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Role of blood cells dynamism on hemostatic complications in low-risk patients with essential thrombocythemia

Andrea Piccin · Michael Steurer · Manfred Mitterer · Elisabeth Maria Blöchl · Luigi Marcheselli · Irene Pusceddu · Alessandra Marabese · Irene Bertozzi · Daisy Corvetta · Maria Luigia Randi · Elena Elli · Enrico Maria Pogliani · Dino Veneri · Omar Perbellini · Mauro Krampera · Enrica Pacquola · Michele Gottardi · Mario Tiribelli · Anna Guella · Barbara Innella · Paolo Vivaldi · Ercole De Biasi · Rosaria Sancetta · Roberta Rocconi · Renato Bassan · Filippo Gherlinzoni · Giovanni Pizzolo · Günther Gastl · Sergio Cortelazzo

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Abstract Patients with essential thrombocythemia (ET) aged less than 60 years, who have not suffered a previous vascular event (low-risk patients), may develop thrombotic or hemorrhagic events. So far, it has not been possible to identify useful markers capable of predicting which of these patients are more likely to develop an event and therefore who needs to be treated. In the present study, we analysed the relationship between vascular complications and longitudinal blood counts of 136 low-risk ET patients taken over a sustained period of time (blood cells dynamism). After a median follow-up of 60 months, 45 out of 136 patients (33 %) suffered 40 major thrombotic and 5 severe hemorrhagic complications. A total number of 5,781 blood counts were collected longitudinally. Thrombotic and hemorrhagic events were studied together (primary endpoint) but also separately (thrombotic alone = secondary endpoint; hemorrhagic alone = tertiary endpoint). The primary endpoint showed no significant association between platelet and WBC count at diagnosis and risk of any event (platelet, $p = 0.797$; WBC, $p = 0.178$), while Hb at baseline did show an association ($p = 0.024$). In the dynamic analysis with Cox regression model, where the blood count values were studied by time of follow-up, we observed that the risk for Hb was 1.49 (95 % CI 1.13–1.97) for every increase of 1 g/dL, and that this risk then marginally decreased during follow-up. WBC was associated with an increased risk at baseline for every increase of 1×10^9/L (hazard ratio (HR) 1.07, 95 % CI 1.01–1.13, $p = 0.034$), the risk was stable during follow-up (HR 0.95, $p = 0.187$ at 60 months). Also, for each increment at baseline of 100×10^9 platelets/L, HR was increased by 1.08 (95 % CI 0.97–1.22, $p = 0.159$) and decreases during follow-up. In conclusion, this study is the first to evaluate

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A. Piccin (✉) · I. Pusceddu · A. Marabese · D. Corvetta · S. Cortelazzo
Department of Haematology, San Maurizio Regional Hospital, Bolzano, South Tyrol, Italy
e-mail: apiccin@gmail.com

A. Piccin · M. Steurer · E. M. Blöchl · G. Gastl
Division of Haematology & Oncology, University Hospital, Innsbruck, Austria

M. Mitterer
Oncohaematology Unit, Meran Hospital, Merano, South Tyrol, Italy

L. Marcheselli
Department of Diagnostic, Clinical and Public Health Medicine, University of Modena and Reggio Emilia, Modena, Italy

I. Bertozzi · M. L. Randi
Department of Internal Medicine, University of Medicine, Padua, Italy

E. Elli · E. M. Pogliani
Department of Haematology, University of Milano Bicocca, Milan, Italy

D. Veneri · O. Perbellini · M. Krampera · G. Pizzolo
Department of Medicine, Section of Hematology, University of Verona, Verona, Italy

E. Pacquola · M. Gottardi · F. Gherlinzoni
Department of Haematology, Cà Foncello Hospital, Treviso, Italy

in ET low-risk patients, the risk of developing a thrombotic/hemorrhagic event considering blood counts over time. Overall our study shows that the risk changes over time. For example, the risk associated with WCC is not linear as previously reported. An interesting new finding is that PLT and even Hb contribute to the risk of developing vascular events. Future treatments should take into consideration these findings and aim to control all parameters over time. We believe this early study may help develop a dynamic analysis model to predict thrombosis in the single patient. Further studies are now warranted to further validate our findings.

Keywords Essential thrombocythemia · Low-risk · Thrombosis · Hemorrhage

Introduction

Essential thrombocythemia (ET) is a clonal disorder characterized by the abnormal proliferation of megakaryocytes followed by uncontrolled increase of circulating platelets (PLT) [1]. Patients with ET have a relatively benign prognosis [2], but thrombotic and hemorrhagic events may indeed occur over time, leading to severe morbidity [3–5]. Previous studies by Cortelazzo et al. [6–8] showed that age >60 years and previous thrombotic or hemorrhagic events are strongly associated with a higher risk of developing further events. Conversely, the so-called low-risk ET group (young patients without previous vascular events), may also develop thrombotic or hemorrhagic events during follow-up. However, agreement is lacking about the role of blood counts in this risk. A relationship between high WBC (>11 $\times$ 10^9/L) and the risk of thrombosis has been shown, [9, 10] but these findings have been contradicted by other authors [11–13]. Recently, Passamonti et al. re-examined this issue using a dynamic model based on two sequential evaluations of WBC count within 2 years from diagnosis, and find that patients with an increase greater than one-third of the baseline WBC count, have a significantly higher risk of developing thrombosis [14]. A correlation between elevated WBC count during follow-up and risk of thrombosis has also been reported by Campbell et al. [15], in high-risk ET patients (PT-1 trial). These findings prompted us to assess the role of blood cell values and the risk of thrombotic and hemorrhagic events due to their changes over time in a large group of low-risk ET patients, followed up for a long period of observation. Moreover, we also repeated our statistical analysis using the new IPSET risk classification (low, intermediate and high).

M. Tiribelli
Department of Haematology,
Azienda Ospedaliero-Universitaria, Udine, Italy

A. Guella · B. Innella · P. Vivaldi
Department of Haematology, Santa Chiara Hospital, Trento, Italy

E. De Biasi
Department of Haematology, Camposampiero Hospital, Padua, Italy

R. Sancetta · R. Rocconi · R. Bassan
Department of Haematology, Venice Hospital, Venice, Italy

Patients and methods

Patients

Patient data from 1981 to 2011 was retrospectively obtained from nine Haematology Departments in Northern Italy and the Department of Haematology at Innsbruck University of Medicine in Austria. All patients were enrolled in a consecutive manner at all centers, and were all at first diagnosis. A database ad hoc was created and shared between all centers. 130 patients had a diagnosis of low-risk ET according to the 2008 WHO criteria (all cases had bone marrow biopsy performed), primary myelofibrosis was also excluded [16]. Another six cases were diagnosed according to the PVSG criteria. All patient data was collected anonymously. Approval for this multi-centre study was obtained from the Ethics Committee of the leading centre.

Patients undergoing treatment with hydroxyurea or aspirin were excluded from this study.

Risk category

- WHO risk groups: low-risk patients were defined as <60 years of age without prior thrombotic or hemorrhagic events [7], and a platelet count <1,500 $\times$ 10^9/L with or without cardiovascular risk factors such as diabetes, hypertension, hypercholesterolemia and smoking or *JAK-2* mutational status [16, 17].
- IPSET risk groups: we also performed a secondary analysis with the updated IPSET criteria giving to each patient a score as follows: Age >60 years (1 point), history of thrombosis (2 points), presence of cardiovascular risk factors (diabetes, hypertension, smoking = 1 point) and presence of the JAK2 V617F mutation (2 points).

Using this model, we determined three thrombosis risk groups: low risk for thrombosis (total score zero or 1), intermediate risk (score 2) and high risk (score > 2).

Blood counts

Longitudinal blood counts, including white blood count (WBC), platelet count and haemoglobin (Hb) concentration, were recorded from the date of diagnosis (entry time) until the development of an event or up to 5 years of follow-up (exit time).

Events

Events were defined as thrombotic events (stroke, transient ischemic attack, myocardial infarction, peripheral artery thrombosis, deep or splenic vein thrombosis and pulmonary embolism), and hemorrhagic events. Moreover, hemorrhagic events requiring transfusion >2 units, or causing a decrease in Hb to >2 g/dL were defined as major hemorrhagic events, while all others were defined as minor hemorrhagic events.

End points

Because ET may cause either hemorrhagic or thrombotic events, we considered the primary end point to be any thrombotic or hemorrhagic event. Subsequently, we re-analysed all data looking at thrombotic events only, this represents the secondary endpoint of our study.

Data collection and statistical analysis

We defined as fixed covariates the variables recorded at baseline only (entry time), and as varying covariates, the consecutive blood counts recorded longitudinally during 5 years of follow-up, or until an event occurred (exit time) [11]. Binary covariates were reported as a proportion, and comparisons at baseline were made using χ^2 test, where appropriate, or Fisher's exact test. Continuous covariates were expressed by means of median and interquartile range (IQR), and compared with the Mann–Whitney test. The association between blood counts recorded longitudinally and time at blood sampling (in years), were analysed according to a linear regression analysis, using a linear regression with random factors (random effect). This was necessary because consecutive blood count measurements were taken on the same subject, similar to what was suggested by recent studies [16]. In the linear mixed model, the association between the time at sampling (in years), and the blood count, was adjusted for age at control, and gender. Furthermore, WBC and platelet counts were transformed in logarithmic form [Log_{10}] to allow a Gaussian distribution. The event-free survival (EFS) of the primary end points of arterial or venous thrombosis, or serious haemorrhage, was defined from the date of diagnosis to the date of an event, or the date of the last blood count (censored cases). EFS of the primary endpoints cumulative probability was estimated by means of the Kaplan–Meier method [18, 19]. Comparison between curves of EFS of the primary end points was performed using the log-rank test [20]. If the curves crossed, we used the Renyi type test in addition to the log-rank test [21].

To assess the effect of the blood count covariates, we used the Cox proportional hazard model [22].

We fitted a Cox proportional hazards regression model, stratified by type of event, following two approaches: (i) using restricted cubic spline basis for the blood count predictor, and (ii) using time-dependent covariates [23]. Blood count covariates were measured with the time of follow-up (time scale months) in logarithmic form [ln_e (1 + month)]. All regression coefficients and hazard ratios (HR) were reported together with their confidence interval at 95 % (95 % CI) or standard error (SE). As this study was a retrospective analysis, we did not calculate a sample size. For all tests, a two-sided p value < 0.05 was considered significant, to demonstrate a moderate strength of evidence against the null hypothesis. This level of probability is helpful for providing clinically useful advice. All statistical analysis was performed using the statistical package Stata 10.1 SE (College Station, TX 77845 USA).

Results

Patients

From 1981 to 2011, a total of 136 consecutive patients with low-risk ET were diagnosed in ten cancer centres. 130 were diagnosed according to the 2008 WHO criteria (bone marrow biopsy performed), while another six cases were diagnosed according to the PVSG criteria. Their demographic and baseline characteristics are reported in Table 1. The median age was 49 years (range 18–59 years), and the M/F ratio was 0.50.

Blood counts

During a median follow-up of 60 months (range 1–360), a total of 2,661 longitudinal blood counts of 136 low-risk ET patients were analysed. Regarding the blood count at diagnosis, the median value for Hb was 14.2 g/dL (range 11.7–17.9), WBC count was 8.2×10^9/L (range 4.4–24), and the platelet count was 718×10^9/L (range 451–1,806).

Other risk factors

Other risk factors included: diabetes ($n = 2/113$, 2 %), hypertension ($n = 22/122$; 18 %), hypercholesterolemia ($n = 16/91$; 18 %), hypertriglyceridemia ($n = 8/114$;

7 %), active smoker ($n = 18/74$; 24 %) and *JAK2V617F* mutation ($n = 63/106$; 59 %). Also, 70 patients (81 %) were in the IPSET low-risk group.

Vascular events and risk factors

After a median follow-up of 80 months (range 1–360 months), 45 out of 136 patients (33 %) had 40 thromboses including: 3 strokes, 11 transient ischemic attacks, 12 myocardial infarctions, 2 peripheral arterial thromboses, 2 deep and 7 splanchnic vein thromboses, 2 pulmonary embolisms, 1 acute coronary syndrome and 5 major hemorrhagic events (4 nasal bleeding and 1 gastrointestinal bleeding). The median EFS of the primary end points was 204 months (95 % CI 164–240 months), and the overall estimated risk of the primary end points at 5 and 10 years was 14 % (95 % CI 9–24 %) and 30 % (95 % CI 20–45 %), respectively (Fig. 1). No statistical difference was found between the estimated risk of arterial and venous thrombosis at 5 and 10 years ($p = 0.238$) (data not shown). Moreover, neither cardiovascular risk factors (hypertension, $p = 0.519$; hypercholesterolemia, $p = 0.149$, hypertriglyceridemia, $p = 0.915$ and smoke, $p = 0.123$), nor *JAK-2* mutational status ($p = 0.463$, Renyi test), influenced the rate of vascular complications. Regarding blood counts, we did not find a significant association between platelet and WBC count at diagnosis and thrombotic/hemorrhagic events (platelet, $p = 0.797$; WBC, $p = 0.178$) or thrombosis (platelet, $p = 0.850$ and WBC, $p = 0.256$). Instead, Hb at baseline showed an association with thrombosis/hemorrhage ($p = 0.024$) and thrombosis ($p = 0.006$).

Adopting the newly established IPSET criteria, there were 70 patients with low risk and 66 patients with intermediate risk, however, IPSET did not show an association with thrombotic/hemorrhagic events ($p = 0.656$) or thrombosis only ($p = 400$).

Table 1 Patient characteristics

Variable	*N*	Median (range)
Age, years	136	49 (18–59)
Hemoglobin, g/dL	136	14.2 (11.7–17.9)
WBC 10^9/L	136	8.2 (4.4–23.8)
Platelets 10^9/L	136	718 (451–1,806)
	N	*n* (%)
Gender, male	135	45 (33)
JAK2 V617F mutation	106	63 (59)
Smokers	74	18 (24)
Hypercholesterolemia	91	16 (18)
Diabetes	113	2 (2)
Hypertension	122	22 (18)
Hypertriglyceridemia	114	8 (7)
IPSET Score: low (0–1)	136	70 (51)

Risk: defined according to IPSET score (Barbui et al., Blood, 2012)
WBC white blood count

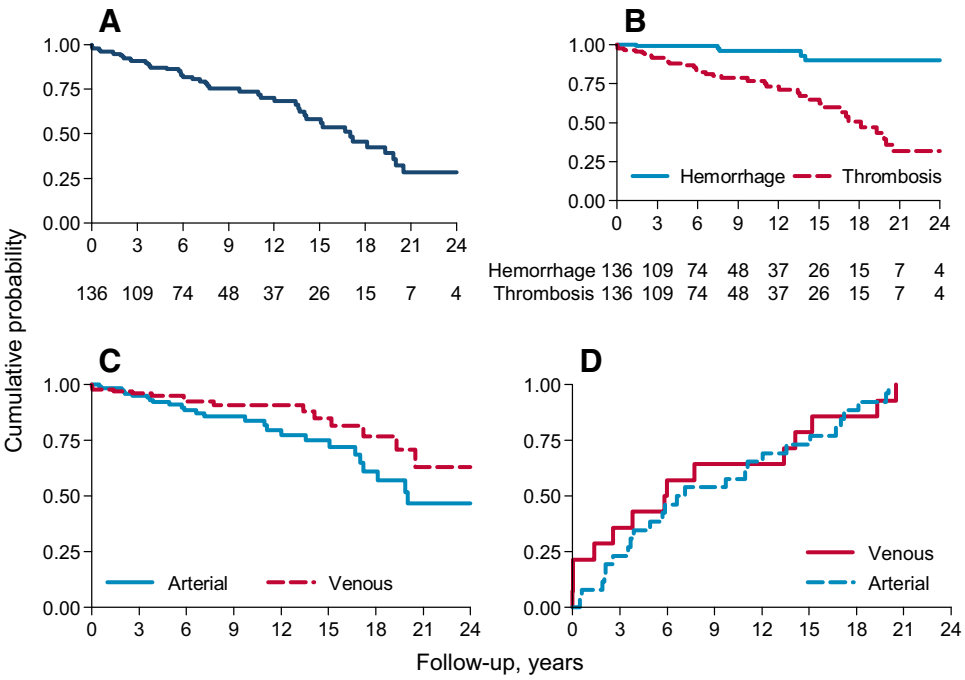

Fig. 1 Cumulative event free survival (EFS, median 204 months) of our low risk ET patients with both thrombotic/hemorrhagic events (**a**). Figure **b** shows the EFS separated for hemorrhagic ($n = 5$) and thrombotic events. No statistical difference was is found between the estimated risk of arterial and venous thrombosis at 5 years (**c**). Figure **d** shows cumulative distribution of venous and arterial events

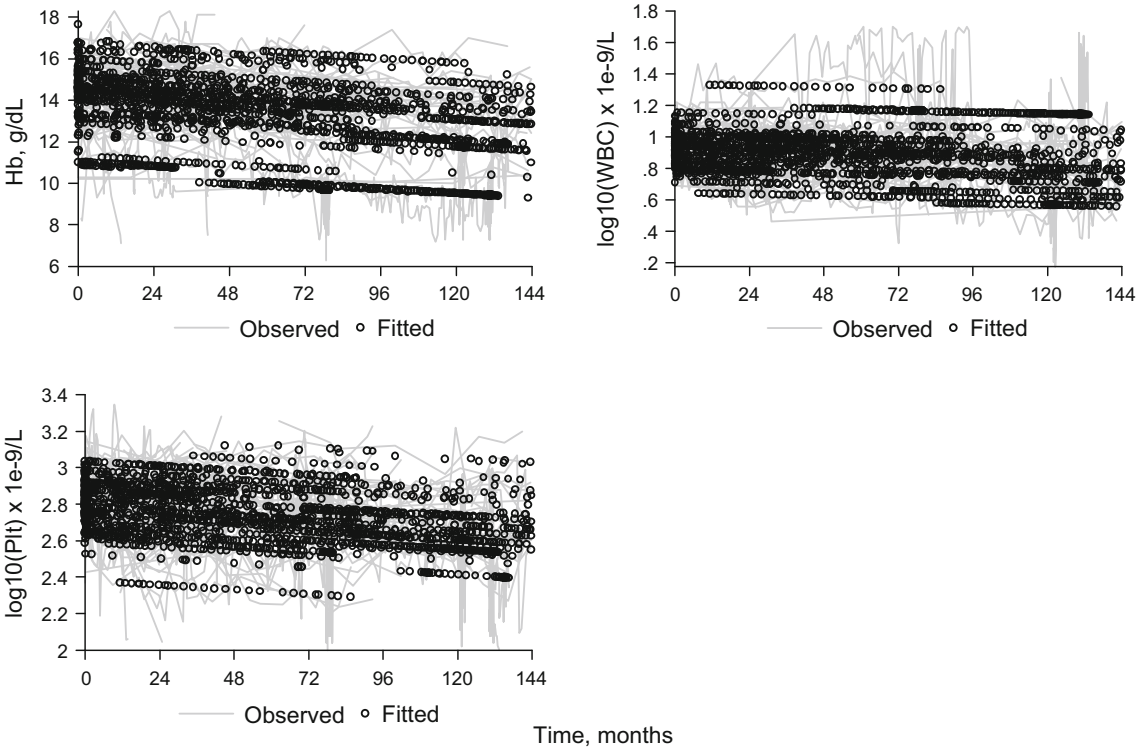

Fig. 2 Shown blood cells dynamism in low risk ET patients with a thrombotic/haemorragic event. *Hb* hemoglobin g/dL, *WBC* white blood cells $\times 10^9$/L in logarithm base 10 and platelets $\times 10^9$/L in logarithm base 10

Risk of developing a thrombotic or hemorrhagic event

A total number of 2,661 blood counts were collected longitudinally in the 136 low-risk patients, with a median time of 1.7 months (range 0.5–7) between each blood sampling. Overall, during the follow-up time, after adjusting for age and gender, Hb values decreased from baseline in a linear manner (−0.11 g/dL by year, $p < 0.001$). The WBC and platelet counts showed a similar trend over follow-up time (-0.09×10^9/L every year, $p = 0.058$ and -26×10^9/L every year, $p < 0.001$), as reported in Fig. 2.

Risk analysis for the primary endpoint (all events)

The cumulative analysis of the relationship between blood count values, modeled by means of restricted cubic spline regression, and vascular thrombotic/hemorrhagic events, evaluated with Cox proportional hazards regression models, shows that the risk increases when platelets are above 1,500–2,000 $\times 10^9$/L ($p = 0.012$), while WBC and Hb do not show an association with vascular events ($p = 0.800$ and $p = 0.194$, respectively) (Fig. 3a–c). From the dynamic analysis with the Cox regression model, where the blood count values were interacted by time of follow-up (Table 2), we observe: (1) the risk of developing a vascular event at baseline for Hb increases is 1.49 (95 % CI 1.13–1.97) for every increase of 1 g/dL, and this risk then marginally decreases during follow-up (HR at 60 months for any increase of 1 g/dL of Hb is 0.81 (95 % CI 0.65–1.03, $p = 0.083$); (2) WBC increases are associated with an increased risk at baseline (HR 1.07, 95 % CI 1.01–1.13, $p = 0.034$) for every increase of 1×10^9/L, the risk is stable during follow-up (HR 0.95, $p = 0.187$ at 60 months); (3) when platelet values and HR were considered, for each increment at baseline of 100×10^9 platelets/L, HR is increased by 1.08 (95 % CI 0.97–1.22, $p = 0.159$), and tend to decrease during follow-up (HR = 0.90, 95 % CI 0.81–1.01, $p = 0.075$ at 60 months) (Fig. 3c–e).

Risk analysis for the secondary endpoint (thrombotic events only)

Cox proportional hazards regression models, confirm an increased risk of thrombosis when platelets counts are above 1,500–1,700 $\times 10^9$/L ($p = 0.022$) (Fig. 4a–c). According to the model with the time-depending covariates

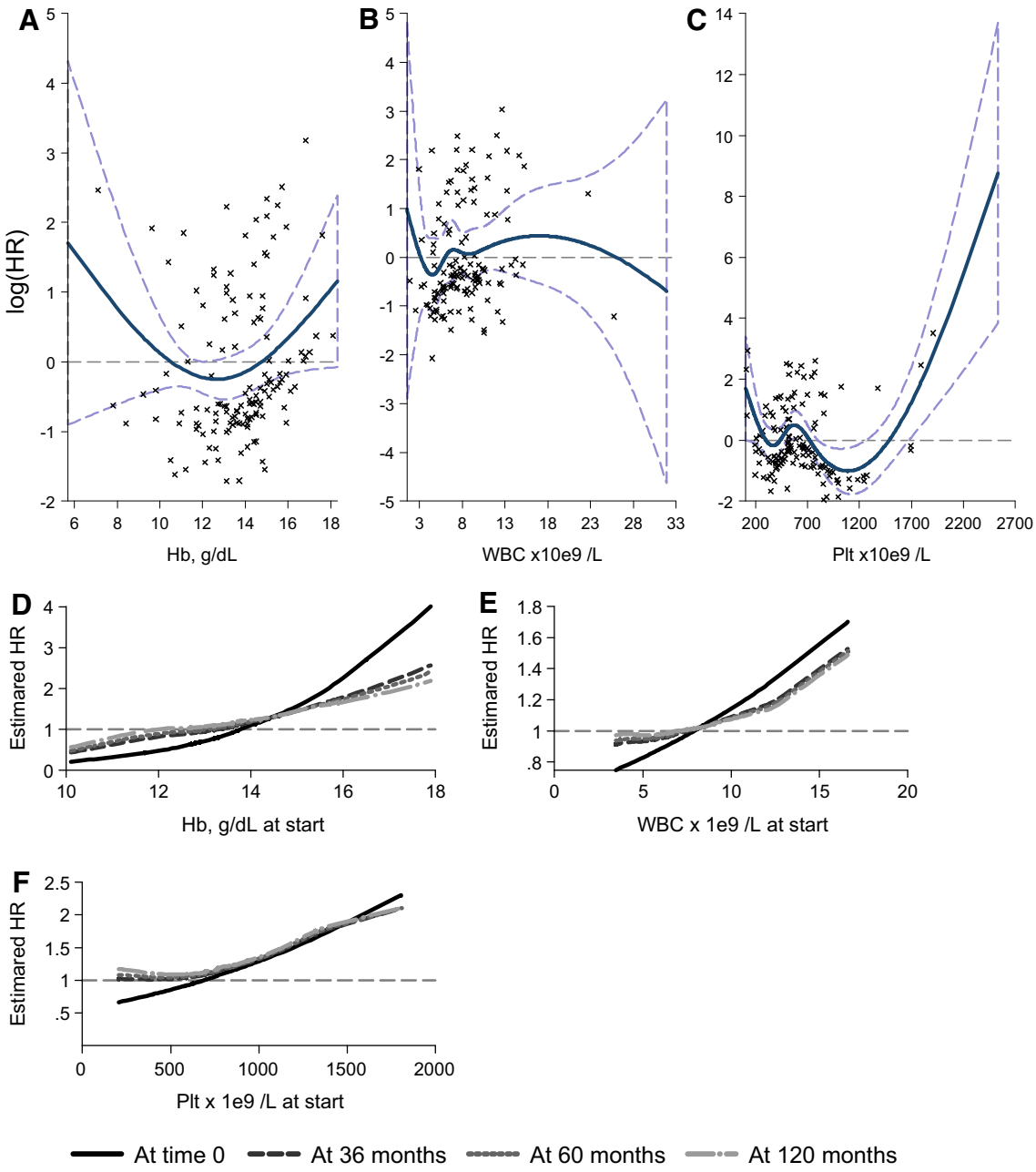

Fig. 3 Primary endpoint risk analysis cumulative thrombotic/hemorraghic events. Shown cumulative dynamic hazard risk (HR) valuated with Cox proportional hazards regression models by restricted cubic spline for Hb (**a**), WBC (**b**) and platelets (**c**). The *blue line* represents expected log (HR); *dashed lines* represent 95 % CI, cumulative estimated hazard ratio (HR) from Cox PH dynamic model. The HR for any covariates was estimated varying Hb (**d**) or WBC (**e**) or platelets (**f**) at time of follow-up of zero, 3, 5 and 10 years

(Table 3), the analysis confirms an association between Hb and WBC and the risk of developing a thrombotic event at baseline (HR = 1.64, $p = 0.001$ and HR = 1.08, $p = 0.010$, respectively). The risk of thrombosis is reduced during the follow-up for Hb (by any increase of Hb of 1 g/dL at 60 months, HR = 0.78, 95 % CI 0.61–0.99, $p = 0.048$) (Fig. 4c–e).

Risk analysis for the tertiary endpoint (hemorrhagic events only)

The results of the tertiary endpoint (hemorrhagic events only) analysis show an increased risk of haemorrhage related to the increasing of WBC up to 13000 × 10^9/L ($p = 0.004$). Conversely, a linear decreasing risk of

Table 2 Cumulative Dynamic Cox regression model for thrombosis/hemorrhagic events ($n = 136$, $n = 45$ events)

Univariate analysis	Coef.	SE	*P*	HR	95 % CI
Gender, male	0.429	0.320	0.179	1.54	0.82–2.88
Prior treatment	−0.348	0.352	0.324	0.71	0.35–1.41
Age/10	0.050	0.117	0.665	1.05	0.84–1.32
Risk group interim. vs low	0.142	0.300	0.637	1.15	0.64–2.08
(Hb-14) at diagnosis	0.398	0.142	0.005	1.49	1.13–1.97*
(Hb-14) × ln(months + 1)	−0.050	0.029	0.083	0.81	0.65–1.03**
(WBC-8.0) at diagnosis	0.063	0.030	0.034	1.07	1.01–1.13*
WBC × ln(month + 1)	−0.012	0.009	0.187	0.95	0.88–1.02**
(Plt-700)/100 at diagnosis	0.081	0.057	0.159	1.08	0.97–1.22*
(Plt-700)/100 × ln(month + 1)	−0.024	0.014	0.075	0.90	0.81–1.01**

Hb hemoglobin g/dL, *WBC* white blood cell count 10^9/L, *PLT* platelet count 10^9/L, *ln* natural logarithm (basis *e*); $e = 2.71828 \ldots$ Euler constant

* For every increase of 1 g/dL in hemoglobin or 1×10^9/L in WBC or 100×10^9/L in Plt at time of follow-up equal to zero

** For every increase of 1 g/dL in Hb or 1×10^9/L in WBC or 100×10^9/L in Plt at 60 months follow-up

haemorrhage is seen with a decrease of the platelet count ($P = 0.031$).

Analysis adopting the newly published IPSET criteria

Adopting the newly established IPSET criteria, low-risk patients were 73 in number, while those with *intermediate*-risk were 63. Interestingly, when either thrombotic or hemorrhagic events are considered, Hb and WBC at diagnosis show a statistically significant correlation with the risk of developing an event (respectively, HR 1.49, $p = 0.005$; HR 1.08, $p = 0.034$). However, because the number of hemorrhagic events was small ($n = 5$), we repeated the study looking only at the thrombotic events, and in this case only WBC at diagnosis correlates with the probability of developing an event (HR 1.08, $p = 0.010$). Overall the trend between the low-risk patients identified by the WHO criteria is similar to the trend of the low-risk group identified with the IPSET criteria.

Discussion

Low-risk ET patients may develop thrombotic and hemorrhagic events, which carry severe morbidity and mortality risks. For these reasons, we aimed to identify predictors of thrombotic and hemorrhagic events. The uniqueness of our study was to run a dynamic analysis with a Cox regression model, where the blood count values were studied by time of follow-up.

The estimate of thrombosis-free survival at 5 years is superior in our study compared with the high-risk group, 85 versus 72 %, respectively. The rate of hemostatic complications in our study cannot be directly compared with other studies because of the wide range of reported vascular events (ranging from 7.6 to 25 %) [12, 14, 24]. This discrepancy between different study findings may be related to the use of different selection criteria, which allowed the inclusion of subgroups, which represented higher risk categories, such as those with early/prefibrotic myelofibrosis [24], cardiovascular risk factors or JAK2 mutation. In fact, the newly proposed International Prognostic Score of thrombosis in ET (IPSET-thrombosis) includes only 48 % of young and asymptomatic patients in the new low-risk group, while the remaining 52 % are included in the intermediate-risk group or in a small fraction of the high-risk group (5 %), thus confirming the heterogeneity of the previously defined low-risk category of ET patients [25].

Our findings are also in keeping with previous reports showing that arterial and venous thrombosis are prevalent, as only 4 % of patients suffered a major hemorrhage [12, 24]. In our study, the analysis of possible risk factors for vascular events examined at diagnosis reveals that neither blood count, nor cardiovascular risk factors nor JAK2 mutations are predictive for thrombosis or hemorrhage during follow-up. Carobbio et al. suggest an association between leukocytosis at diagnosis and thrombosis [10]. However, other studies do not show that platelet or leukocyte counts at presentation are predictive of subsequent vascular events [12, 14]. In contrast with the observations of a recent study [26], but in keeping with the findings of an earlier study on a similar population [12], multivariate analysis excludes an association between either cardiovascular risk factors or JAK2 mutation and subsequent vascular events.

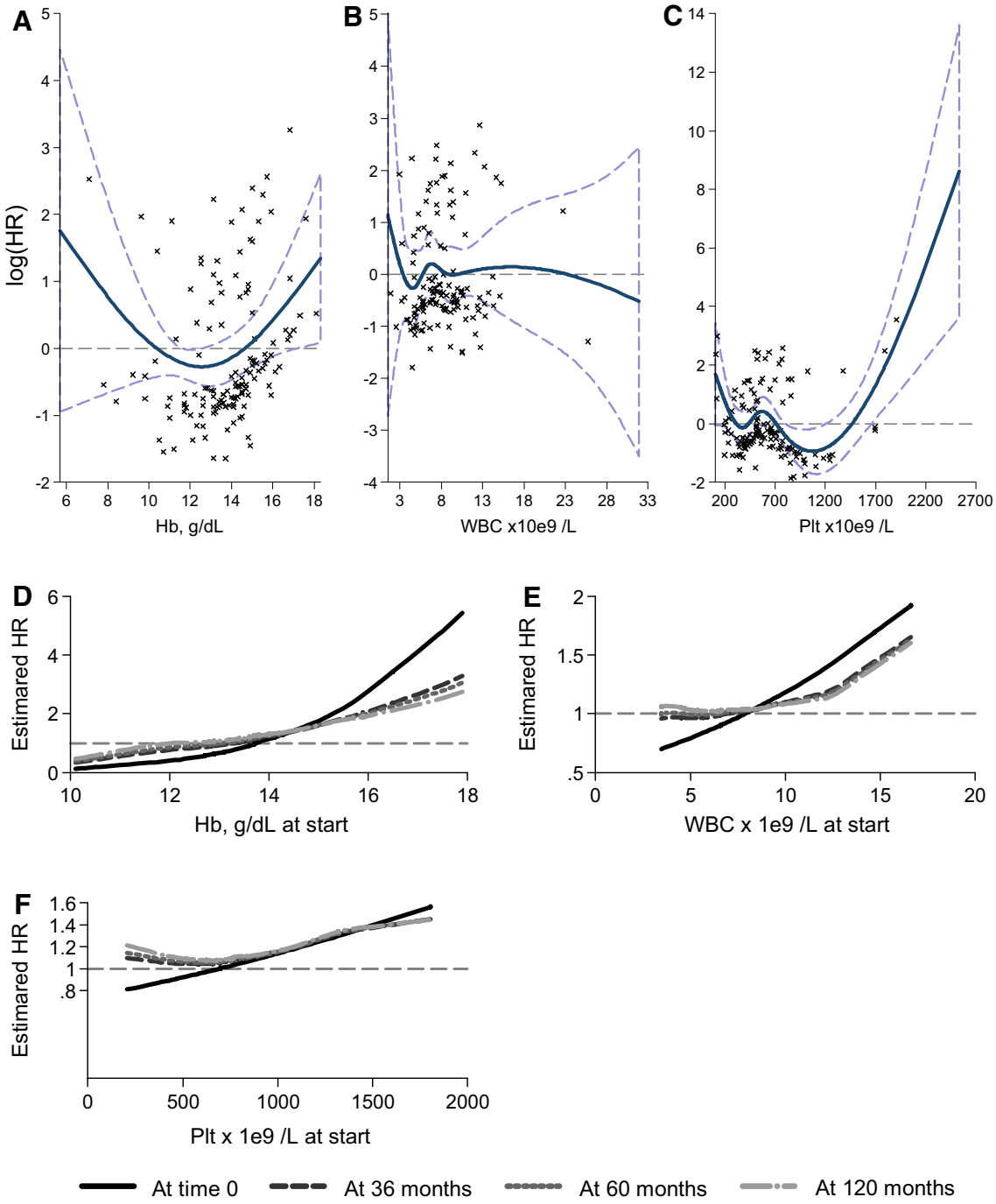

Fig. 4 Secondary endpoint risk analysis: Thrombotic events only. Shown the logarithm of the hazard risk [log(HR)] valuated with Cox proportional hazards regression models by restricted cubic spline related to thrombotic events only for hemoglobin (**a**), WBC (**b**) and platelets (**c**). The *blue line* represents expected log(HR); *dashed lines* represent 95 % CI. The estimated hazard ratio (HR) from Cox PH dynamic model for thrombotic events only was estimated varying Hb (**d**) or WBC (**e**) or platelets (**f**) at time of follow-up of zero, 3, 5 and 10 years

The results of the cumulative analysis conducted on combined thrombotic and hemorrhagic events (primary endpoint), explain at least in part, how cell dynamism contributes to vascular events in ET. Previous studies have always considered thrombotic and hemorrhagic events independently.

Recent findings on ET pathophysiology suggest that not only blood cell variations, but also qualitative blood cells

Table 3 Cumulative Dynamic Cox regression model for only thrombosis events ($n = 136$, $n = 40$ thrombosis)

Univariate analysis	Coef.	SE	*P*	HR	95 % CI
Gender, male	0.521	0.337	0.122	1.68	0.87–3.26
Prior treatment	−0.513	0.363	0.157	0.60	0.29–1.22
Age/10	0.070	0.124	0.576	1.07	0.84–1.37
Risk group interim. vs low	0.178	0.318	0.576	1.19	0.64–2.23
(Hb-14) at diagnosis	0.493	0.148	0.001	1.64	1.22–2.19*
(Hb-14) × ln(1 + months)	−0.060	0.030	0.048	0.78	0.61–0.99**
(WBC-8.0) at diagnosis	0.078	0.030	0.010	1.08	1.02–1.15*
WBC × ln(1 + month)	−0.020	0.010	0.060	0.92	0.85–1.00**
(Plt-700)/100 at diagnosis	0.042	0.065	0.517	1.04	0.92–1.18*
(Plt-700)/100 × ln(1 + month)	−0.018	0.015	0.242	0.93	0.82–1.05**

Hb hemoglobin g/dL, *WBC* white blood cell count 10^9/L, *PLT* platelet count 10^9/L, *ln* natural logarithm (basis *e*); $e = 2.71828 \ldots$ Euler constant

* For every increase of 1 g/dL in hemoglobin or 1 × 10^9/L in WBC or 100 × 10^9/L in Plt at time of follow-up equal to zero

** For every increase of 1 g/dL in Hb or 1 × 10^9/L in WBC or 100 × 10^9/L in Plt at 60 months follow-up

alterations, contribute to events. For this reason, we believe that thrombosis and hemorrhage are only the epiphenomenon of these changes, and we evaluated all events together (primary endpoint), as well as independently for thrombosis only (secondary endpoint), or for haemorrhage only (tertiary endpoint).

This primary endpoint analysis shows that a higher WBC clearly increases the risk of cardiovascular events, and similarly for PLT counts, (for each increment of PLT of 100×10^9 platelets/L, HR was increased by 1.08 $p = 0.159$). However, this risk is reduced over time.

The secondary endpoint analysis (thrombotic events only), shows that neither WBC nor PLT increases significantly affect the risk of thrombosis. Another finding of the secondary endpoint analysis, is that the risk of developing a thrombotic event increases by 34 % (95 % CI 7–69 %), for each increase of 1 g/dL of Hb after 60 months of follow-up. These results contradict previous reports by Campbell et al. [15], who find a linear relationship between WBC count increases during follow-up and risk of thrombosis. However, Campbell's study did not consider a model with the time-depending covariates, using only a cubic spines one. Also, the maximum follow-up was for only 60 days, while in our study it was for 60 months. Moreover, the discrepancies between the Campbell study and our study might be due to the different inter-study population. In fact, our series included only low-risk untreated ET patients, while the patients considered in the Cambpell study were those from the PT-1 study that were high-risk patients, who underwent cytoreduction. The finding of an association between Hb increase and thrombosis has also been reported by Gangant N in low-risk ET patients [12]. This further suggests that the findings from the Campbell study are not valid for low-risk ET patients. We do not have a clear explanation for this finding, however, patients with ET often have a high HCT and Hb levels, as ET and polycythemia vera may overlap. However, information on patient hematocrit was lacking.

The results of the tertiary endpoint analysis (hemorrhagic events only), shows that an increased risk of haemorrhage is related to the increasing of the WBC levels, while a linear decreasing risk of haemorrhage is seen with decreasing platelet counts. However, we feel that these finding should be considered with caution because of the low number of hemorrhagic events.

Overall our study shows that the risk of developing any hemorragic or thrombotic events changes over time. Thus, the risk associated with WCC increases is not linear as previously reported. The interesting new finding is that PLT and even Hb somehow contribute to the risk of developing any vascular event. At the present, we cannot explain these findings, and moreover, we cannot explain why risk of thrombosis or haemorrhage changes over time. We can speculate that some patients may be developing myelofibrosis (MF), and that these changes over time may simply reflect the developing of a new disease phenotype (moving from ET towards MF). Moreover, the slight risk reduction seen over time with high PLT counts may be attributed to a certain degree of Acquired von Willebrand disease (avWD), which might balance the overall risk of thrombosis, without being strong enough to induce haemorrhage. Future treatments should take into consideration these findings, and aim to a control of blood parameters over time. We believe this early study may help develop a dynamic analysis model to predict thrombosis in any single patient. Further studies are now warranted to validate our findings.

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Conflict of interest None.

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