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Contribution of cardiovascular risk factors in the thrombotic complications of essential thrombocythaemia: a Hungarian single-institute retrospective analysis

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Abstract. – OBJECTIVE: Essential thrombocythaemia (ET) is a myeloproliferative neoplasm in which there is an increased risk of thrombotic complications. The conventional thrombosis risk assessment of these patients is based on an age over 60 and a history of thrombosis. The aim of this report is to analyse the contribution of cardiovascular risk (CV) factors as possible additional thrombotic risk factors in the thrombotic complications seen in ET.

PATIENTS AND METHODS: One hundred and one ET patients (72 females and 29 males with a median age of 61 years) were enrolled between 1999 and 2011. Mann-Whitney and multivariate binary logistic regression tests were performed. The Kaplan-Meier method followed by the log-rank test was used to evaluate the probability of thrombosis-free survival.

RESULTS: The presence of one or two or more CV risk factors significantly increased the risk of thrombosis. Separately, the contribution of high blood pressure and hyperlipidaemia proved to be influential, whereas tobacco use, diabetes mellitus and obesity were not significant. Significant differences were revealed in the probability of thrombosis-free survival between patients without CV risk factors and those with at least one CV risk factor, and between those with at most one CV risk factor and those with two or more CV risk factors.

CONCLUSIONS: On the basis of the results on the current cohort, it is suggested that CV risk factors may influence the thrombotic complications in ET.

Key Words:
Thrombosis, Cardiovascular risk factors, Hypertension, Hyperlipidaemia, JAK2 V617F mutation, Essential thrombocythaemia, Myeloproliferative neoplasm.

Introduction

Essential thrombocythaemia (ET) is a chronic Philadelphia chromosome-negative myeloproliferative neoplasm, characterized by the excessive proliferation of megakaryocytes in the bone-marrow and the overproduction of circulating platelets in the periphery[1]. The most important complications that can exert a major effect on the morbidity of ET patients are thrombo-haemorrhagic events. The risk of major arterial (e.g. myocardial infarction, ischaemic stroke or a transient ischaemic attack) and venous thrombotic events (e.g. cerebral sinus and venous thrombosis or deep venous thrombosis) ranges between 11% and 25%[2-10]. The main goal of the current therapy in ET is prevention of the thrombotic complications, tailored to the currently accepted thrombosis risk management strategy of ET[11]. This is based on only two risk factors (an age over 60 and a history of thrombosis). Besides the accepted and well-known risk factors in the thrombosis-risk assessment of ET, other possible additional thrombotic risk factors are under active investigaton, such as leukocytosis, cardiovascular (CV) risk factors, the *JAK2 V617F* mutation and the relationship between vascular events and oxidative status parameters[6-8,12-17]. Although CV risk factors are "traditional" ones, their contributions to the thrombotic complications in ET have been investigated only recently[13].

The main object of our study was an analysis of the contributions of the main CV risk factors (high blood pressure, hyperlipidaemia, tobacco use, diabetes mellitus and obesity) to the thrombotic complications of the patients diagnosed with ET.

Patients and Methods

Patients and Data Collection

Between 1999 and 2011, 101 ET patients (72 females and 29 males with a median age of 61

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years, range 20-95 years) diagnosed at the 2nd Department of Internal Medicine and Cardiology Centre were enrolled. Through use of the clinical centre data files, all the haematological results on these patients were reviewed with the approval of the Regional and Institutional Human Medical Biological Research Ethics Committee. Informed consent was not required. The study was conducted according to the Declaration of Helsinki.

The haematological management strategy was based on risk-oriented recommendations: antiplatelet therapy if it was necessary for the low-risk patients (age <60 years, without a prior thrombotic event), and a cytoreductive drug (hydroxyurea) alone or in combination with antiplatelet medication for the high-risk patients (age >60 years and/or with a prior thrombotic event)[18]. The main demographic and clinicohaematological characteristics of the enrolled patients are presented in Table I.

Laboratory Methods

Routine blood analysis with automated blood count equipment was performed as part of the diagnostic protocol. For screening of the *JAK2 V617F* mutation by an allele-specific PCR method, DNA was isolated from EDTA-stabilized peripheral blood samples[19].

Statistical Analysis

Data were collected with Microsoft Office Excel, and statistical analysis was performed with Statsoft Statistica v 9.1 (Statsoft) and SPSS 17 software (IBM).

Mann-Whitney and multivariate binary logistic regression tests were performed in the cases of the presence or absence of thrombotic events after the diagnosis of ET, comparing the overall and partial effects of series variables: 1) CV risk factors: hypertension (>140/80 mmHg), tobacco use, diabetes mellitus, hyperlipidaemia (hypercholesterolaemia or hypertriglyceridaemia or both), and obesity (BMI >30 kg/m^2), 2) the *JAK2 V617F* mutation, 3) leukocytosis, 4) conventional risk factors in ET: age >60, or a history of thrombosis, and 5) therapy: cytoreductive (hydroxyurea) and antiplatelets.

Due to the relatively low number of patients, besides the usual 5% level of significance, we also considered the 10% level.

The Kaplan-Meier method was used, followed by the log-rank test, to evaluate the probability of the thrombosis-free survival of the patients in this cohort[20]. ET patients without CV risk factors were compared with ET patients with at least one CV risk factor, and ET patients with at most one CV risk factor were compared with ET patients with two or more CV risk factors.

Table I. Demographic and clinicohaematological characteristics of the study population.

Characteristics of the study population	Data
Males (N, [%])	29 (28.7)
Females (N, [%])	72 (71.3)
Age at diagnosis, median (years) (range)	61 (20-95)
Median leukocyte count at diagnosis (giga/L) (range)	9.4 (4.5-34)
Median platelet count at diagnosis (giga/L) (range)	664 (78-2240)
Prior major* vascular events	38
Follow-up major* vascular complications	16
JAK2 V617F-positive cases (N, [%])	61 (60.4)
Conventional risk factors in ET	
Age > 60 years (N, [%])	52 (51.5)
Prior thrombotic events	38
Distribution of cardiovascular risk factors (N, [%])	
High blood pressure	47 (46.5)
Hyperlipidaemia	13 (12.9)
Diabetes mellitus	7 (6.9)
Tobacco use	15 (14.9)
Obesity (BMI > 30 kg/m^2)	16 (15.8)
Treatment (N, [%])	
Antiplatelets	43 (42.6)
Hydroxyurea (alone or in combination with antiplatelets)	42 (41.6)

*Major thrombotic events were cerebrovascular (ischaemic stroke or a transient ischaemic attack), cardiovascular (myocardial infarction) and venous thrombotic events (deep vein thrombosis or a pulmonary embolism); microvascular events were excluded from the analyses.

Table II. Mann-Whitney test and multivariate binary logistic regression results relating to the overall and partial effects on the probability of thrombotic events of the newly suggested risk factors, conventional risk factors and administered therapy in the comparison of ET patient subgroups who had or who had not suffered thrombotic events in the follow-up period.

Comparison of ET patients who had or who had not suffered thrombotic events in the follow-up period				
	Mann-Whitney univariate analysis	Multivariate binary logistic regression analysis		
Variables	*p*	*p*	*odds ratio*	*95% CI*
Newly suggested but not conventional risk factors in ET				
Cardiovascular risk factors				
High blood pressure	*0.092	0.280	2.174	0.531-8.899
Hyperlipidaemia	**0.011	*0.097	3.511	0.797-15.470
Tobacco use	0.545	0.971	0.971	0.193-4.890
Diabetes mellitus	0.965	0.806	0.735	0.063-8.555
Obesity	0.634	0.821	0.835	0.175-3.990
The presence of two or more CV risk factors	**0.025	*0.089	2.862	0.852-9.614
The presence of only one CV risk factor	*0.096		ND	
JAK2 V617F mutation	0.651	0.903	1.083	0.301-3.891
Leukocytosis White blood cell count at least 11.1 giga/L	0.525		ND	
Conventional risk factors				
Prior thrombotic events	*0.066	0.170	2.406	0.686-8.437
Age > 60 years	0.877	0.763	0.815	0.217-3.067
Therapy				
Hydroxyurea	0.319		ND	
Antiplatelets	0.730		ND	

Significant differences at 10% are marked by *; at 5% by **; CV: cardiovascular risk factors; ND: not determined.

Results

The presence of one CV risk factor (univariate: $p = 0.096$) or two or more CV risk factors (univariate: p=0.025; multivariate: odds ratio: 2.862, 95% CI 0.852-9.614; p=0.089 at 10%) at the time of the haematological diagnosis of ET significantly increased the risk of thrombotic events during the haematological follow-up period. There was a significant overall association between an enhanced thrombotic tendency and a high blood pressure (univariate: p=0.092; multivariate: odds ratio: 2.174, 95% CI 0.531-8.899; p=0.280), and significant partial and overall effects in the case of hyperlipidaemia (univariate: p=0.011; multivariate: odds ratio: 3.511, 95% CI 0.797-15.47; p=0.097 at 10%). Tobacco use (univariate: p=0.545; multivariate: odds ratio: 0.971 95% CI 0.193-4.890; p=0.971), diabetes mellitus (univariate: p=0.965; multivariate: odds ratio: 0.735 95% CI 0.063-8.555; p=0.806) and obesity (univariate: p=0.634; multivariate: odds ratio: 0.835, 95% CI 0.175-3.990; p=0.821) were not associated significantly with an increased risk of thrombosis (Table II).

As concerns the age, the *JAK2 V617F* mutation and leukocytosis, univariate and/or multivariate statistical analyses revealed non-significant tendencies from the aspects of the presence of the *JAK2 V617F* mutation (univariate: p=0.651; multivariate: odds ratio: 1.083, 95% CI 0.301-3.891; p=0.903) and an age >60 years (univariate: p=0.877; odds ratio: 0.815, 95% CI 0.217-3.067; p=0.763). The role of leukocytosis at the time of the haematological diagnosis of ET (white blood cell count at least 11.1 giga/L) in futher thrombotic complications was likewise not significant (univariate: p=0.525). However, the partial effect of the reported prior vascular events in the possible prediction of further thrombosis was significant (univariate: p=0.066; multivari-

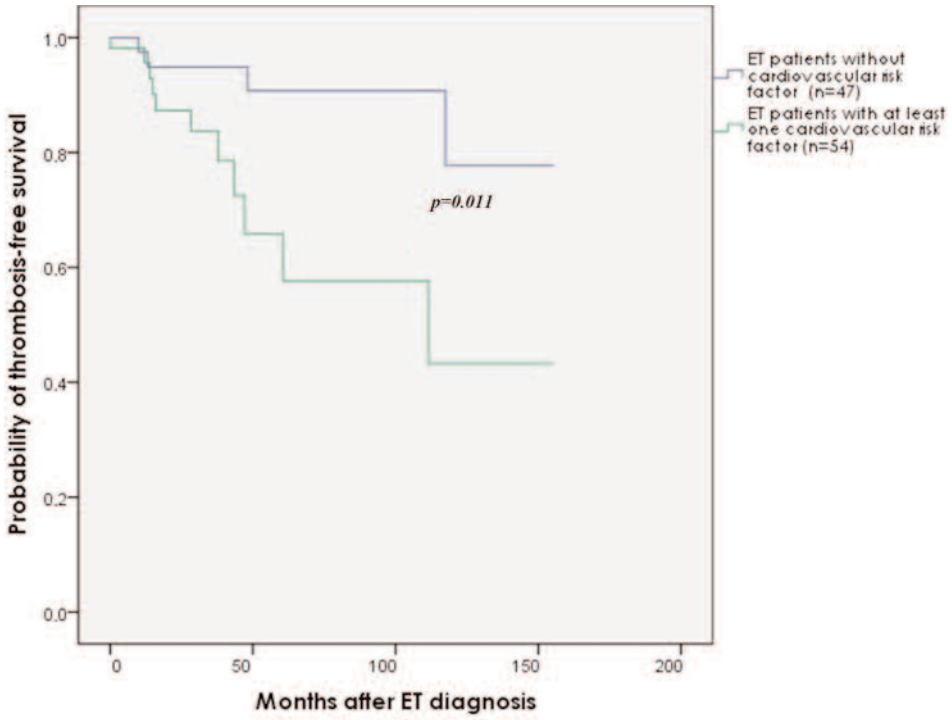

Figure 1. Probability of thrombosis-free survival in the haematological follow-up period in the subgroups of ET patients without CV risk factor and ET patients with at least one CV risk factor.

ate: odds ratio: 2.406, 95% CI 0.686-8.437; p=0.170) (Table II).

The administered therapy was included in the analysis in order to consider its potential influence on the thrombotic events in the follow-up period. Differences were observed between the patients treated with cytoreductive (univariate: p=0.319) or antiplatelet therapy (univariate: p=0.730) in the two subgroups, depending on the presence or absence of major thrombotic events, but these differences were not significant (Table II). A limitation of our study is its retrospective design. The question arises as to whether the antithrombotic treatment and cytoreductive therapy affect the prognosis, with the differences observed possibly being underestimated.

To compare the thrombosis-free survival of the patients in the presence or absence of the investigated CV risk factors, Kaplan-Meier curves and log rank tests (Mantel-Cox) were utilized. A significant difference was observed between the thrombosis-free survival of the observed ET patients without CV risk factors (n=47) and those with at least one CV risk factor (n=54) (p=0.011) (Figure 1). A significant difference was also seen between the ET patients with at most one CV risk factor (n=77) and those with two or more CV risk factors (n=24) (p=0.002) (Figure 2).

Discussion

There is an increased risk of arterial (e.g. myocardial infarction, ischaemic stroke or a transient ischaemic attack) and venous thrombotic events (e.g. deep venous thrombosis) in ET[6,11]. The currently accepted thrombosis risk management strategy of ET is based on only two risk factors (an age over 60 years and a prior thrombotic event)[13,18,21]. For a more accurate thrombosis risk-guided management, the predictive potential of the *JAK2 V617F* mutation and CV risk factors is considered to be significant, while the role of leukocytosis at the time of ET diagnosis are still controversial[6-8,12-15,22-25]. In a large multicentric study, the contribution of at least one CV risk factor, i.e hypertension and/or diabetes and/or active tobacco use, was suggested[8]. Furthermore, the new score system, IPSET (International Prognostic Score of Thrombosis for ET), has already incorporated the CV risk factors[13]. In contrast, the 2015 annual clinical updates in

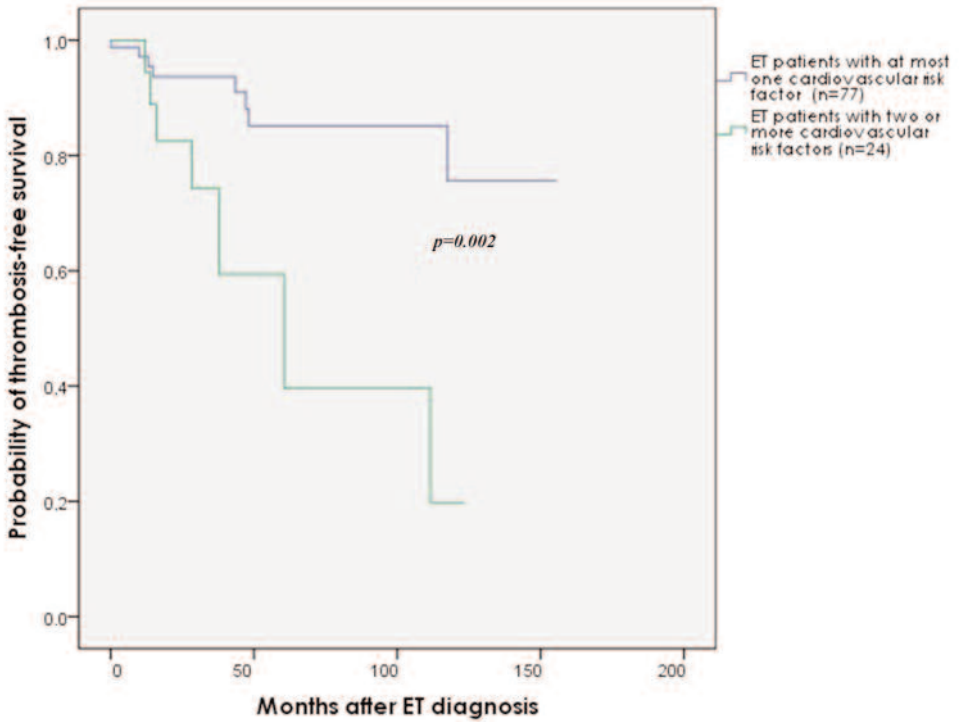

Figure 2. Probability of thrombosis-free survival in the haematological follow-up period in the subgroups of ET patients with at most one CV risk factor and ET patients with two or more CV risk factors.

haematological malignancies still does not consider changes in the currently used classical two-categorical risk stratification model[11]. The results on the current patient population demonstrated that the presence of one, or two or more CV risk factors significantly increased the risk of thrombosis during the course of the neoplasm. Our detailed statistical analyses revealed that the most important CV risk factors contributing to an enhanced thrombotic tendency were a high blood pressure and hyperlipidaemia. The probability of thrombosis-free survival was also significantly different between the currently analysed patients without CV risk factors and those with at least one CV risk factor, and between patients with at most one CV risk factor and those with two or more CV risk factors. The current study did not confirm that the presence of leukocytosis at the time of ET diagnosis contributes significantly to subsequent thrombotic complications. No significant impact of the *JAK2 V617F* mutation in the subsequent thrombotic events (even with a consideration of the CV risk factors) could be proven in this cohort. The role of this mutation in the thrombotic complications of ET was analyzed in detail earlier, but without the contributions of CV risk factors[16].

Conclusions

We suggest that identification and consideration of the CV risk factors are important for a more accurate thrombosis risk-guided management.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW (eds). WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Oxford University Press, 2008. ISBN 978-92-832-2431-0.

2) Barbui T, Thiele J, Passamonti F, Rumi E, Boveri E, Ruggeri M, Rodeghiero F, d'Amore ES, Randi ML, Bertozzi I, Marino F, Vannucchi AM, Antonioli E, Carrai V, Gisslinger H, Buxhofer-Ausch V, Mullauer L, Carobbio A, Gianatti A, Gangat N, Hanson CA, Tefferi A. Survival and disease progression in essential thrombocythemia are significantly influenced by accurate morphologic diagnosis: an international study. J Clin Oncol 2011; 29: 3179-3184.

3) Chim CS, Kwong YL, Lie AK, Ma SK, Chan CC, Wong LG, Kho BC, Lee HK, Sim JP, Chan CH, Chan JC, Yeung YM, Law M, Liang R. Long-term outcome of 231 patients with essential thrombocythemia: prognostic factors for thrombosis, bleeding, myelofibrosis, and leukemia. Arch Intern Med 2005; 165: 2651-2658.

4) 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.

5) Tefferi A, Thiele J, Orazi A, Kvasnicka HM, Barbui T, Hanson CA, Barosi G, Verstovsek S, Birgegard G, Mesa R, Reilly JT, Gisslinger H, Vannucchi AM, Cervantes F, Finazzi G, Hoffman R, Gilliland DG, Bloomfield CD, Vardiman JW. Proposals and rationale for revision of the World Health Organization diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis: recommendations from an ad hoc international expert panel. Blood 2007; 110: 1092-1097.

6) Falanga A, Marchetti M. Thrombotic disease in the myeloproliferative neoplasms. Hematology Am Soc Hematol Educ Program 2012; 2012: 571-81.

7) Tefferi A, Elliott M. Thrombosis in myeloproliferative disorders: prevalence, prognostic factors, and the role of leukocytes and JAK2V617F. Semin Thromb Hemost 2007; 33: 313-320.

8) Carobbio A, Thiele J, Passamonti F, Rumi E, Ruggeri M, Rodeghiero F, Randi ML, Bertozzi I, Vannucchi AM, Antonioli E, Gisslinger H, Buxhofer-Ausch V, Finazzi G, Gangat N, Tefferi A, Barbui T. Risk factors for arterial and venous thrombosis in WHO-defined essential thrombocythemia: an international study of 891 patients. Blood 2011; 117: 5857-5859.

9) Bazzan M, Tamponi G, Schinco P, Vaccarino A, Foli C, Gallone G, Pileri A. Thrombosis-free survival and life expectancy in 187 consecutive patients with essential thrombocythemia. Ann Hematol 1999; 78: 539-543.

10) Wolanskyj AP, Schwager SM, McClure RF, Larson DR, Tefferi A. Essential thrombocythemia beyond the first decade: life expectancy, long-term complication rates, and prognostic factors. Mayo Clin Proc 2006; 81: 159-166.

11) Tefferi A, Barbui T. Polycythemia vera and essential thrombocythemia: 2015 update on diagnosis, risk-stratification and management. Am J Hematol 2015; 90: 162-173.

12) Tefferi A. Polycythemia vera and essential thrombocythemia: 2012 update on diagnosis, risk stratification, and management. Am J Hematol 2012; 87: 284-293.

13) Barbui T, Finazzi G, Carobbio A, Thiele J, Passamonti F, Rumi E, Ruggeri M, Rodeghiero F, Randi ML, Bertozzi I, Gisslinger H, Buxhofer-Ausch V, De Stefano V, Betti S, Rambaldi A, Vannucchi AM, Tefferi A. Development and validation of an International Prognostic Score of thrombosis in World Health Organization-essential thrombocythemia (IPSET-thrombosis). Blood 2012; 120: 5128-5133.

14) Tefferi A, Barbui T. Personalized management of essential thrombocythemia-application of recent evidence to clinical practice. Leukemia 2013; 27: 1617-1620.

15) Lee HS, Park LC, Lee EM, Lee SJ, Shin SH, Im H, Do KM, Kim EJ, Ye BJ, Song MK, Kim SH, Lee SM, Lee WS, Kim YS. Incidence rates and risk factors for vascular events in patients with essential thrombocythemia: a multicenter study from Korea. Clin Lymphoma Myeloma Leuk 2012; 12: 70-75.

16) Posfai E, Marton I, Kiraly PA, Kotosz B, Kiss-Laszlo Z, Szell M, Borbenyi Z. JAK2 V617F, MPL, and CALR mutations in essential thrombocythaemia and major thrombotic complications: a single-institute retrospective analysis. Pathol Oncol Res 2015 Jan 10. [Epub ahead of print].

17) Durmus A, Mentese A, Yilmaz M, Sumer A, Akalin I, Topal C, Alver A. Increased oxidative stress in patients with essential thrombocythemia. Eur Rev Med Pharmacol Sci 2013; 17: 2860-2866.

18) Barbui T, Barosi G, Grossi A, Gugliotta L, Liberato LN, Marchetti M, Mazzucconi MG, Rodeghiero F, Tura S. Practice guidelines for the therapy of essential thrombocythemia. A statement from the Italian Society of Hematology, the Italian Society of Experimental Hematology and the Italian Group for Bone Marrow Transplantation. Haematologica 2004; 89: 215-232.

19) Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, Vassiliou GS, Bench AJ, Boyd EM, Curtin N, Scott MA, Erber WN, Green AR, Cancer Genome P. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 2005; 365: 1054-1061.

20) AB.Hill, editor. A short textbook of medical statistics. London: Hodder and Stoughton, 1984; p. 170.

21) Passamonti F, Caramazza D, Mora B, Casalone R, Maffioli M. It is time to change thrombosis risk assessment for PV and ET? Best Pract Res Clin Haematol 2014; 27: 121-127.

22) Birgegard G. Pharmacological management of essential thrombocythemia. Expert Opin Pharmacother 2013; 14: 1295-1306.

23) Barbui T, Barosi G, Birgegard G, Cervantes F, Finazzi G, Griesshammer M, Harrison C, Hasselbalch HC, Hehlmann R, Hoffman R, Kiladjian JJ, Kroger N, Mesa R, McMullin MF, Pardanani A, Passamonti F, Vannucchi AM, Reiter A, Silver RT, Verstovsek S, Tefferi A, European L. Philadelphia-negative classical myeloproliferative neoplasms: critical concepts and management recommendations from European LeukemiaNet. J Clin Oncol 2011; 29: 761-770.

24) Tefferi A. Polycythemia vera and essential thrombocythemia: 2013 update on diagnosis, risk-stratification, and management. Am J Hematol 2013; 88: 507-516.

25) Andrikovics H, Szilvasi A, Meggyesi N, Kiraly V, Halm G, Lueff S, Nahajevszky S, Mikala G, Sipos A, Lovas N, Csukly Z, Matrai Z, Tamaska J, Tordai A, Masszi T. [Role of the activating mutation Val617Phe of Janus kinase 2 gene in myeloproliferative diseases and significance of its detection]. Orv Hetil 2007; 148: 203-210.