Correlation of blood counts with vascular complications in essential thrombocythemia: analysis of the prospective PT1 cohort

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Running title:
Blood counts in essential thrombocythemia
ABSTRACT

Essential thrombocythemia, a myeloproliferative neoplasm, is associated with increased platelet count and risk of thrombosis or hemorrhage. Cytoreductive therapy aims to normalize platelet counts, despite minimal association between platelet count and complication rates. Evidence is increasing for correlation between white cell count and thrombosis but prospective data are lacking. We studied the relationship between vascular complications and 21,887 longitudinal blood counts in a prospective, multicenter cohort of 776 ET patients. After correction for confounding variables, no association was seen between blood counts at diagnosis and future complications. However, platelet count outside normal range during follow-up was associated with immediate risk of major hemorrhage (p=0.0005) but not thrombosis (p=0.7). Elevated white cell count during follow-up correlated with thrombosis (p=0.05) and major hemorrhage (p=0.01). These data imply that the aim of cytoreduction in essential thrombocythemia should be to keep the platelet count, and arguably the white cell count, within the normal range. This study is registered at http://isrctn.org as #72251782.
INTRODUCTION

The myeloproliferative neoplasms (MPNs) comprise several chronic hematological malignancies with overlapping clinical and molecular features\(^1\). Essential thrombocythemia (ET), the most prevalent of the MPNs, is characterized by raised platelet counts, increased risk of hemorrhage and thrombosis, both arterial and venous, and long-term risk of transformation to myelofibrosis or acute leukemia. Current practice aims to reduce platelet counts in ET to the normal range in high-risk patients\(^2,3\). However, observational studies have consistently failed to show a relationship between elevated platelet count, at diagnosis or during follow-up, and thrombosis risk in this disorder\(^4-6\), and it is therefore not clear what the target platelet count should be. Furthermore, there is evidence that leukocytosis at diagnosis is a more powerful prognostic factor for complications in ET and the related MPN, polycythemia vera (PV)\(^4,5,7,9\). These findings have generated considerable controversy and debate about the role of blood count variables in risk stratification\(^4,10\).

Here, we analyse the relationship between blood counts and complications in the PT-1 trial, the largest multicenter randomized study performed in ET. This cohort of patients has been extensively characterized through molecular studies\(^11-14\), histological analysis\(^15,16\) and long-term clinical follow-up\(^17\). Longitudinal blood counts have been collected over many years.

METHODS

The PT-1 trial has relevant ethics and MHRA approvals in all participating countries. Patients with ET were entered into one of three PT-1 studies.
depending on their risk of vascular complications. High-risk patients were treated with aspirin plus either hydroxyurea or anagrelide on a randomized basis\textsuperscript{17}. Intermediate-risk ET patients aged 40-60 years with no vascular risk factors were randomized to either hydroxyurea plus aspirin or aspirin alone. The low-risk study is a cohort study of aspirin therapy alone in patients less than 40 years with no vascular risk factors. Details of the trials have been previously described\textsuperscript{17}. The trial opened in 1997, and involves UK, Ireland, France, Australia and New Zealand. The high-risk arm closed in 2003 with the other two arms still open to recruitment. The cohort here comprises 776 patients who have been genotyped for the \textit{JAK2} V617F mutation\textsuperscript{13}, followed prospectively for median 36 months (range, 2-87 months). Data from 21,887 full blood counts performed during follow-up were available.

We fitted semi-parametric Cox proportional hazards regression models with a penalized cubic spline basis for the blood count predictor\textsuperscript{18}. Only the first end-point event per patient was included. All models included sex, \textit{JAK2} status, randomized treatment and past history of the relevant end-point as covariates. End-points were thrombosis (deep or splanchnic vein thrombosis, pulmonary embolism, myocardial infarction, acute coronary syndrome, stroke, transient ischemic attack, or peripheral artery thrombosis) and major hemorrhage (intracranial, or requiring transfusion >2 Units, or causing drop in hemoglobin >2g/dL). The hypothesis of interest was whether inclusion of blood count variables into the model improved the fit, assessed with likelihood ratio tests. The relationship between complications and blood count data during follow-up was studied by treating blood count as a time-dependent covariate, with each
individual’s follow-up broken into distinct, potentially discontinuous, periods of time linked to the nearest blood count immediately preceding, up to 60 days maximum\textsuperscript{19}. Also, age and transformation to myelofibrosis were included as time-dependent covariates. Most high-risk patients remained on randomized therapy (88\% of blood count intervals for hydroxyurea; 76\% for anagrelide), with no material difference in results if treatment received is included in the model instead of randomization allocation.

**RESULTS & DISCUSSION**

In previous studies, analyses of blood counts and risk of complications have generally either assumed the relationship to be linear or divided the continuous blood count variable arbitrarily at specific cut-points. However, the relationship may well be non-linear and is unlikely to be discontinuous at the cut-points chosen. To circumvent these issues, we fitted a flexible semi-parametric family of curves, known as cubic splines\textsuperscript{18}, to model the relationship between blood count variables and risk of complications in 776 patients with ET, after correction for known confounding factors. Overall, 58 patients in the cohort had at least one thrombosis and 31 had a major hemorrhage (supplementary table 1).

We found no significant association between blood counts at diagnosis and future risk of thrombosis (platelets, \(p=0.4\); WCC, \(p=0.6\); Hb, \(p=1.0\)), major hemorrhage (platelets, \(p=0.6\); WCC, \(p=0.6\); Hb, \(p=0.6\)) or transformation to leukemia, myelodyplasia or myelofibrosis (platelets, \(p=0.5\); WCC, \(p=0.9\); Hb, \(p=0.8\)). Some studies in ET have demonstrated an association between
leukocytosis at diagnosis and later complications\textsuperscript{7,20}, but not other studies\textsuperscript{21}. Reasons for the discrepancies among these studies include differences in statistical methodology, length of follow-up and play of chance.

Using data from 21,887 full blood counts collected longitudinally in the 776 patients, we assessed whether blood counts during follow-up were associated with vascular complications in the 60 days after the date of the blood count (supplementary table 2). Risk of thrombosis, comprising both arterial and venous thrombosis, was not significantly associated with platelet count during follow-up (p=0.4, figure 1A). The fitted curve shows that the risk (hazard ratio) is relatively flat throughout the range of platelet counts, although confidence intervals are wide at platelet counts over 1000x10\textsuperscript{9}/L. In contrast, we found a significant association between white cell count and risk of thrombosis (p=0.03, figure 1B), with a nearly linear relationship and no particular threshold at which the risk begins to increase. We found no association between hemoglobin concentration and risk of thrombosis (p=0.8, figure 1C).

Interestingly, we found a U-shaped curve for the relationship between platelet count and risk of major hemorrhage (p=0.0005, figure 2A). There is strong evidence for an association between hemorrhage and a low platelet count, with thrombocytopenia here generally resulting from excessive cytoreduction. Although confidence intervals are wide, those time periods in which platelet counts were above the normal range (>450x10\textsuperscript{9}/L) as opposed to within normal limits were also associated with an increased risk risk of hemorrhage (HR, 3.7; 95\% CI, 1.7-8.2; p=0.001). The curve for white cell count also showed a U-shaped
pattern \( p=0.01 \); figure 2B). Finally, a low hemoglobin concentration also correlated with major hemorrhage \( p<0.0001 \), figure 2C). It is likely, however, that this association represents patients with an acute hemorrhage on the background of chronic blood loss, rather than a potentially causal link between low hemoglobin and hemorrhage.

Taken together, these data indicate that platelet count in ET correlates with the immediate risk of major hemorrhage but not thrombosis. Strikingly, the risk of major hemorrhage is at its lowest with a platelet count in the normal range, and therefore provides evidence for the recommendation that the goal of cytoreductive therapy in this condition should be to keep the platelet count in the normal range. Additionally, we have shown a linear association between white cell count during follow-up and risk of thrombosis, as previously documented. There is much biological evidence that white cells contribute to thrombosis in these conditions, and our data support this. There has been some debate about whether normalizing white cell count should be an additional aim of cytoreductive therapy in ET. Our data show a clear correlation, but do not necessarily imply that the adverse risk associated with high white cell counts is modifiable by therapy. The strength and consistency of the observational data do, however, strongly support formal testing of the hypothesis in a randomized controlled trial.

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**AUTHOR CONTRIBUTIONS**

PJC, CM, PAB, GB and KW participated in study concept, study design and collection of data. The statistical analysis was performed by PJC under the supervision of KW. PJC, JJK, CF, CNH and ARG co-ordinate and oversee the multicenter prospective PT-1 trial.

**CONFLICT OF INTEREST**

The authors have no conflicts of interest to declare.
REFERENCES


FIGURE LEGENDS

Figure 1. Relationship between blood counts during follow-up and risk of thrombosis. The curve of best fit is shown as a thick green line with the 95% confidence intervals for the curve shown as thin green lines. A hazard ratio of 1 (dashed line) indicates no increased risk of thrombosis, whereas >1 indicates increased risk. Counts at which events occurred are marked with an orange line above the x axis; counts at which no events occurred are marked with black ticks. Curves are shown for platelet count (A), white cell count (B) and hemoglobin concentration (C).

Figure 2. Relationship between blood counts during follow-up and risk of major hemorrhage. The curve of best fit is shown as a thick green line with the 95% confidence intervals for the curve shown as thin green lines. A hazard ratio of 1 (dashed line) indicates no increased risk of thrombosis, whereas >1 indicates increased risk. Counts at which events occurred are marked with an orange line above the x axis; counts at which no events occurred are marked with black ticks. Curves are shown for platelet count (A), white cell count (B) and hemoglobin concentration (C).
Figure 2

A

Estimated hazard ratio

Platelets (x10^9/L)

B

Estimated hazard ratio

WBC (x10^9/L)

C

Estimated hazard ratio

Hb (g/dL)

p<0.001

p=0.02

p<0.001
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