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Reticulin Accumulation in Essential Thrombocythemia: Prognostic Significance and Relationship to Therapy

Peter J. Campbell, **David Bareford**, **Wendy N. Erber**, **Bridget S. Wilkins**, **Penny Wright**, **Georgina Buck**, **Keith Wheatley**, **Claire N. Harrison**, and **Anthony R. Green**
Department of Haematology, University of Cambridge; Departments of Haematology and Histopathology, Cambridge University Hospitals National Health Service Foundation Trust, Cambridge; Cancer Genome Project, Wellcome Trust Sanger Institute, Hinxton; Department of Haematology, Russells Hall Hospital, Dudley; Birmingham Clinical Trials Unit, University of Birmingham, Birmingham; Departments of Histopathology and Haematology, St Thomas' Hospital, London; and the Clinical Trial Service Unit, Oxford, United Kingdom

Abstract

Purpose—Essential thrombocythemia (ET) manifests substantial interpatient heterogeneity in rates of thrombosis, hemorrhage, and disease transformation. Bone marrow histology reflects underlying disease activity in ET but many morphological features show poor reproducibility.

Patients and Methods—We evaluated the clinical significance of bone marrow reticulin, a measure previously shown to have relatively high interobserver reliability, in a large, prospectively-studied cohort of ET patients.

Results—Reticulin grade positively correlated with white blood cell ($P = .05$) and platelet counts ($P = .0001$) at diagnosis. Elevated reticulin levels at presentation predicted higher rates of arterial thrombosis (hazard ratio [HR], 1.8; 95% CI, 1.1 to 2.9; $P = .01$), major hemorrhage (HR, 2.0; 95% CI, 1.0 to 3.9; $P = .05$), and myelofibrotic transformation (HR, 5.5; 95% CI, 1.7 to 18.4; $P =$.

Corresponding author: Anthony Green, FRC Path, F Med Sci, Department of Haematology, Cambridge Institute for Medical Research, University of Cambridge, Hills Rd, Cambridge CB2 2XY, United Kingdom; arg1000@cam.ac.uk..
D.B., W.N.E., and B.S.W. contributed equally to the manuscript.

AUTHOR CONTRIBUTIONS

Conception and design: Peter J. Campbell, David Bareford, Wendy N. Erber, Bridget S. Wilkins, Claire N. Harrison, Anthony R. Green
Provision of study materials or patients: Peter J. Campbell, Claire N. Harrison, Anthony R. Green
Collection and assembly of data: Peter J. Campbell, David Bareford, Wendy N. Erber, Bridget S. Wilkins, Penny Wright, Georgina Buck, Keith Wheatley, Claire N. Harrison, Anthony R. Green
Data analysis and interpretation: Peter J. Campbell, David Bareford, Wendy N. Erber, Bridget S. Wilkins, Georgina Buck, Keith Wheatley, Claire N. Harrison, Anthony R. Green
Manuscript writing: Peter J. Campbell, Anthony R. Green
Final approval of manuscript: Peter J. Campbell, David Bareford, Wendy N. Erber, Bridget S. Wilkins, Penny Wright, Georgina Buck, Keith Wheatley, Claire N. Harrison, Anthony R. Green

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0007) independently of known risk factors. Higher reticulin levels at diagnosis were associated with greater subsequent falls in hemoglobin levels in patients treated with anagrelide ($P < .0001$), but not in those receiving hydroxyurea ($P = .9$). Moreover, serial trephine specimens in patients randomly assigned to anagrelide showed significantly greater increases in reticulin grade compared with those allocated to hydroxyurea ($P = .0003$), and four patients who developed increased bone marrow reticulin on anagrelide showed regression of fibrosis when switched to hydroxyurea. These data suggest that patients receiving anagrelide therapy should undergo surveillance bone marrow biopsy every 2 to 3 years and that those who show substantially increasing reticulin levels are at risk of myelofibrotic transformation and may benefit from changing therapy before adverse clinical features develop.

Conclusion—Our results demonstrate that bone marrow reticulin grade at diagnosis represents an independent prognostic marker in ET, reflecting activity and/or duration of disease, with implications for the monitoring of patients receiving anagrelide.

INTRODUCTION

The three major myeloproliferative disorders (MPDs) are essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF).[1-3] They share numerous phenotypic similarities, underscored by the discovery that an acquired mutation in the tyrosine kinase, *JAK2*, is found in all three diseases.[1-4] ET is characterized by thrombocytosis and associated with increased risk of arterial and venous thrombosis and hemorrhage. The bone marrow shows megakaryocyte proliferation and varying degrees of reticulin fibrosis.[5,6] ET is genetically heterogeneous, with 50% to 60% of patients carrying the *JAK2* V617F mutation and having distinct phenotypic features reminiscent of PV,[7-9] with an additional 5% carrying a mutated thrombopoietin receptor, *MPL*.[10-13]

Historically, PMF has been viewed as a distinct disease entity and it has been suggested that histologic criteria can be used to distinguish ET from the early phases of PMF.[5] We have previously assessed the role of bone marrow histology in the diagnosis of ET[6] and found that many morphological features, described as important for patient classification, show poor interobserver reliability and are not sufficiently robust to allow reproducible identification of disease subgroups. PMF is clinically indistinguishable from postpolycythemic or post-thrombocythemic myelofibrosis and it has been suggested that PMF represents patients presenting in accelerated phase of an unrecognized prior MPD.[14] According to this concept, ET and PMF form a biologic continuum with a patient's phenotype reflecting multiple factors such as disease duration and genetic background.

Reticulin grade is one morphological feature that shows strong inter-observer reproducibility and correlates with many of the other histological features associated with fibrotic progression of ET, such as new bone formation, dysplastic and clustered megakaryocytes and increased cellularity.[9] However, the relationship between the subclinical increases in reticulin seen in ET and clinical outcome has not been studied using prospective methodology.

PATIENTS AND METHODS

Study Population

The PT-1 trials enrolled newly diagnosed and previously treated patients, age ≥18 years, who met the Polycythemia Vera Study Group (PVSG) criteria for ET (Appendix Table A1, online only).[15] We have re-evaluated this cohort of patients using the 2001 WHO diagnostic criteria.[6] The current analysis, however, is based on PVSG criteria due to the poor performance of the WHO classification on our patients[6] and the need for hydroxyurea-

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anagrelide comparisons to be on an intention-to-treat basis. The trials consist of three multicenter studies, including the completed high-risk PT-1 trial[16] in which patients were randomly assigned to receive aspirin with either hydroxyurea or anagrelide. Secondly, the National Cancer Research Initiative study for intermediate-risk patients (no high-risk features and age 40 to 60 years) is an ongoing random assignment between aspirin alone or hydroxyurea plus aspirin. Thirdly, the National Cancer Research Initiative study for low-risk patients (no high-risk features and age < 40 years) is an ongoing observational cohort of patients receiving aspirin alone. Follow-up procedures and definitions of end points have been described previously.[16] Events occurring before January 31, 2006, notified by June 30, 2006, were analyzed, and follow-up is 99% complete. The median follow-up is 68 months from trial entry.

Bone Marrow Trephine Specimens

On patient registration, we requested bone marrow trephine specimens from all patients, although biopsy was not a requirement of trial entry. In 2003, before closure of the high-risk trial, we invited follow-up trephines to be performed if patients consented. Trephine biopsy sections were stained in a single laboratory using hematoxylin and eosin and Gordon and Sweets' silver stain for reticulin.

Trephine sections were assessed by three hematopathologists, as previously described.[6] Assessment was performed independently, with knowledge only of the age and sex of the patient. No information was available to the observers on treatment allocation or patient outcomes, and follow-up trephine specimens were mixed in with the diagnostic samples.

A total of 636 trephine specimens were received from 1,022 patients enrolled. Poor quality samples were excluded. The statistical analysis of prognosis is based on a set of 361 high-quality trephine specimens taken at diagnosis. Of these, 250 (69.3%) enrolled in the trial within 3 months of diagnosis, and for the other 31% of patients, the median duration of disease was 13 months at trial entry. Of the 361 patients with diagnostic trephines, *JAK2* status[7] was available for 311; this is the set of patients analyzed in Presenting Features section of the Results. Analysis of the relationship between reticulin levels and response to therapy used the 299 patients with diagnostic trephines and known *JAK2* status who entered the high-risk trial.

Ninety-seven patients enrolled in the high-risk trial had sequential trephine biopsies. Initial trephine biopsies in these patients were all performed at or preceding trial entry, but were not required to be from diagnosis.

Reticulin grade was scored using a scale[17] from 0 to 4: 0, almost complete absence of fibers; 1, few scattered fibers, predominantly around stromal vessels; 2, incomplete meshwork of randomly orientated fibers, relatively few intersections; 3, more dense and complete meshwork, still with randomly orientated fibers but with many intersections; 4, denser meshwork still, with organization of fibers into parallel arrays and areas within which organization of these parallel fibers into thicker bands is found.

Statistical Analysis

The reticulin grade for each trephine specimen was defined as the median of the scores from the hematopathologists. The associations between reticulin grade and diagnostic features were assessed by linear regression and χ^2 tests for trend. Cox proportional hazards models were used for multivariate assessment of the effects of reticulin on end points, with predetermined sets of covariates included in the models, as listed in the footnotes to Table 1. For the survival analyses, patients with grade 0 reticulin were included with grade 1, and grade 4 with grade 3, due to small numbers in grades 0 and 4, although results were not

altered when analyzed on the full 0 to 4 scale, or when patients with grade 4 were excluded from analysis.

To model response to therapy in patients in the high-risk trial, mixed effects models were fitted to blood counts at 3-month intervals treating time as a categoric variable, with an Autoregressive-Moving Average(1,1) model of within-subject correlation.[18] Age, sex, and *JAK2* status were covariates. Analyses were performed on intention-to-treat basis.

To analyze the change in reticulin over time, a linear mixed effects model was fitted. Fixed effects were the number of years from diagnosis, treatment allocation, and the hematopathologist giving the score. Where a score from one of the hematopathologists was missing at one of the time points, the score for the other time point from that observer was deleted. Interindividual differences in the rate of change in reticulin were included as a random effect, subject to within-subject continuous autoregressive(1) correlation. The analysis was undertaken on an intention-to-treat basis.

RESULTS

Presenting Features: Reticulin Grade Correlates With Clinical and Laboratory Features at Diagnosis and Is Independent of JAK2 Status

The relationships of *JAK2* status, reticulin grade at diagnosis, and presenting blood counts were studied in 311 patients with ET enrolled in the PT-1 trials. A full range of reticulin scores was seen, with grades 1 and 2 being particularly frequent, although nearly 20% of patients had a median reticulin grade of 3 after central review (Fig 1A). Fifteen patients had a reticulin grade of 4 on central review but lacked other features needed for a diagnosis of primary myelofibrosis; results are not materially altered for subsequent analyses if these patients are excluded. There was no difference in the distribution of reticulin grade between V617F-positive and V617F-negative patients.

Hemoglobin levels at diagnosis did not show significant changes as reticulin grade increased ($P = .4$), and the relationship was independent of V617F status ($P = .2$ for interaction; Fig 1B). Unexpectedly, both white cell and platelet counts showed significant increases as reticulin grade increased ($P = .05$ and $P = .0001$, respectively), occurring equally in both V617F-positive and V617F-negative patients ($P = .9$ and $P = .8$ for interaction; Figs 1C, 1D). The higher white cell and platelet counts were associated with increased levels of granulocytic and megakaryocytic cellularity in the bone marrow ($P < .0001$; Figs 1E, 1F).

These data demonstrate that increasing reticulin grade at diagnosis was associated with steadily more pronounced abnormalities in granulopoiesis and megakaryopoiesis. Both *JAK2*-positive and *JAK2*-negative patients showed these patterns, suggesting that reticulin accumulation reflects the operation of similar biologic mechanisms in both subtypes of ET.

Response to Therapy: Anagrelide, but Not Hydroxyurea, Is Associated With a Progressive Fall in Hemoglobin, Worse in Patients With Higher Reticulin Grades

To assess any association between reticulin grade at diagnosis and response to therapy, we studied follow-up blood counts from 299 patients in the high-risk PT-1 study for whom *JAK2* genotype data and diagnostic trephine specimens were available.

For patients allocated to hydroxyurea, there was an initial decrease in hemoglobin after trial entry, which subsequently stabilized (Fig 2A). The extent of this decrease was not associated with reticulin at diagnosis ($P = .9$). In contrast, for patients randomly assigned to anagrelide, higher grades of reticulin at diagnosis predicted significantly greater initial falls in hemoglobin levels with a previously unrecognized subsequent progressive decline ($P <$.

0001; Fig 2B). The difference between patients randomly assigned to hydroxyurea or anagrelide was statistically significant ($P = .01$). The effect of reticulin on hemoglobin appeared to widen with time ($P = .07$), but was independent of *JAK2* status ($P = .2$).

The effects of reticulin on platelet count were also different depending on whether patients were randomly assigned to anagrelide or hydroxyurea ($P = .04$; Figs 2C, 2D). In patients treated with anagrelide, there was no association between follow-up platelet counts and reticulin grade at diagnosis ($P = .9$; Fig 2D). In contrast, for patients treated with hydroxyurea, the association between reticulin grade and platelet count at diagnosis was maintained during follow-up ($P = .02$; Fig 2C). This effect was independent of *JAK2* status ($P = .5$). White cell count, dose of hydroxyurea, and dose of anagrelide during follow-up were not significantly correlated with reticulin grade at diagnosis (data not shown).

In summary, therapy with anagrelide, but not hydroxyurea, was associated with a progressive fall in hemoglobin, and this effect was more severe in those patients presenting with higher levels of reticulin. These results raise the possibility that patients with higher levels of reticulin who are treated with anagrelide may progress more quickly toward myelofibrosis, compared with those who receive hydroxyurea.

Clinical Outcome: Higher Reticulin Grade at Presentation Is Associated With Increased Complication Rates

To assess whether reticulin grade at diagnosis carries prognostic significance, we studied the rate of clinical complications following trial entry in the 361 patients with presentation bone marrow trephine biopsies available for analysis (Table 1; Fig 2).

On multivariate analysis, increased reticulin grade at diagnosis was not significantly associated with poorer overall survival (Fig 3A; Table 1) or with rates of venous thrombosis. Rates of arterial thrombosis (Fig 3B), major hemorrhage (Fig 3C), and myelofibrotic transformation (Fig 3D) were, however, significantly elevated in patients with higher reticulin grades in diagnostic trephines, even after correction for potential confounding factors (Table 1). For arterial thrombosis, risk of thrombosis was approximately 80% increased for every additional grade of reticulin (hazard ratio [HR], 1.8; 95% CI, 1.1 to 2.9; $P = .01$). The risk of major hemorrhage was approximately twice as high for each 1 grade increase in reticulin (HR, 2.0; 95% CI, 1.0 to 3.9; $P = .05$) and there was also a strong association between presenting reticulin grade and transformation to myelofibrosis (HR, 5.5; 95% CI, 1.7 to 18.4; $P = .0007$).

Anagrelide Is Associated With Greater Increases in Reticulin Over Time Than Hydroxyurea

To assess whether anagrelide was associated with greater progression of reticulin fibrosis over time than hydroxyurea, we studied serial trephine specimens from patients entered in the high-risk PT-1 trial. In 2003, before closure of the high-risk study, patients entered in the trial were invited to undergo a follow-up bone marrow trephine biopsy. A total of 97 patients had pretrial entry and follow-up trephines, 41 randomly assigned to hydroxyurea and 56 to anagrelide. There were no differences between the patients allocated to hydroxyurea and those allocated to anagrelide in reticulin grade of the pretrial entry trephine specimens (mean grade, 1.52 *v* 1.53, respectively; $P = .9$). The median time between biopsies was 4 years, and was not significantly different between the two groups ($P = .6$). The statistical analysis was performed on an intention-to-treat basis, correcting for differences in the length of follow-up.

Most patients randomly assigned to hydroxyurea showed little change in the level of reticulin over time (Fig 4A), with an average increase of 0.03 grades of reticulin per year (95% CI, −0.05 to 0.11 grades/year). In marked contrast, many patients randomly assigned

to anagrelide showed substantial increases in reticulin burden over time, with levels rising an average 0.23 grades per year (95% CI, 0.16 to 0.30 grades/year; Fig 4B). The difference between anagrelide and hydroxyurea in the change in reticulin over time was highly significant ($P = .0003$).

This difference between anagrelide and hydroxyurea remained statistically significant if each hematopathologist's reticulin scores were analyzed individually; if patients with reticulin grades of 3 to 4 on the pretrial entry specimen were excluded; if the analysis only included patients in whom the earlier trephine specimen was taken at diagnosis; or if only patients who were newly diagnosed at trial entry were analyzed.

Interestingly, patients with more pronounced increases in reticulin between pretrial entry and follow-up trephines showed significantly greater falls in hemoglobin levels during follow-up ($P < .0001$) and significantly greater rises in white cell count overtime ($P < .0001$).

These data suggest that anagrelide is associated with a greater accumulation of reticulin than hydroxyurea, and that this correlates with adverse changes in blood counts.

Reticulin Fibrosis Associated With Anagrelide Therapy May Be Reversible on Hydroxyurea

The preceding sections raise the question of whether progression of reticulin fibrosis on anagrelide is reversible on hydroxyurea. We studied serial biopsies from four patients who were randomly assigned to receive anagrelide and were switched to hydroxyurea therapy at the cessation of the trial. None showed clinical evidence of myelofibrotic transformation, and the treatment change was decided after the recommendation from the steering committee to switch high-risk patients on anagrelide to hydroxyurea. Biopsies were performed at trial entry, immediately before cessation of anagrelide, and after 18 to 24 months of hydroxyurea treatment.

Of the four patients, three showed an increase in reticulin grade from 0 to 1 to 3 to 4 while on anagrelide therapy (Fig 4C). After switching to hydroxyurea, these three patients all decreased to grade 2 reticulin. Despite the small numbers, the difference between the change in reticulin while on anagrelide (mean change, 2.4 grades) and on hydroxyurea (mean change, −1.4 grades) was statistically significant ($P = .04$). Representative photomicrographs of the three serial trephine specimens from two of the patients are shown in Figure 5.

DISCUSSION

It has been recognized for some years that patients with chronic phase ET show extensive heterogeneity in the levels of bone marrow reticulin at diagnosis, but the clinical significance of this has not been well documented. Herein we have studied the importance of bone marrow reticulin in patients with ET entered in the PT-1 trials. These patients represent a large and carefully characterized prospective cohort, with rigorously conducted evaluation of clinical and histological data, detailed treatment history, and well-documented molecular status.

In normal individuals, the level of bone marrow reticulin is generally grade 0 to 1, occasionally 2, at the iliac crest,[17] and our findings therefore indicate that many patients with ET have accumulated elevated reticulin levels even by the time of diagnosis. Reticulin levels correlate with increases in the white cell count, platelet count, and marrow granulocytic and megakaryocytic cellularity. Furthermore, reticulin grade independently predicts the future risk of arterial thrombosis, major hemorrhage, and myelofibrotic transformation. Taken together, our data are consistent with the proposal that variable degrees of reticulin accumulation reflect the interplay between genetic background, disease

duration, clonal burden, and the acquisition of additional genetic lesions. The prognostic significance of reticulin grade may therefore result from its strong correlation with fundamental facets of MPD biology, such as disease aggressiveness and clonal dominance.

Our results suggest that reticulin grade provides useful prognostic information beyond that captured by other established risk factors, and additionally aids the individualization of risk assessment and therapeutic decisions. Ultimately, this could lead to the development of a prognostic model which identifies a group of ET patients older than age 60 years with minimal reticulin fibrosis and no other risk factors who may not benefit from cytoreductive therapy, although such a change in clinical practice would require supportive evidence from a randomized trial. In contrast to previous retrospective studies of histology in ET,[19,20] we have not shown a significant association between reticulin and overall survival, probably reflecting methodologic differences. As opposed to the previous studies,[19,20] our data were collected prospectively, the survival analysis is genuinely multivariate, correcting for multiple risk factors, and full treatment history is available. The lack of survival difference, coupled with the fact that there were relatively small numbers of complications even at higher levels of reticulin, supports the concept that patients presenting with isolated thrombocytosis but elevated reticulin have a relatively benign prognosis, and should be diagnosed and treated as ET rather than, say, cellular phase myelofibrosis.

We find that in patients treated with anagrelide, reticulin levels increase by an average of 1 grade every 4 to 5 years, albeit with extensive interindividual variation. This rate is similar to the findings of a recents equential biopsy study of ET patient streated with anagrelide.[21] In contrast, patients randomly assigned to the hydroxyurea arm showed, on average, significantly lower increases in reticulin over time. These data are consistent with our previous observation that clinically overt myelofibrotic transformation occurs more frequently in those randomly assigned to anagrelide.[16] It is impossible on the basis of these data to determine whether hydroxyurea suppresses the natural history of fibrotic progression in ET or whether anagrelide promotes reticulin accumulation, a question that could only be answered by comparison with an untreated cohort. Our results also show that therapy with an agrelide, but not hydroxyurea, was associated with an unexpected slow, progressive decline in hemoglobin levels, which may be due to both early dilutional effects from plasma volume expansion and late effects on erythropoiesis.[22]

The increase in reticulin fibrosis with anagrelide therapy, and its potential reversibility, implies that patients on anagrelide should have surveillance bone marrow trephines performed every 2 to 3 years, with cross-over to alternative agents should reticulin levels increase. Intriguingly, these data suggest that progression of fibrosis is not an immutable end point of the disease, raising the hope that JAK2 inhibitors may also be able to reverse fibrosis and perhaps other features of disease acceleration.

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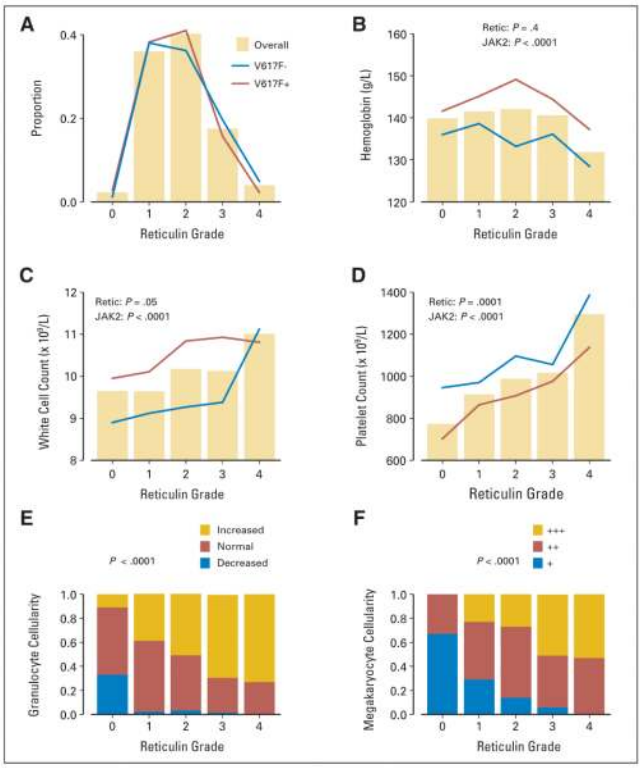

Fig 1.

Association of reticulin grade at diagnosis with presentation laboratory features. (A) Histogram showing the frequency distribution of reticulin grades in the cohort of patients with essential thrombocythemia studied. The red and blue lines show the frequency distributions for *JAK2*-positive and *JAK2*-negative patients, respectively. (B) Mean hemoglobin levels across reticulin grades for the cohort as a whole (beige bars), as well as *JAK2*-positive patients (red line) and *JAK2*-negative patients (blue line) separately. (C) Mean white blood cell counts across reticulin grades for the cohort as a whole (beige bars), as well as *JAK2*-positive patients (red line) and *JAK2*-negative patients (blue line) separately. (D) Mean platelet counts across reticulin grades for the cohort as a whole (beige bars), as well as *JAK2*-positive patients (red line) and *JAK2*-negative patients (blue line) separately. (E) Distribution of bone marrow granulocyte cellularity by reticulin grade for the cohort. (F) Distribution of bone marrow megakaryocyte cellularity by reticulin grade for the cohort. Retic, reticulin; (+), mild increase; (++), moderate increase; (+++), severe increase.

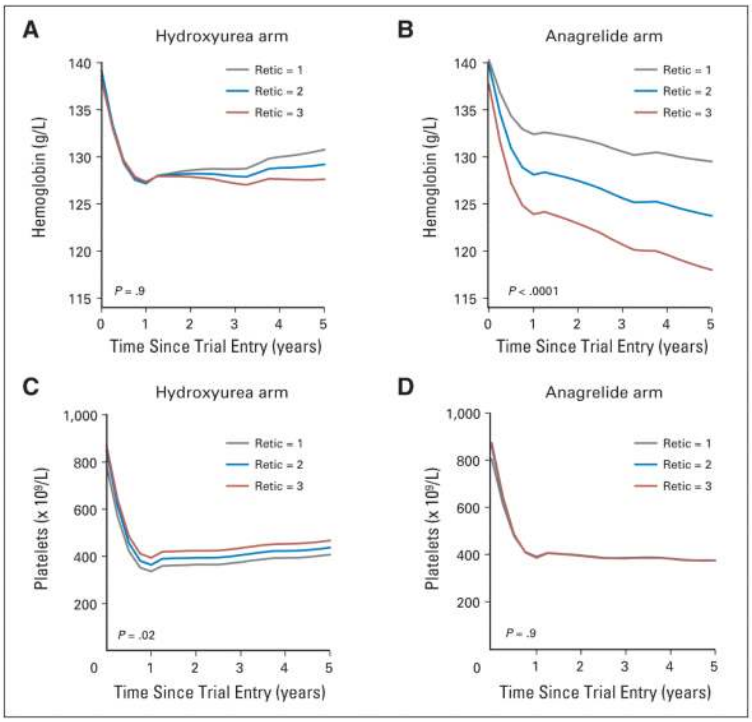

Fig 2.

Response of blood counts to therapy with hydroxyurea and anagrelide analyzed by bone marrow reticulin (retic) levels at presentation. (A) Mean hemoglobin levels by reticulin grade over time since trial entry, predicted by linear mixed effects modeling, in patients randomly assigned to receive hydroxyurea plus aspirin therapy. (B) Mean hemoglobin levels by reticulin grade over time since trial entry, predicted by linear mixed effects modeling, in patients randomly assigned to receive anagrelide plus aspirin therapy. (C) Mean platelet counts by reticulin grade over time since trial entry, predicted by linear mixed effects modeling, in patients randomly assigned to receive hydroxyurea plus aspirin therapy. (D) Mean platelet counts by reticulin grade over time since trial entry, predicted by linear mixed effects modeling, in patients randomly assigned to receive anagrelide plus aspirin therapy.

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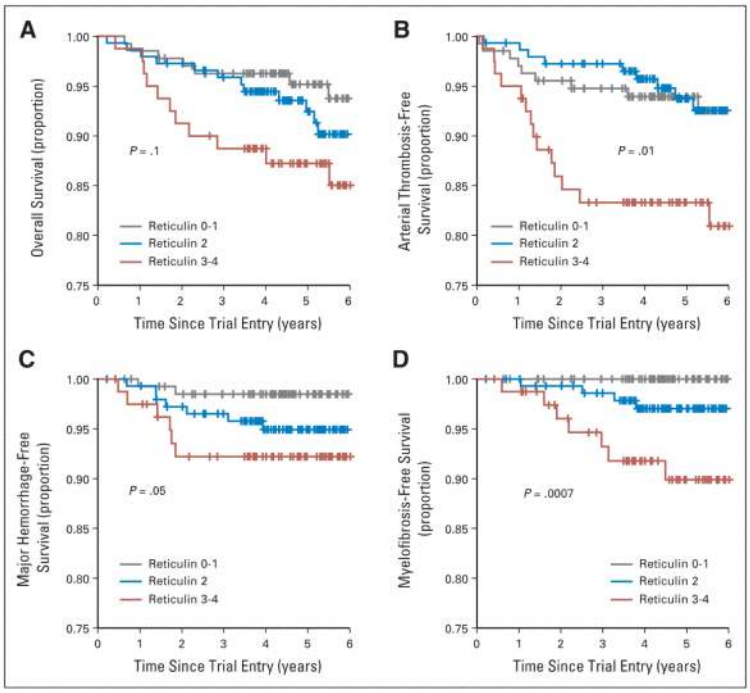

Fig 3.

Complication rates analyzed by bone marrow reticulin grade at diagnosis. Kaplan-Meier curves are shown by reticulin grades 0 to 1 (gray line), 2 (blue line), and 3 to 4 (red line) for (A) overall survival, (B) survival from arterial thrombosis, (C) survival from major hemorrhage, and (D) survival from myelofibrotic transformation. Censoring points of individual subjects are marked by vertical lines on the survival curves.

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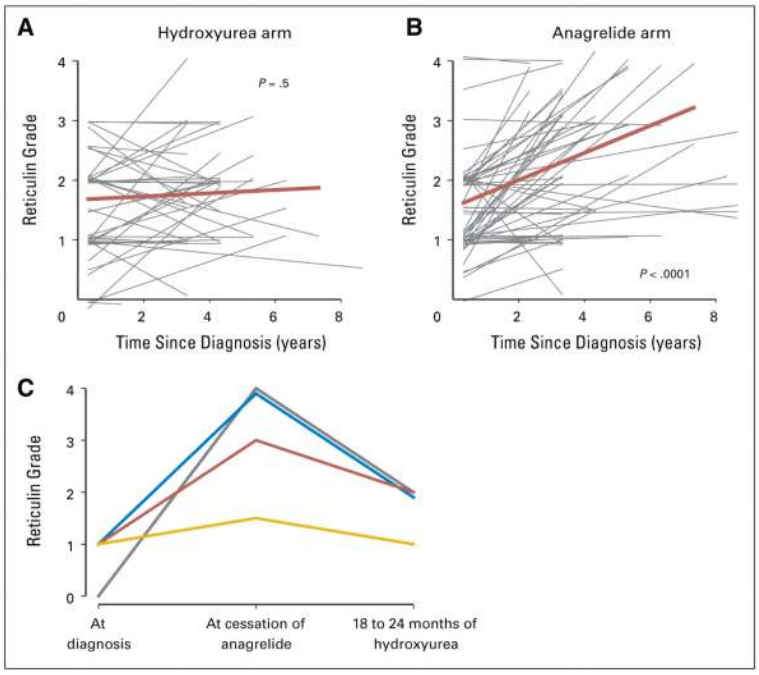

Fig 4.

Progression of reticulin levels over time analyzed by treatment random assignment in high-risk essential thrombocythemia (ET) patients. (A) Change in reticulin over time in sequential biopsies from 41 patients randomly assigned to receive hydroxyurea plus aspirin (gray lines), together with the mean change over time (thick red line) predicted by linear mixed effects models. (B) Change in reticulin over time in sequential biopsies from 56 patients randomly assigned to receive anagrelide plus aspirin (gray lines), together with the mean change over time (thick red line) predicted by linear mixed effects models. (C) Change in reticulin levels in four high-risk ET patients switched from anagrelide to hydroxyurea therapy at the end of the high-risk PT-1 trial.

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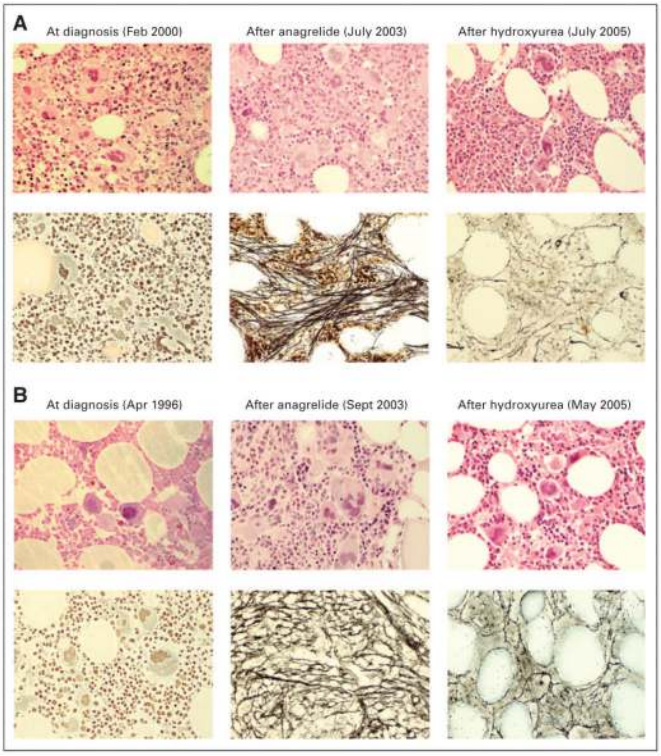

Fig 5.

Serial trephine biopsies in (A, B) two patients switched from anagrelide to hydroxyurea therapy at the end of the high-risk PT-1 trial. The top panels for each patient are stained with hematoxylin and eosin, and the bottom panels are stained with Gordon and Sweets' silver stain for reticulin. Three time points for each patient are shown: at diagnosis, at the time of switching from anagrelide to hydroxyurea, and after 18 to 24 months on hydroxyurea. Note that the trephine biopsies taken at diagnosis for each patient were embedded in resin, whereas the biopsies for the later two time points were paraffin embedded. The staining for reticulin was done for all six trephine sections in the same run.

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Table 1

End Point Events by Reticulin Grade

	Reticulin Grade								
	0/1		2		3/4		Multivariate Analysis		
Parameter	**No.**	**%**	**No.**	**%**	**No.**	**%**	**Hazard Ratio***	**95% CI**	***P***
No. of patients	135		146		80				
Death†	9	6.7	14	9.6	12	15.0	1.4	0.9 to 2.2	.1
Arterial thrombosis‡	9	6.7	10	6.8	14	17.5	1.8	1.1 to 2.9	.01
Myocardial infarct	3		3		4				
Unstable angina	2		0		2				
Stroke	4		6		4				
Transient ischemic attack	2		1		5				
Other	0		0		1				
Venous thrombosis§	3	2.2	3	2.1	0	0	0.6	0.2 to 2.1	.4
Deep vein thrombosis	1		2		0				
Pulmonary embolism	2		2		0				
Major hemorrhage‖	2	1.5	7	4.8	6	7.5	2.0	1.0 to 3.9	.05
GI	2		2		4				
Intracranial	0		1		1				
Other	0		4		1				
Transformation#	1	0.7	6	4.1	8	10.0	2.9	1.3 to 6.7	.005
Acute myeloid leukemia/ myelodysplasia#	1	0.7	3	2.1	1	1.3	1.5	0.4 to 5.8	.5
Myelofibrosis#	0	0	4	2.7	7	8.8	5.5	1.7 to 18.4	.0007

*Hazard ratio refers to the relative increase in hazard associated with a 1 grade increase in reticulin.

†Multivariate analysis included age, history of arterial thrombosis, history of venous thrombosis, diagnostic white blood cell count, and study/drug allocation. The analysis used left censoring to correct for disease duration before trial entry.

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‡Multivariate analysis included age, history of arterial thrombosis, diagnostic white blood cell count, and study/drug allocation. The analysis used left censoring to correct for disease duration before trial entry.

§Multivariate analysis included age, history of venous thrombosis, diagnostic white blood cell count, and study/drug allocation. The analysis used left censoring to correct for disease duration before trial entry.

¶Multivariate analysis included age, history of hemorrhage, diagnostic white blood cell count, and study/drug allocation. The analysis used left censoring to correct for disease duration before trial entry.

//Multivariate analysis included age, history of hydroxyurea therapy (before trial entry), history of other cytotoxic therapy (before trial entry), diagnostic white blood cell count, and study/drug allocation. The analysis used left censoring to correct for disease duration before trial entry.

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