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The haematocrit and platelet target in polycythemia vera

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Summary

Polycythemia vera (PV) is a chronic myeloproliferative disorder whose major morbidity and mortality are thrombohaemorragic events and progression to acute leukaemia or myelofibrosis. Whether the haematocrit and platelet count predict such complications remains unclear. The European Collaboration on Low-dose Aspirin in Polycythemia Vera prospective study included 1638 PV patients. A total of 164 deaths (10%), 145 (8·85%) major thrombosis and 226 (13·8%) total thrombosis were encountered during 4393 person-years follow-up (median 2·8 years). In time-dependent multivariable analysis, a haematocrit in the evaluable range of 40–55% was neither associated with the occurrence of thrombotic events, mortality nor with haematological progression in the studied population. The haematocrit of patients in the highest and lowest deciles at baseline was maintained within a narrow interval of haematocrit values ranging from 40% to 47% throughout follow-up. High platelet count was associated with a lower progression rate to acute leukaemia/myelofibrosis, whereas it had no significant relationship with thrombotic events or mortality. Our findings do not suggest that the range of haematocrit (<55%) and platelet counts ($<600 \times 10^9$/l) we encountered in our population had an impact on the outcome of PV patients treated by current therapeutic strategies.

Keywords: polycythemia vera, thrombosis, haematocrit, platelet count.

Polycythemia vera (PV) is a chronic myeloproliferative disorder characterised by panmyelosis, splenomegaly, a predisposition to venous and arterial thrombosis, and a possible progression to myelofibrosis, and acute leukaemia (Spivak, 2002; Schafer, 2006). In PV, the proliferation of a multipotent haematopoietic progenitor cell leads to the accumulation of red cells, white cells and platelets. The dominant feature of PV and the 'sine qua non' for its diagnosis remains erythrocytosis, which is also regarded as the main cause for the most frequent and serious complications of PV, namely thrombosis and haemorrhagic episodes (Spivak, 2002; Schafer, 2006).

Current PV treatment recommendations are to maintain the haematocrit <45% in males, <42% in females, <36% during pregnancy and the platelet count below 400×10^9/l (Spivak, 2002; Schafer, 2006), based on earlier evidence suggesting a proportional increase of thrombotic events with high haem-

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atocrit and platelet count (Pearson & Wetherley-Mein, 1978; Schafer, 2006). An aggressive management of these haematologic variables is thus widely practiced, despite the lack of solid data backing this recommendation (Prchal, 2003; Schafer, 2006).

The European Collaboration on Low-dose Aspirin in Polycythemia Vera (ECLAP) study was recently concluded (Landolfi & Marchioli, 1997; Landolfi *et al*, 2004; Finazzi *et al*, 2005; Marchioli *et al*, 2005). This large, prospective multicentre project included 1638 PV patients and provides a unique opportunity for a comprehensive reassessment of the prognostic value of haematocrit and platelet count relative to thrombotic events and/or progression to acute leukaemia and myelofibrosis. The aim of this analysis was to evaluate haematocrit and platelet count as possible predictors of thrombotic and/or haematological complications in patients with PV.

Patients and methods

Patients

The characteristics of the ECLAP study have been described previously (Landolfi & Marchioli, 1997; Spivak *et al*, 2003; Landolfi *et al*, 2004; Marchioli *et al*, 2005). Briefly, all patients with the most current or conventional diagnosis of PV [diagnosed according to the criteria established by the PV Study Group (PVSG) (Berk *et al*, 1995) or by Pearson & Messinezy, 1996] were included in a prospective study with no exclusion with respect to age, therapy or duration of disease. Treatment strategies had to comply with the recommendation of maintaining the haematocrit below 45% and platelet count $<400 \times 10^9$/l. Data regarding clinical outcomes, treatments and laboratory values during the prospective follow-up were recorded at 12, 24, 36, 48 and 60 months of follow-up. The mean duration of the disease at entry and the duration of the follow-up were 5·0 and 2·7 years respectively.

The aims of the current study were to determine whether haematocrit and platelet count in the ECLAP study may suggest a 'specific target value' to be maintained during the course of the disease or, in other words, if treatment strategies should be more or less aggressive to control the disease. We also attempted to assess if this 'specific target value' (if existent) would impact on thrombotic and neoplastic events.

Outcome events

Outcome events were total mortality, major thrombosis (i.e. non-fatal myocardial infarction, stroke, deep venous thrombosis, pulmonary embolism or cardiovascular death), total thrombosis (i.e. major thrombosis plus transient ischaemic attacks, peripheral artery thrombosis or superficial thrombophlebitis), haematological transformation (i.e. leukaemia and myelodysplasia), and myelofibrosis. Myocardial infarction was defined as at least two of the following: chest pain of typical intensity and duration; ST-segment elevation or depression of 1 mm or more in any limb lead of the electrocardiogram, of 2 mm or more in any precordial lead, or both; and at least a doubling in cardiac enzymes. Stroke was defined as unequivocal signs or symptoms of a neurological deficit, with sudden onset and duration of more than 24 h. Diagnosis had to be confirmed by computed tomography, magnetic resonance imaging or by autopsy. Deep venous thrombosis was defined as a typical clinical picture with positive instrumental investigation (phlebography, ultrasonography, impedance plethysmography and computed tomography at unusual sites). In case of a suspected recurrence in a site of previous deep venous thrombosis, the diagnosis could be accepted if the instrumental test showed extension or recurrence of thrombosis as compared with previous testing. Pulmonary embolism was defined by a positive pulmonary angiogram, a high probability ventilation–perfusion scan or evidence of pulmonary embolism at necropsy. Cardiovascular death included: documented diagnosis of myocardial infarction or stroke in the absence of any other evident cause, sudden death, death from heart failure, and all deaths classified as being cardiovascular in nature. A transient ischaemic attack was defined as the abrupt onset of unilateral motor or sensory disturbance, speech defect, homonymous hemianopsia, constructional apraxia or transient monocular blindness that resolved completely in <24 h. Diagnosis and classification of leukaemia and myelodysplasia were established using the French–American–British Cooperative Group criteria (Bennett *et al*, 1982, 1985). Myelofibrosis was defined as the development of leucoerytroblastic blood picture, in the presence of splenomegaly, corroborated with a bone marrow biopsy showing diffuse bone marrow fibrosis.

The validation of causes of death, as well as thrombotic and haemorrhagic events was ensured by an *ad hoc* committee of expert clinicians. Each event was validated independently by two evaluators, and any disagreement was solved by the chairperson of the study.

Statistical analysis

Cox proportional hazards models were used to evaluate risk, with censoring at first event, death or last follow-up visit up to December 2002. Covariates were chosen based on biological plausibility as confounders and associations with exposure and outcome in the present population. Multivariate models were evaluated unadjusted, adjusted for age and gender, and further adjusted for other potential confounding factors.

Data were explored using multivariate time-dependent analysis so as to assess whether the level of exposition to a factor that had been recorded in the last clinical visit before the outcome event of interest was associated with the probability of having that event during follow-up. Time-varying covariates were used to update information on haematocrit, white blood cell, and platelet counts, and other risk factors at 1, 2, 3, 4 and 5 years. Where appropriate, the substitution of the missing data for incomplete repeated measures was done with the last

value carried forward. Indicator variables were used for missing data on baseline covariates; values were otherwise carried forward for missing time-varying covariates. Some covariates (age at recruitment, gender, time from diagnosis to enrolment, thrombotic or hemorrhagic events prior to recruitment, history of hypertension, claudication or erythromelalgia) were determined only at baseline, whereas others were updated during follow-up (smoking habits, diabetes mellitus, total blood cholesterol, splenomegaly, immature cells, haematocrit, platelet and leucocyte counts, therapeutical interventions such as phlebotomy, interferon, hydroxycarbamide, antiplatelet agents and anticoagulants; as well as myelosuppressive therapy that included ^{32}P, busulphan, chlorambucil, and pipobroman). For the variables updated during follow-up visits, the last measurement before an event was considered in the time-dependent analysis. Haematocrit and platelet count were tested as continuous variables, median values, approximate tertiles, quintiles, and deciles depending on the number of events in each analysis (i.e. robustness of the model).

Since evolution to either myelofibrosis or leukaemia could be the cause and not the result of the modification of blood parameters collected during follow-up, a multivariate analysis using values measured at baseline was used to assess whether the level of exposition for a potential-risk predictor captured at enrolment could be a statistically significant marker of increased probability of myelofibrosis or haematological transformation during follow-up.

Tests for trend were calculated by assigning the median value of each category and evaluating this as a continuous variable. Stratified analyses were used to assess for effect modification, with significance evaluated using likelihood-ratio testing and multiplicative (exposure times covariate) interaction terms. Analyses were performed with SAS 9.1 software (SAS Inst., Cary, NC, USA). All probability values are two-tailed; $P < 0{\cdot}05$ were considered significant.

Results

The baseline characteristics of the cohort are shown in Table I. Six hundred and thirty-three (39%) patients had a prior history of thrombosis, which was an arterial thrombotic event in three quarters of the cases (Marchioli *et al*, 2005). Cerebrovascular events accounted for two-thirds of arterial thrombosis, while deep venous thrombosis represented approximately 40% of vein thromboses. A positive history of bleeding was present in 8·1% of patients of which 79 (4·8%) was major bleeding (gastrointestinal, 4·1%; intracranial, 0·7%). Patients with haematocrit values ≤45% at baseline were significantly more likely to have intermittent claudication and to have had a prior thrombotic or haemorrhagic event when compared with subjects with higher haematocrit levels.

The proportion of patients with the target haematocrit ≤45% was approximately 40% at baseline, 48% at 12 months, 49% at 24 months, 49% at 36 months, 47% at 48 months and 46% at the end of the study period (Fig 1A). The range of haematocrit levels was maintained at relatively low values throughout the study, with only 10% of the patients having a haematocrit above 50%. With PV treatment, the absolute difference of haematocrit levels at baseline between the highest and the lowest decile groups decreased during the whole follow-up period from 24% to about 5–7% (Fig 1B). Median platelet

Table I. Baseline characteristics of the European Collaboration on Low-dose Aspirin in Polycythemia Vera population according to tertiles of haematocrit and platelet count.

	Haematocrit (%)			Platelet count (×10^9/l)			
	≤45 (n = 556)	46–50 (n = 530)	>50 (n = 345)	≤300 (n = 592)	301–500 (n = 622)	>500 (n = 407)	Total (n = 1638)
Females	50·9	37·7	33·9	33·8	47·0	48·2	42·5
Age at diagnosis, years (mean ± SD)	61·5 (12·8)	60·2 (13·4)	59·9 (12·9)	59·0 (13·3)	61·0 (12·8)	61·5 (13·5)	60·4 (13·2)
Age at recruitment, years (mean ± SD)	67·3 (12·0)	65·0 (12·6)	63·3 (12·9)	64·4 (12·9)	66·2 (12·3)	65·5 (12·7)	65·4 (12·7)
Years from diagnosis of PV to enrolment (mean ± SD)	5·8 (5·0)	4·8 (4·8)	3·4 (4·3)	5·4 (5·3)	5·2 (4·9)	4·0 (4·5)	5·0 (5·0)
Prior bleeding	10·1	7·6	5·8	8·3	8·8	6·9	8·1
Prior thrombosis	45·1	38·7	29·9	35·0	40·5	41·8	38·6
Erythromelalgia	5·4	5·3	6·4	3·0	4·5	9·6	5·3
Intermittent claudication	6·7	3·6	3·2	5·4	4·0	4·7	4·7
Smoke	10·3	13·6	15·9	15·4	10·6	12·8	12·8
Hypertension	42·6	40·2	39·1	40·4	38·1	40·5	39·5
Diabetes mellitus	6·5	7·4	7·0	6·9	7·9	6·6	7·1
Splenomegaly	38·3	45·7	55·9	41·1	45·8	49·1	44·7
Immature cells (any type or number)	4·3	4·2	7·5	4·9	5·6	4·4	5·1
Packed cell volume (l/l) (mean ± SD)	0·415 (0·036)	0·476 (0·014)	0·556 (0·044)	0·467 (0·061)	0·470 (0·063)	0·480 (0·065)	0·472 (0·063)
Platelet count (×10^9/l) (mean ± SD)	384 (198)	385 (197)	444 (243)	214 (55·0)	391 (59·2)	678 (185)	398 (208)

PV, polycythemia vera.

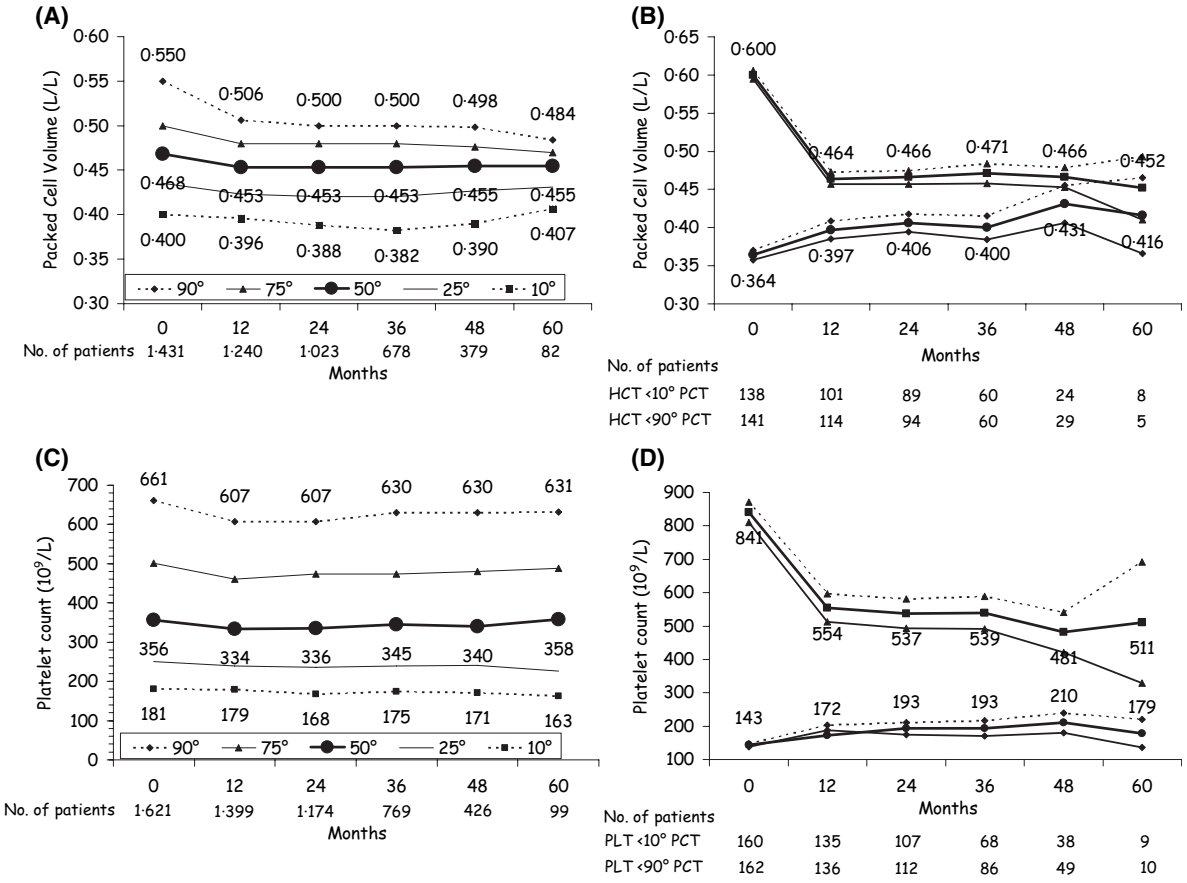

Fig 1. Distribution (percentiles) of haematocrit values and platelet count at baseline and during follow-up of 1638 polycythemia vera patients enrolled in the European Collaboration on Low-dose Aspirin in Polycythemia Vera study. (A) Haematocrit levels distribution in the whole study population (each time point is shown independently from the other ones). (B) Haematocrit control during follow-up by selecting patients in the highest and lowest deciles of haematocrit at recruitment. (C) Platelet count at baseline and during follow-up in the whole study population (each time point is shown independently from the other ones). (D) Platelet count control during follow-up by selecting patients in the highest and lowest deciles of platelet count at recruitment.

count was 356×10^9/l at baseline. Thirty-six per cent of patients had levels higher than 400×10^9/l at 12 months, and 62% had platelets below this cut-off at 60 months (Fig 1C and D).

Analysis of mortality, major and total thrombosis and bleeding

In a follow-up of 4·393 person-years (median 2·8 years), a total of 164 deaths (10%), 145 (8·9%) major thrombosis, 226 (13·8%) total thrombosis, and 35 (2·1%) major bleeds were observed. At multivariate analysis, major thrombosis was associated with age above 65 years, history of thrombosis [hazard ratio (HR), 1·74; 95% CI: 1·21–2·51, $P = 0·0031$], arterial hypertension, and claudication (data not shown). Total thrombosis was significantly associated with age above 65 years, history of thrombosis and inversely related to antiplatelet therapy. Total mortality was significantly associated with age above 65 years, history of thrombosis, antiplatelet therapy, diabetes, smoking, prior bleeding and splenomegaly (data not shown).

Haematocrit was neither related to any of the thrombotic outcomes nor to bleeding events in univariate and multivariate analyses. The risk for major thrombosis remained similar across haematocrit deciles (Table II and Fig 2A), with analogous results for total thrombosis (Table II and Fig 2B), and mortality (Table II and Fig 2C). When compared to patients with haematocrit levels ≤45%, those with haematocrit above 45% had a comparable risk of death (HR 0·85; 95% CI: 0·60–1·31, $P = 0·3761$), major thrombosis (HR 0·94; 95% CI: 0·65–1·36, $P = 0·7396$) or total thrombosis (HR 0·97; 95% CI: 0·72–1·30, $P = 0·8171$). The results of unadjusted analyses for haematocrit levels did not change meaningfully in the four progressively adjusted, time-dependent predictive models (Table II).

As shown in Fig 3B and C, platelet count was neither significantly associated with thrombotic events nor with total mortality. Major thrombotic events occurred in 8·3% of patients with a platelet count at baseline above 400×10^9/l vs. 9·3% of those with lower platelet levels (HR 0·96; 95% CI: 0·66–1·38, $P = 0·8107$).

Table II. Time-dependent multivariate analysis on the relative risk of major thrombosis, total thrombosis and death among 1638 men and women with polycythemia vera.

	Haematocrit (%)		Platelet count ($\times 10^9$/l)	
Hazard ratio (95% CI) *P*-value	46–50 ($n = 530$)	>50 ($n = 345$)	301–500 ($n = 622$)	>500 ($n = 407$)
Major thrombosis ($n = 145$)				
(1) Unadjusted	0·80 (0·55–1·17) 0·2480	0·88 (0·53–1·48) 0·6320	0·86 (0·60–1·24) 0·4183	0·82 (0·53–1·28) 0·3794
(2) Age- and gender adjusted	0·87 (0·60–1·28) 0·4890	0·96 (0·57–1·62) 0·8759	0·88 (0·61–1·27) 0·5028	0·85 (0·54–1·32) 0·4600
(3) +Disease duration-, prior thrombosis- and prior haemorrhage adjusted	0·88 (0·60–1·29) 0·5043	1·04 (0·61–1·75) 0·8941	0·84 (0·58–1·21) 0·3460	0·78 (0·50–1·22) 0·2834
(4) +RF-, and comorbidity adjusted	0·84 (0·57–1·24) 0·3796	0·98 (0·57–1·67) 0·9364	0·77 (0·53–1·13) 0·1782	0·64 (0·39–1·03) 0·0656
(5) +Cytoreductive- and antithrombotic treatment adjusted	0·89 (0·60–1·34) 0·5844	1·04 (0·61–1·78) 0·8884	0·78 (0·53–1·15) 0·2099	0·67 (0·41–1·09) 0·1099
Total thrombosis ($n = 226$)				
(1) Unadjusted	0·86 (0·64–1·16) 0·3173	0·76 (0·50–1·16) 0·1992	0·91 (0·68–1·21) 0·5131	0·80 (0·56–1·14) 0·2199
(2) Age- and gender adjusted	0·93 (0·68–1·25) 0·6130	0·82 (0·53–1·25) 0·3487	0·91 (0·68–1·22) 0·5264	0·81 (0·57–1·16) 0·2556
(3) +Disease duration-, prior thrombosis- and prior haemorrhage adjusted	0·94 (0·70–1·28) 0·7030	0·90 (0·59–1·38) 0·6257	0·86 (0·64–1·16) 0·3277	0·75 (0·52–1·07) 0·1120
(4) +RF-, and comorbidity adjusted	0·89 (0·66–1·21) 0·4699	0·84 (0·54–1·30) 0·4259	0·80 (0·59–1·09) 0·1598	0·64 (0·44–0·95) 0·0249
(5) +Cytoreductive- and antithrombotic treatment adjusted	0·98 (0·71–1·34) 0·8752	0·91 (0·59–1·42) 0·6888	0·85 (0·62–1·15) 0·2906	0·70 (0·48–1·04) 0·0801
Death ($n = 164$)				
(1) Unadjusted	0·74 (0·52–1·04) 0·0858	0·54 (0·30–0·97) 0·0399	1·04 (0·74–1·47) 0·8040	0·90 (0·59–1·38) 0·6291
(2) Age- and gender adjusted	0·85 (0·60–1·22) 0·3806	0·63 (0·35–1·14) 0·1263	1·08 (0·77–1·52) 0·6646	0·95 (0·62–1·45) 0·8042
(3) +Disease duration-, prior thrombosis- and prior haemorrhage adjusted	0·88 (0·61–1·25) 0·4664	0·71 (0·39–1·28) 0·2531	1·03 (0·73–1·46) 0·8581	0·90 (0·59–1·38) 0·6333
(4) +RF-, and comorbidity adjusted	0·82 (0·57–1·17) 0·2706	0·64 (0·35–1·18) 0·1509	0·90 (0·63–1·28) 0·5509	0·69 (0·44–1·09) 0·1125
(5) +Cytoreductive- and antithrombotic treatment adjusted	0·91 (0·63–1·32) 0·6258	0·68 (0·37–1·26) 0·2209	0·92 (0·64–1·32) 0·6555	0·72 (0·45–1·15) 0·1677

Risk estimates are given as hazard ratio (95% CI) *P*-value. RF, risk factor (reference categories: haematocrit ≤45%, $n = 556$; platelet count ≤300 × 10^9/l, $n = 592$).

Model 1: haematocrit or platelet count (three categories each, in two separate analyses). Model 2: model 1 + age (four categories), gender. Model 3: model 2 + time from polycythemia vera diagnosis to recruitment (five categories), thrombotic or haemorrhagic events prior to recruitment (yes/no). Model 4: model 3 + smoking (yes/no), history of diabetes (yes/no), hypertension (yes/no), claudicatio intermittens (yes/no), erythromelalgia (yes/no), splenomegaly (yes/no), circulating immature cells (yes/no), leucocyte count (tertiles), total blood cholesterol (tertiles), haematocrit or platelet count (three categories each, in the pertinent model). Model 5: model 4 + phlebotomy use (yes/no), interferon use (yes/no), hydroxycarbamide use (yes/no), antiplatelets use (yes/no), anticoagulants use (yes/no), ^{32}P use (yes/no), busulphan use (yes/no), chlorambucil use (yes/no), and pipobroman use (yes/no).

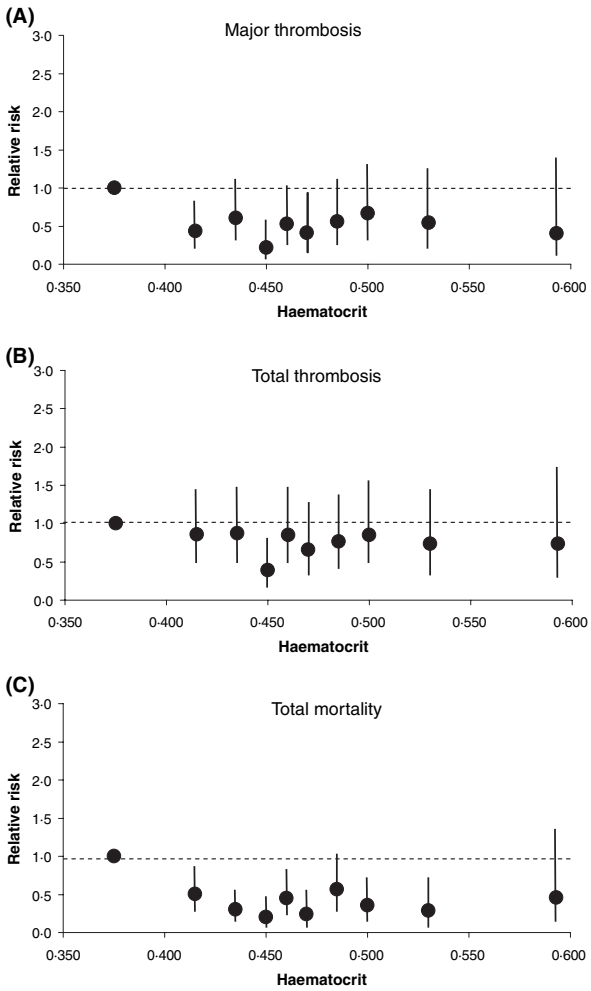

Fig 2. Time-dependent multivariable analysis for (A) major thrombosis, (B) total thrombosis, and (C) total mortality according to deciles of haematocrit. Circles with vertical bars indicate hazard ratios along with their 95% CI. Reference category denotes lowest decile. When the 95% CI (vertical line) crosses the line of no effect (horizontal line) the results are not statistically significant.

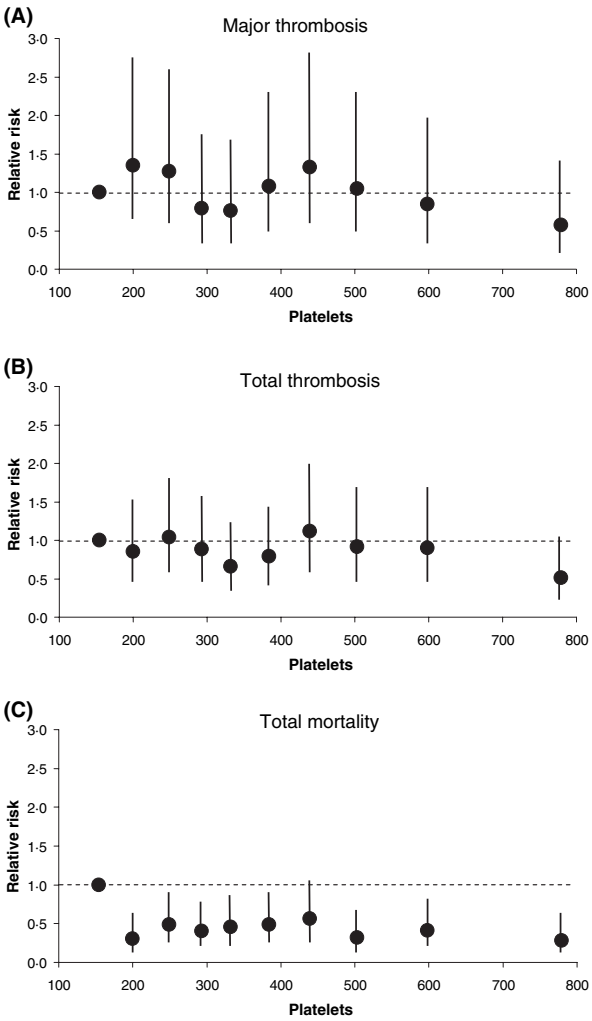

Fig 3. Time-dependent multivariable analysis for (A) major thrombosis, (B) total thrombosis, and (C) and total mortality according to deciles of platelet count. Circles with vertical bars indicate hazard ratios along with their 95% CI. Reference category denotes lowest decile. When the 95% CI (vertical line) crosses the line of no effect (horizontal line) the results are not statistically significant.

Age, disease duration and history of bleeding were the only variables significantly associated with the risk of total bleeding during follow-up. A history of previous bleeding was correlated with subsequent major bleeding (data not shown). There was no association between haematocrit or platelet count and total bleeding or major bleeding events.

Analysis of haematological transformation

There were 22 (1·3%) cases of acute leukaemia and 38 (2·3%) myelofibrosis. Age ≥70 years and cytoreductive drugs (other than hydroxycarbamide and interferon) predicted the risk of leukaemia, whereas a long disease duration was significantly associated with an increased risk of developing myelofibrosis (Marchioli *et al*, 2005).

High haematocrit was not associated with progression to leukaemia, whereas haematocrit values above the third tertile (above 50%) seemed to predict a higher risk for myelofibrosis (HR 1·84; 95% CI: 0·71–4·79, $P = 0·2101$) (Table III). Patients with a platelet count in the second (301–500 $\times 10^9$/l) or third category (above 500 $\times 10^9$/l), respectively, had a 54% (HR 0·46; 95% CI: 0·21–1·02, $P = 0·0550$) and 66% (HR 0·34; 95% CI: 0·12–0·97, $P = 0·0431$) lower risk of myelofibrosis when compared to those with platelets in the reference category (platelet count ≤300 $\times 10^9$/l). However, when we assessed the prognostic role of the haematocrit and platelet count measured at baseline, no statistically significant association could be found.

Discussion

The present analysis of outcome events during follow-up with the more recent laboratory data obtained before the occur-

Table III. Multivariable analysis on the relative risk of haematological transformation and myelofibrosis among 1638 men and women with polycythemia vera using baseline values and time-varying covariates.

	Haematocrit (%)		Platelet count ($\times 10^9$/l)	
Hazard ratio (95% CI) *P*-value	46–50 ($n = 530$)	>50 ($n = 345$)	301–500 ($n = 622$)	>500 ($n = 407$)
Haematological transformation ($n = 22$)				
Baseline analysis	2·18 (0·73–6·55) 0·1632	0·74 (0·17–3·23) 0·6914	0·55 (0·18–1·65) 0·2852	0·39 (0·09–1·60) 0·1904
Time-dependent analysis	1·36 (0·48–3·86) 0·5645	0·43 (0·05–3·81) 0·4482	0·14 (0·03–0·62) 0·0101	0·64 (0·18–2·31) 0·4966
Myelofibrosis ($n = 38$)				
Baseline analysis	0·71 (0·31–1·62) 0·4152	1·35 (0·54–3·39) 0·5236	1·32 (0·58–3·01) 0·5081	0·97 (0·35–2·70) 0·9458
Time-dependent analysis	0·56 (0·23–1·35) 0·1951	1·84 (0·71–4·79) 0·2101	0·46 (0·21–1·02) 0·0550	0·34 (0·12–0·97) 0·0431

Haematocrit or platelet count (three categories each, in two separate analyses), adjusted for age (three categories), gender, time from polycythemia vera diagnosis to recruitment (five categories), thrombotic or haemorrhagic events prior to recruitment (yes/no), smoking (yes/no), history of diabetes (yes/no), hypertension (yes/no), claudicatio intermittens (yes/no), erythromelalgia (yes/no), splenomegaly (yes/no), circulating immature cells (yes/no), leucocyte count (tertiles), total blood cholesterol (tertiles), haematocrit or platelet count (three categories each, in the pertinent model), phlebotomy use (yes/no), interferon use (yes/no), hydroxycarbamide use (yes/no), antiplatelets use (yes/no), anticoagulant use (yes/no), ^{32}P use (yes/no), busulphan use (yes/no), chlorambucil use (yes/no), and pipobroman use (yes/no) (reference categories: haematocrit ≤45%, $n = 556$; platelet count ≤300×10^9/l, $n = 592$).

rence of that same event and its results does not support a prognostic value of haematocrit in PV complications, namely thrombosis events, haematological progression and myelofibrosis. While significantly higher at baseline, the haematocrit of patients in the highest decile was conservatively reduced by treatment, to levels comparable with the median value of haematocrit during follow-up. The same phenomenon, although in the opposite direction, was observed for patients in the lowest decile of haematocrit at baseline. With the extremes of haematocrit distribution maintained throughout follow-up, within a narrow interval around 45% (Fig 1B), high haematocrit was neither found as a significant predictor of death, thrombotic events nor haematological progression. Our findings suggest that high platelet count might be associated with a decreased risk of haematological transformation and myelofibrosis.

Thrombosis, myelofibrosis and acute leukaemia frequently complicate the course of PV (Spivak, 2002; Spivak *et al*, 2003). The relevance of the haematocrit and platelet count in predicting any of these outcomes has not been clearly established in prospective studies. According to a recent survey conducted among North America haematologists, there seems to be little consensus and high variability over the control of the haematocrit and/or thrombocytosis in PV (Streiff *et al*, 2002). Such heterogeneity in current clinical practice might possibly reflect the uncertainty over the benefit of strict haematocrit and/or thrombocytosis control.

Mortality, major and total thrombosis, and bleeding

Based on some initial observations suggesting a higher risk of thrombosis at moderately increased haematocrit levels, it has been advised that the haematocrit should be maintained below 45% in males and 42% in females (Pearson & Wetherley-Mein, 1978; Spivak *et al*, 2003). The haematocrit could increase the risk of thrombosis by several mechanisms, such as raising blood viscosity, impacting on nitrous oxide level or by enhancing platelet–vessel wall interactions (Spivak, 2002; Schafer, 2006). Data in support of an association between elevated haematocrit and thrombotic events, however, have not been always concordant (Wehmeier *et al*, 1991; Berk *et al*, 1995).

The PVSG-01, the largest prospective PV cohort together with the ECLAP, included 431 patients (Berk *et al*, 1995). In the PVSG-01, no haematologic parameter measured at the closest observation prior to the thrombotic event was associated with increased risk of thrombosis. In addition, patients of the PVSG protocol developed thrombotic complications when the haematocrit was reasonably well controlled by phlebotomy or myelosuppression. Other studies suggested that haematocrit did not correlate with thrombosis in Chuvash polycythemia (Gordeuk *et al*, 2004; Gordeuk & Prchal, 2006), while large studies of patients with polycythemia of high altitude or resulting from Eisenmenger syndrome and other cyanotic heart diseases do not support the haematocrit as the only factor causing thrombosis (Thorne, 1998; Vongpatanasin *et al*, 1998; Prchal & Beutler, 2005). Although haematocrit levels could be not strictly correlated with red blood cell mass, which has been suggested to be the real causative factor associated with the risk of thrombosis in PV (Spivak, 2002), our findings are in agreement with these previous results and do not support a predictive value of haematocrit for death or thrombotic events in patients receiving current antithrombotic and cytoreductive treatments, the latter allowing the haematocrit level to be maintained below 45% in half of PV patients and below 50% in more than 90% of PV subjects.

Several differences might explain the conflicting data between the initial observations (Pearson & Wetherley-Mein,

1978) and later studies. First, previous studies included a limited number of patients who did not receive an adequate control of cardiovascular risk factors, antihypertensive and antiplatelet therapy, and cytoreductive therapy as in the ECLAP. Moreover, methodological limitations, such as the use of univariate analysis and of not taking into account the dependency of observations, might have biased some previous conclusions (Pearson & Wetherley-Mein, 1978).

A correlation between haematocrit and cardiovascular disease has been previously reported in patients without PV (Sorlie *et al*, 1981; Erikssen *et al*, 1993; Brown *et al*, 2001; Irace *et al*, 2003). Differences in study populations or the use of haematocrit measurements at inclusion, rather multiple determinations and a time-dependent analysis, and a univariate analysis of the data may partially explain the contrasting results.

Despite the widespread belief that thrombotic tendency in PV may be related to thrombocytosis, no study to date, either prospective or retrospective, has demonstrated a significant correlation between platelet number or function and thrombosis (Spivak, 2002; Schafer, 2006). In the PSVG study, platelet counts at the nearest times before the thrombotic events did not predict thrombosis (Berk *et al*, 1995). Accordingly, we did not find any association between platelet count and thrombotic events. Neither the currently proposed target of 400×10^9/l nor any of the platelet count deciles predicted a higher risk of thrombosis. In our study, the platelet count remained relatively high during the whole study period, which seems to suggest that current PV treatment does not primarily aim at lowering the platelet count (Fig 3).

Platelet activation, rather than platelet count, might be an important determinant of thrombotic events in PV. While no specific platelet abnormality seems to correlate to an increased thrombotic risk, platelet activation, as indicated by increased thromboxane B formation, has been described in PV (Landolfi *et al*, 1992; Murphy, 1995). Accordingly, the trial component of the ECLAP study recently showed a significant 60% reduction of the combined endpoint of non-fatal myocardial infarction, non-fatal stroke or death from cardiovascular causes in PV patients assigned to aspirin, when compared with those receiving placebo (Landolfi *et al*, 2004).

Recently, a point mutation (V617F) in *JAK2* has been described in 70–95% of PV patients (Schafer, 2006) and preliminary data suggest a pivotal role of this mutation in the PV phenotype. New biomarkers, such as *JAK2*, may prove useful in future but more extensive research is needed to clarify their predictive role and their utility as surrogate endpoints.

A high platelet count has been associated with a haemorrhagic diathesis in patients with PV and the literature has rather consistently showed that a reduction of platelet count with myelosuppressive therapy reduces the bleeding rate (Chien & Gallik, 1995; Schafer, 2006). In the PSVG-05 trial, high platelet count tended to be associated with a higher risk of haemorrhage not thrombosis (Berk *et al*, 1995). A higher bleeding risk at high platelet count could be explained by von Willebrand factor deficiency caused by an increased clearance through platelet-dependent mechanisms. Our study does not confirm a pro-haemorrhagic tendency in patients with a high platelet count, although the proportion of patients with extreme thrombocytosis was very low.

Haematological transformation and myelofibrosis

The prognostic value of the haematocrit or platelet count for PV progression to leukaemia/myelofibrosis has not been investigated. In the current analysis, we found no association between haematological progression and haematocrit whereas there was a trend for a higher risk of myelofibrosis at haematocrit levels above the highest tertile (HR 1·84; 95% CI: 0·71–4·79).

Haematological complications seemed to occur more frequently in patients with a low platelet count. During follow-up, in the time-dependent analysis, patients with platelets $\leq 300 \times 10^9$/l had a more than threefold and twofold higher risk of developing acute leukaemia (HR 3·69; 95% CI: 1·31–10·45, $P = 0{\cdot}0138$) and myelofibrosis (HR 2·40; 95% CI: 1·18–4·87, $P = 0{\cdot}0157$), respectively, than for higher platelet counts. Such an association, however, could be due to the development of haematological transformation, although a non-statistically significant high rate of haematological malignancy was evident at baseline in subjects with a low platelet count. The association between haematocrit or platelet count and haematological transformation remains unclear. It could be speculated that high haematocrit and/or low platelets identify a subgroup of patients with a more aggressive form of the disease who are more likely to develop haematological complications. However, the relatively low absolute number of cases of leukaemia–myelofibrosis in the ECLAP study, while comparable with previous trials (Finazzi *et al*, 2005), does not enable firm conclusions to be drawn. Thus, the present findings have to be taken with caution and need confirmation in large prospective trials.

In summary, the results of the present analysis seem not to support a prognostic importance of haematocrit and platelet count in PV and challenge the need for an aggressive control of these parameters in patients with PV for the prevention of thrombohaemorrhagic complications. The current findings, together with currently available evidence, underscore the lack of specific therapeutic targets in the management of PV.

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