**Abstract.** – **OBJECTIVE:** In polycythaemia vera (PV) and essential thrombocythaemia (ET), the life expectancy of the patients is greatly affected by thrombotic events. An investigation was performed of the potential association of PV/ET, and thrombotic complications with cardiovascular (CV) risk factors, a leukocyte count at the haematological diagnosis > 11.1 G/L, and the JAK2V617F mutation.

**PATIENTS AND METHODS:** In the period 1998-2011, 128 women with a median age of 62 years were enrolled.

**RESULTS:** The risk of thrombotic events before the diagnosis was 32.8% (42/128), while in the follow-up period it was 10.2% (13/128). The difference in the probability of thrombosis-free survival between those with at most one CV risk factor and those with two or more CV risk factors was significant ($p = 0.005$). The presence of two or more CV risk factors (univariate: $p = 0.011$; multivariate: relative risk: 4.728, 95% CI 1.312-17.040; $p = 0.018$) significantly increased the risk of thrombosis. Univariate analyses revealed that high blood pressure ($p = 0.001$), hyperlipidaemia ($p = 0.005$) and cigarette smoking ($p = 0.051$) were associated with a significantly higher risk of thrombosis. Analyses of the influence of the leukocyte count (univariate: $p = 0.424$; multivariate: relative risk: 1.407, 95% CI 0.359-5.307; $p = 0.624$) and the JAK2V617F mutation (univariate: $p = 0.367$; multivariate: relative risk: 1.428, 95% CI 0.316-6.460; $p = 0.643$) on subsequent thrombotic complications resulted in a non-significant tendency.

**CONCLUSIONS:** Female patients who display CV risk factors (high blood pressure, hyperlipidaemia and/or cigarette smoking) and PV or ET may well be at a higher risk of thrombotic events and require special consideration as concerns as the prevention and management of thrombotic events.

**Key Words:** JAK2 V617F, Acquired thrombophilia, Chronic myeloproliferative disorder, Essential thrombocythaemia, Polycythaemia vera, Thrombosis, Cardiovascular risk factors, Woman, High blood pressure, Hyperlipidaemia.

**Introduction**

Congenital thrombophilias have been demonstrated to be risk factors for venous thrombosis, while acquired thrombophilias such as antiphospholipid antibody syndrome, hyperhomocysteaemia and polycythaemias (e.g. polycythaemia vera (PV)) may increase the risk of both arterial and venous thrombosis\(^1-3\). PV and essential thrombocythaemia (ET) are listed by the World Health Organization as chronic Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs), but they can be regarded as rare acquired thrombophilias\(^4-6\). In PV and ET, the risk of thrombohaemorrhagic complications (which ranges between 12% and 39%) is considered to be one of the most important key predictors of the otherwise quite normal life expectancy in these patients\(^7-9\). Thrombotic events related to MPNs may affect the arteries (e.g. myocardial infarction, ischaemic stroke or a transient ischaemic attack) or the veins (e.g. visceral vein thrombosis, cerebral sinus and venous thrombosis, deep vein thrombosis or a pulmonary embolism). In younger patients, with a female predominance, the most common cause in splanchn-
nic venous thrombosis, in approximately 50% of Budd-Chiari syndrome cases and in 25% of portal vein thrombosis cases is associated with the presence of an MPN\textsuperscript{7,10-15}. Both arterial and venous thrombotic events may develop in the preclinical phase of the MPN or during the haematological follow-up period\textsuperscript{7,16,17}. Additional possible risk factors have recently been investigated with a view to improving the prediction of thrombotic events in MPNs, with therapeutic strategies based on the thrombotic risk\textsuperscript{7,9,18,20}. The 2013 updated risk stratification data for thrombosis in PV and ET not only consider the well-known roles of advanced age and prior thromboses, but additionally suggest the importance of the main cardiovascular (CV) risk factors (smoking, hypertension and diabetes mellitus) and JAK2 V617F mutation positivity. But to date CV risk factors have not been integrated in the widely used risk-guided management of ET or PV. It is still based only on two risk factors (age and a prior thrombotic event), and classifies the patients into low- (age < 60 years, without a prior thrombotic event) and high-risk (age > 60 years and/or with a prior thrombotic event) categories\textsuperscript{9,21}.

In the relevant literature, there are only limited data concerning the investigation of thrombotic complications separately in female ET and PV patients and, we therefore, set out to investigate the prevalence of cardiovascular (myocardial infarction), cerebrovascular (ischaemic stroke or transient ischaemic attack) and venous thrombotic events before and after the haematological diagnosis of PV and ET in a female population. A further aim was to evaluate the impact of major CV factors (hypertension, cigarette smoking, diabetes mellitus, obesity and hyperlipidaemia), leukocytosis and the presence of the JAK2 V617F mutation on the occurrence of thrombotic events after the haematological diagnosis among a female population of PV and ET patients. In the current report, we evaluate the contributions of the CV risk factors in thrombotic complications of ET/PV as previously reported in multicentric studies; and besides the overall association, we separately analyse the predictive potential of tobacco use, hypertension, diabetes mellitus, and additionally hyperlipidaemia and obesity. We also analyse the thrombosis-free survival in the case of the CV risk factors, which is not common in the relevant literature.

**Patients and Methods**

**Patients and Data Collection**

Patients were selected from the chronic MPN DNA bank of the Institute of Medical Genetics. A review of 561 DNA analysis results revealed 56 PV and 72 ET female patients with a median age of 62 years (range: 14-95 years) diagnosed at the 2nd Department of Internal Medicine between 1998 and 2011 (Table I, Figures 1 and 2). In the view of the wide age range of 14-95 years, the CV risk factors, the distribution of the diagnosis of ET and PV and thrombotic complications during the follow-up period is depicted as a function of the patients’ age in Figure 3. Patients who used either oral contraceptives or oral HRT were included in the analysis since there was no significant difference in the distribution of data in terms of the presence of CV risk factors.

**Table I.** Main characteristics of the study population.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, total no.</td>
<td>128</td>
</tr>
<tr>
<td>PV</td>
<td>56</td>
</tr>
<tr>
<td>ET</td>
<td>72</td>
</tr>
<tr>
<td>Median follow-up (months)</td>
<td>43.63 (0.59-180)</td>
</tr>
<tr>
<td>Median age at diagnosis</td>
<td>62 (14-95)</td>
</tr>
<tr>
<td>Median red blood cell count at diagnosis</td>
<td>4.95 (2.66-10.81)</td>
</tr>
<tr>
<td>Median platelet count at diagnosis</td>
<td>518 (65-2240)</td>
</tr>
<tr>
<td>Conventional risk factors</td>
<td></td>
</tr>
<tr>
<td>Age &gt; 60</td>
<td>72 (56.3%)</td>
</tr>
<tr>
<td>Prior thrombotic events</td>
<td>42 events (in 37 patients, 28.9%)</td>
</tr>
<tr>
<td>JAK2 V617F positivity (%)</td>
<td>84 (65.63%)</td>
</tr>
<tr>
<td>Treatment, patient no. (%)</td>
<td></td>
</tr>
<tr>
<td>No treatment</td>
<td>23 (17.97%)</td>
</tr>
<tr>
<td>Antithrombotic treatment</td>
<td>48 (37.50%)</td>
</tr>
<tr>
<td>Hydroxyurea (alone or in combination with antiplatelets)</td>
<td>55 (42.97%)</td>
</tr>
<tr>
<td>Phlebotomy (haematocrit target &lt; 45%)</td>
<td>23 (17.97%)</td>
</tr>
</tbody>
</table>
Figure 1. Distribution of thrombotic complications before ET/PV diagnosis and in the follow-up period. Abbreviations: TIA, transient ischaemic attack; MI, myocardial infarct.

Excluded from the study. Information relating to an inherited thrombophilic state was not reported. Further data were collected from the medical data files, and all the haematological results were reviewed. The cardiovascular (myocardial infarction), cerebrovascular (ischaemic stroke or a transient ischaemic attack) and other venous thrombotic events (such as deep vein thrombosis or pulmonary embolism thromboses, and cerebral sinus and venous thromboses) before and after the haematological diagnosis were collected.

To evaluate the impact of major CV risk factors on thrombotic events, hypertension (> 140/80 mmHg), cigarette smoking, diabetes mellitus, obesity (BMI > 30 kg/m²) and hyperlipidaemia (hypercholesterolaemia or hypertriglyceridaemia or both) were also assessed in each patient. The haematological management strategy was based on the current risk-oriented recommendations. Low-risk patients participated in anti-platelet
therapy if it was necessary, while high-risk patients received cytoreductive drugs (e.g. hydroxyurea) alone or in combination with antiplatelet therapy\textsuperscript{21}. Phlebotomy was recommended for patients diagnosed with PV at low risk and before the cytoreductive treatment in high risk PV patients to reach the recommended target haematocrit level of 0.40 and 0.45. The study was approved by the Regional and Institutional Human Medical Biological Research Ethics Committee. No informed consent was required. The study was conducted according to the Declaration of Helsinki.

**Laboratory Methods**

Routine blood analysis with automated blood count equipment was performed as part of the diagnosis protocol. Genomic DNA was isolated from EDTA-stabilized peripheral blood samples and screened for JAK2 V617F with an allele-specific PCR method\textsuperscript{22}.

**Statistical Analysis**

Data were collected with Microsoft Office Excel, and statistical analyses were performed with Statsoft Statistica v 9.1 and SPSS 20.0.0.2 software (SPSS Inc., Chicago, IL, USA). Multivariate binary logistic regression was used to estimate the partial effects of age, prior thrombotic events, leucocytosis at haematological diagnosis, the JAK2 V617F mutation, and the presence of two or more CV risk factors on the probability of thrombotic events, and the potential influence of cytoreductive (hydroxyurea) and antiplatelet therapy on the subsequent thrombotic events after the haematological diagnosis. For an evaluation of the impact of treatment, we additionally computed the difference in

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**Figure 3.** Distribution by age of (A) diagnosed ET and PV cases, (B) cardiovascular risk factors, and (C) thrombotic complications, during the follow-up period. Abbreviations: ET: essential thrombocythaemia; PV: polycythaemia vera; CV: cardiovascular.
the number of thrombotic events after and before the diagnosis for each patient. We tested the relationship between the treatment and the change in the number of thrombotic events by Fisher’s exact test, Somers’ D test and the significance test of Spearman’s coefficient.

The overall effects of the major CV risk factors (hypertension, cigarette smoking, diabetes mellitus, obesity and hyperlipidaemia) on the thrombotic events and the effect of the JAK2 V617F mutation on thrombotic events after the haematological diagnosis were investigated by means of the Mann-Whitney test in subgroups of patients who had or had not suffered thrombotic events. The Kaplan-Meier method was carried out, followed by the log-rank test to evaluate the probability of thrombosis-free survival of the patients in the cohort. In this analysis, the data on patients with at most one CV risk factor and those with two or more CV risk factors were compared. \( p < 0.05 \) was considered statistically significant.

**Results**

JAK2 V617F positivity was proven in 84 patients (65.63%). A total of 55 thrombotic events were recorded in the history of 37 patients with 42 events (32.8%) before the clinical diagnosis of the neoplasms: ischaemic stroke or a transient ischaemic attack in 13 patients (10.16%), myocardial infarction in 15 patients (11.72%), and venous thrombotic events (deep vein thrombosis, portal vein thrombosis or splenic vein thrombosis) in 14 patients (10.94%). During the haematological follow-up period, 13 (10.2%) new thrombotic events were recorded: ischaemic stroke or a transient ischaemic attack in 11 patients (8.59%) and a venous thrombotic event (pulmonary embolism) or a CV event in one patient each (0.78%) (Figure 1). 70 patients (54.7%) exhibited high blood pressure, 17 patients (13.3%) hyperlipidaemia and, 17 patients (13.3%) diabetes mellitus, and 15 patients (11.7%) were cigarette smokers. 96 women (75%) had a normal body weight, and the remaining 32 (25%) were obese (BMI > 30 kg/m²) (Figure 2).

Mann-Whitney univariate tests and multivariate binary logistic regression were run to compare two subgroups, distinguished by the presence or absence of major thrombotic events. This revealed that the presence of two or more CV risk factors (univariate: \( p = 0.011 \); multivariate: relative risk: 4.728 95% CI 1.312-17.040; \( p = 0.018 \) significantly increased the risk of thrombotic events during the follow-up period of the PV/ET neoplasms. Statistical analyses demonstrated a significant overall association between an enhanced thrombotic tendency and high blood pressure (\( p = 0.001 \)), hyperlipidaemia (\( p = 0.005 \)) and tobacco use (\( p = 0.051 \)), whereas diabetes mellitus (\( p = 0.635 \)) and obesity (\( p = 0.866 \)) were not associated with a significantly increased risk of thrombosis. Besides the JAK2 V617F mutation (univariate: \( p = 0.367 \); multivariate: relative risk: 1.428, 95% CI 0.316-6.460; \( p = 0.643 \)) and the well-known standard risk factors (univariate: \( p = 0.816 \) multivariate: relative risk: 0.848 95% CI 0.239-3.004; \( p = 0.798 \)) (Table II), leukocytosis and the potential influence of the therapy applied on the thrombotic events in the follow-up period was analysed.

Analyses of the cases with a leukocyte count > 11.1 G/L measured at the time of the haematological diagnosis resulted in a non-significant tendency from the aspect of further thrombotic complications in the follow-up period (univariate: \( p = 0.424 \); multivariate: relative risk: 1.407, 95% CI 0.359-5.507; \( p = 0.624 \) (Table II).

In the two subgroups distinguished by the presence or absence of major thrombotic events among the patients treated with antiplatelets (univariate: \( p = 0.94 \) or with antiplatelets with hydroxyurea (univariate: \( p = 0.405 \) and phlebotomy in PV (univariate: \( p = 0.614 \); multivariate: relative risk: 0.798, 95% CI 0.172-3.710; \( p = 0.773 \)), no significant effect on the presence of thrombosis in the follow-up period was observed. Although the tendency observed in the case of phlebotomy was non-significant, the multivariate analysis relative risk data indirectly suggest that phlebotomy may have a beneficial effect on thrombotic complications in the follow-up period. We, additionally, computed the difference in the number of thrombotic events after and before the diagnosis for each patient and, then, tested the relationship between the treatment and the change in the number of thrombotic events by means of Fisher’s exact test, Somers’ D test and the significance test of Spearman’s coefficient with a view to obtaining a robust result. Overall, when all of the treatments were considered together, there was a significant (Fisher’s exact test \( p \)-value: 0.061, Somers’ D test \( p \)-value: 0.044, Spearman’s coefficient \( p \)-value: 0.044) impact of the treatment in decreasing the number of thrombotic events.
However, the results for the separately analysed treatments proved to be heterogeneous, typically with a non-significant decreasing impact ($p$-values in the range $0.194-0.625$).

For further analyses, Kaplan–Meier curves and log rank tests (Mantel-Cox) were run to compare the thrombosis-free survival of the patients. A significant difference was observed ($p = 0.005$) between patients with at most one CV risk factor ($n=79$) and those with two or more CV risk factors ($n=47$) (Figure 4).

**Discussion**

In view of the fact that the life expectancy of PV and ET patients is strongly affected by thrombotic events, an investigation of both conventional and additional possible risk factors of thrombotic events in women appears to be of appreciable importance.

Multicentric investigations (mostly in the period 2010-2013), have led to the view that additional thrombotic risk factors should also be considered in the currently accepted thrombosis risk-guided management $^{7,9,16,24,30}$ The roles of the JAK2 V617F mutation and cardiovascular risk factors (at least one CV risk factor, hypertension and/or diabetes and/or active tobacco) are believed to be the most important, while the potential role of the leukocyte count measured at diag-

### Table II

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mann-whitney univariate analysis</th>
<th>Multivariate binary logistic regression analysis</th>
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</thead>
<tbody>
<tr>
<td>At least one CV risk factor present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High blood pressure</td>
<td>0.001**</td>
<td>ND</td>
</tr>
<tr>
<td>Hyperlipidaemia</td>
<td>0.005**</td>
<td>ND</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>0.051*</td>
<td>ND</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.635</td>
<td>ND</td>
</tr>
<tr>
<td>Obesity</td>
<td>0.866</td>
<td>ND</td>
</tr>
<tr>
<td>Two or more CV risks factors present</td>
<td>0.011**</td>
<td>0.018** 4.728 1.312 17.040</td>
</tr>
<tr>
<td>Conventional MPN risk factors</td>
<td>0.816</td>
<td>0.798 0.848 0.239 3.004</td>
</tr>
<tr>
<td>advanced age (over 60) or a prior thrombotic event</td>
<td>0.367</td>
<td>0.643 1.428 0.316 6.460</td>
</tr>
<tr>
<td>JAK2 V617F mutation</td>
<td>0.424</td>
<td>0.624 1.407 0.359 5.507</td>
</tr>
<tr>
<td>WBC &gt; 11.1 G/L</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significant differences at 10% are marked by *, and at 5% by **. Abbreviations: CV: cardiovascular risk; ND: not determined; MPN: myeloproliferative neoplasm; WBC: white blood cell count.

**Figure 4.** Probability of thrombosis-free survival in the haematological follow-up period in the observed subgroups. Abbreviation: CV: Cardiovascular.
nosis is still controversial\textsuperscript{7,8,10,14-20}. However, the current widely used risk stratification in the thrombosis risk management strategy of ET/PV is still based on only two risk factors (age and a prior thrombotic event), and classifies the patients into low- (age < 60 years, without a prior thrombotic event) and high-risk (age > 60 years and/or with prior thrombotic event) categories\textsuperscript{21}. Our findings on female patients indicated that CV risk factors, and especially a high blood pressure and cigarette smoking (similarly as in a multicentric female and male MPN population) and hyperlipidaemia, pose a significantly higher risk of thrombotic events after the diagnosis of PV or ET, while in the case of diabetes mellitus we did not find a significant association. In cases in which the presence of two or more CV risk factors was observed, a significantly higher risk of thrombosis was also seen. Moreover, a significant difference was revealed in the thrombosis-free survival between patients with at most one CV risk factor and those with two or more CV risk factors.

The 2013 updated risk stratification for thrombosis in MPN patients emphasized the prognostic role of the presence of the JAK2 V617F mutation\textsuperscript{9}. Our female patients were predominantly (65.63\%) JAK2 V617F mutation-positive, but our results did not indicate that the presence of the JAK2 V617F mutation was statistically associated with an increased prevalence of thrombotic events. Larger studies on female and male ET/PV populations together have indicated that the JAK2 V617F mutation has a significant role in the prediction of subsequent thrombosis in ET/PV, whereas our current findings on a female population were rather paradoxical from this aspect\textsuperscript{25}. The role of the JAK2 V617F mutation in the subsequent thrombotic complications did not proved to be statistically significant in current female population. We suggest that the number of patients involved might be determinative as concerns a clear evaluation of the presence of the JAK2 V617F mutation and the prediction of thrombosis.

Falanga et al\textsuperscript{31} demonstrated that activation of the circulating polymorphonuclear leukocytes occurs in MPNs and plays a possible role in the thrombotic complications of the patients. Some previous studies\textsuperscript{32-34} have indicated an association between leukocytosis at diagnosis and later thrombotic complications, although other reports did not confirm this. Gangat et al\textsuperscript{35} concluded that the leukocyte count at the time of the haema-
tological diagnosis, defined by a level of either 15 or 9.4 G/L did not appear to be predictive of a subsequent thrombotic event, whereas Carobbio et al\textsuperscript{36} considered that the role of leukocytes above a median level of 8.7 G/L in predicting thrombotic complications was evident in untreated low-risk as compared with treated high-risk patients. In our female population, a leukocyte count > 11 G/L at the time of the ET/PV diagnosis did not appear to have an influence on the risk of subsequent thrombosis: only a non-significant difference was observed between the patients who suffered subsequent thrombotic complications and those who did not.

We consider that the issue of MPNs, with special focus on women and thromboses, should not be neglected with regard to other, particularly gynaecological and obstetric complications. In the relevant literature, a registry for observational studies has already been created concerning pregnancy in chronic Philadelphia chromosome-negative MPNs, but only one large study has demonstrated that oral contraceptives are associated with an increased risk of venous thrombosis in one type of MPN, ET, while in PV no data have been reported\textsuperscript{37-39}. These findings suggest that spontaneous abortion or major maternal complications such as pre-eclampsia, postpartum pulmonary embolism and extensive postpartum haemorrhage may occur more frequently in PV than in other MPNs\textsuperscript{38,39}. However, the available evidence relating to oestrogen-based hormone treatment is insufficient either to support or to refute an association between oestrogen-based hormone treatment and the risk of thrombosis in MPN\textsuperscript{37}. In view of its importance, it would be interesting to conduct large-scale studies on female patients with PV or ET from the aspect of the prevention of thrombotic events, particularly through comparisons of the risk status of MPN patients who use or do not use oral contraceptives or HRT.

**Conclusions**

Female MPN patients who display cardiovascular risk factors (especially high blood pressure, hyperlipidaemia and cigarette smoking) may be at a higher risk of thrombotic events and require special consideration in the prevention and management of thrombotic events. The roles of the JAK2 V617F mutation and leukocytosis in the subsequent thrombotic complications did not
proved to be statistically significant in this female population. Due to the relatively low number of enrolled female PV and ET patients in this single-centre study, the findings do not permit definitive recommendations, but call for prospective multicentric studies on female ET and PV populations.

Conflict of Interest
The Authors declare that there are no conflicts of interest.

References


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