coagulation activation markers. *Int J Hematol* 2010; 91:792-8.
30. Nelson ME, Steensma DP. JAK2 V617 in myeloid disorders: what do we know now, and where are we headed? *Leuk Lymphoma* 2006; 47:177-94.
31. Cross NC. Genetic and epigenetic complexity in myeloproliferative neoplasms. *Hematology Am Soc Hematol Educ Program* 2011; 2011:208-14.
32. Jones AV, Silver RT, Waghorn K, Cutis C, Kreil S, Zoi K et al. Minimal molecular response in polycythemia vera patients treated with imatinib or interferon alpha. *Blood* 2006; 107:3339-41.

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