Published in final edited form as:

Appl Immunohistochem Mol Morphol. 2014 October ; 22(9): 663–668. doi:10.1097/PAI.
0000000000000000.

Myelofibrotic transformations of polycythemia vera and essential thrombocythemia are morphologically, biologically and prognostically indistinguishable from primary myelofibrosis

Nikhil Sangle, M.D.^{1,*}, Josh Cook, MD^{1,*}, Sherrie Perkins, M.D., PhD^{1,2}, Carolyn J Teman, M.D.¹, David Bahler, M.D., PhD.^{1,2}, Kimberly Hickman³, Andrew Wilson, M.Stat², Josef Prchal, M.D.^{1,2,3}, and Mohamed Salama, M.D.^{1,2}

¹Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT, USA

²ARUP Institute of Clinical and Experimental Pathology, Salt Lake City, UT, USA

³Hematology, University of Utah Health Sciences Center, Salt Lake City, UT, USA

Abstract

A fraction of polycythemia vera (PV) & essential thrombocythemia (ET) cases will, in time, undergo myelofibrotic transformation. In such cases, fibrosis may mask the diagnostic histological features of the original underlying myeloproliferative neoplasm (MPN). Thus confidently differentiating postfibrotic PV/ET from primary myelofibrosis (PMF) histologically may not be possible.

It is controversial whether post PV/ET myelofibrosis (MF) differs clinicopathologically from PMF, or if these entities are biologically, clinically, and prognostically indistinguishable. To answer this question, we compared multiple candidate biologic, morphologic, and prognostic parameters between 19 postfibrotic ET/PV individuals and 18 PMF individuals. The postfibrotic ET/PV and PMF cases did not differ in regards to clinical outcome, cytogenetic abnormalities, serum lactate dehydrogenase (LDH) level, peripheral blast count, bone marrow morphology, or grade of reticulin fibrosis. Only *JAK2* allele burden differed between the two groups, which was higher in the postfibrotic PV/ET population ($p=0.011$).

Cardinal morphologic features of PMF (i.e. marrow cellularity, intrasinusoidal hematopoiesis, osteosclerosis, etc.) were commonly observed in post-PV/ET MF marrow biopsies and only a minority of post-PV/ET MF marrow biopsies retained diagnostic features of the primary MPN (panmyelosis in PV and megakaryocytic hyperplasia in ET). Our study indicates that PMF and post-PV/ET MF are clinically and biologically indistinguishable.

****Corresponding Authors and Address for Reprints:** Mohamed Salama, M.D., Department of Pathology, Division of Hematopathology, University of Utah Health Sciences Center, and ARUP Laboratories Inc., Salt Lake City, Utah 84132, mohamed.salama@path.utah.edu, Phone: 801/581-5854, Fax: 801/585-3831.

*equally contributing co-first authors

DISCLOSURES

The authors reported no potential conflicts of interest.

Keywords

Myelofibrotic transformation; polycythemia; myelofibrosis; thrombocythemia

INTRODUCTION

Polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are the three classic Philadelphia (Ph) chromosome negative myeloproliferative neoplasms (MPNs).[1] In the terminal stages (spent phase) of PV and ET, significant myelofibrosis (MF) may obscure the histological features of the underlying primary MPN to the degree that individuals with post-PV/ET MF may have bone marrow morphologically indistinguishable from PMF.[2] The morphologic continuum of the fibrosing process, and the inherently subjective nature in grading myelofibrosis contributes to the diagnostic conundrum in differentiating between post-PV/ET MF and PMF.[3] Additionally, it is unclear whether post-PV/ET MF is clinicopathologically distinct from PMF.

We attempt to address this difficult differential diagnosis. Multiple clinical, biologic, prognostic, and bone marrow morphologic features (i.e. degree of marrow fibrosis as determined by quantitative digital image analysis) were studied in well characterized post-PV/ET and PMF individuals in an effort to identify parameters which may aid in discriminating between these two entities.

METHODS

Patient selection

103 cases of Ph chromosome negative MPNs with myelofibrosis diagnosed at our institution between 1981 and 2013 were retrospectively reviewed using 2008 WHO criteria following Institutional Review Board (IRB) approval. 66 cases were excluded due to non-availability of high quality diagnostic material, insufficient clinical followup, or lack of molecular studies. 18 cases of PMF and 19 cases of PV (n=10) and ET (n=9) that developed into post-PV/ET MF were identified.

The original diagnosis was confirmed in all 18 cases of PMF and 10 cases of PV using 2008 WHO criteria. Among the ET cases, original diagnostic material was not available in 2 cases. Pre-fibrotic MF cases were excluded based on clinical findings and 2008 WHO criteria for relative cellular composition of marrow plus megakaryocyte morphology.[1, 5, 6] Post-PV/ET MF cases were defined as individuals with a confirmed diagnosis of PV/ET (again using 2008 WHO criteria) with documented progression to grade 2–3 bone marrow fibrosis (on 0–3 scale) identified by reticulin and trichrome staining, as per the criteria set by the International Working Group for Myelofibrosis Research and Treatment.[4, 7]

Morphologic review and assessment

Morphologic review of the peripheral blood smears, bone marrow aspirates and core biopsies was performed for each case. Two hematopathologists (MS and NS), blinded to the original diagnosis, assessed and quantified key histomorphologic features (i.e.

megakaryocyte clustering, size, and nuclear lobulation, marrow cellularity and composition, intrasinusoidal hematopoiesis). Histomorphologic criteria of megakaryocytes including size, nuclear features and clustering was considered present if noted in at least 10% of the cells.

Digital image analysis was used to objectively grade and quantitate the degree of myelofibrosis in the reticulin-stained bone marrow core biopsies. Additionally, traditional subjective grading of fibrosis was performed in the standard manner aided by reticulin.[4] The slides were digitally scanned with ScanScope®XT system and the reticulin fibrosis was quantified using a color deconvolution algorithm provided in ScanScope® software (Aperio Technologies, Inc., Vista, CA, USA) as previously described.[3] The fibrosis quantification score was defined as the proportion of total hematopoietic area (excluding trabeculae) occupied by reticulin fibers. The objective quantification of fibrosis using digital image analysis was highly correlated with the subjective fibrosis score (Spearman correlation, $R=0.79$) (data not shown).

Molecular analysis

Molecular studies included *JAK2* V617F mutant allele quantitation and *MPL* (W515K/L) mutational status. A quantitative real-time polymerase chain reaction (PCR) based allelic discrimination assay was used to determine the percentage of granulocyte mutant alleles for *JAK2* V617F, as previously described [8].

Flow cytometric analysis

Flow cytometric studies were performed on peripheral blood. Parameters measured included peripheral CD34+ blast percentages of total leukocytes, coexpression frequency of adhesion molecule CXCR4 (CD184), and blast characterization, using CD38, CD45RA and IL-3R α (CD123).

Based on phenotype, circulating myeloid progenitors were categorized as common myeloid progenitors (CMP - CD34/CD38+, CD45RA-, CD123+), granulocyte / macrophage progenitors (GMP - CD34/CD38+, CD45RA+, CD123+) or megakaryocyte / erythrocyte progenitors (MEP - CD34/CD38+, CD45RA-, CD123-).[9] CMP or their downstream progeny subpopulations were considered significant if they represented $\geq 30\%$ of the CD34/CD38 positive cells.

Cytogenetic analysis

Conventional cytogenetic studies were performed in all post-PV/ET MF and PMF individuals.

Clinical outcome

Clinical features, including serum LDH levels and event free survival (EFS) characteristics (i.e. development of vascular thrombosis, death, transformation to acute leukemia, or similar adverse events) in the post PV/ET MF and the PMF groups were assessed.

Statistical analysis

Generalized linear models (GLM) were used when comparing continuous variables. Fisher's exact tests were used for comparing categorical variables. Statistical analyses were performed using the SAS software, Version 9.1 of the SAS system (SAS Institute® Inc., Cary, NC, USA). Results were considered statistically significant if $p < 0.05$. The fibrotic transformation of both PV and ET were grouped together as post-PV/ET MF group for comparison with the PMF group. Combined PV/ET MF were also compared to PMF *JAK2* mutated and PMF *JAK2* nonmutated cohorts.

RESULTS

Objective assessment of the grade of MF using image analysis techniques showed no difference between PMF and fibrotic transformation of PV or ET (Figure 1). Traditional subjective grading of the reticulin stain gave similar results. The original cellular composition (panmyelosis in PV and megakaryocytic hyperplasia in ET) was maintained in 37% of post-PV/ET MF biopsies (Figure 2 & 3); the original megakaryocytic morphology/histotopography was observed in 21% post-PV/ET specimens. The remaining cases in the post-PV/ET MF group showed histomorphologic features of PMF.

Informative cytogenetic studies were available in 8 post-PV/ET MF and 13 PMF individuals. The frequency and complexity of cytogenetic abnormalities were subjectively similar between the two groups (Table 1). The post-PV/ET MF cohort showed a normal karyotype in 5/8 specimens. Non-complex cytogenetic abnormalities nonspecific for MPNs were detected in 2/8 specimens (a 13q deletion and a 1q addition). The single PV/ET MF specimen with complex cytogenetic changes included nonspecific abnormalities commonly reported in MPNs and other hematopoietic disorders (monosomy 7, a 20q deletion, and a 5q deletion). The PMF cohort showed a normal karyotype in 9/13 specimens, two specimens each with simple nonspecific chromosomal abnormalities, and two specimens with complex cytogenetic abnormalities. One of the PMF specimens with complex cytogenetic changes included the recurrent +8, +9, gain of 1q, and loss of 7 abnormalities commonly reported in MPNs and other myeloid disorders.

Flow cytometric studies for circulating myeloid progenitors were available for 13 post-PV/ET MF individuals and 18 PMF individuals. When compared, the peripheral blast count and the relative contribution of CEPs, GMPs, and MEPs to the pool of circulating myeloid precursors in post-PV/ET MF and PMF groups were similar. CMP, MEP and GMP represented >30% of the CD34+/CD38+ population in 11/13, 6/13, and 2/13 of post-PV/ET and 18/18, 7/18 and 1/18 PMF specimens, respectively (Table 2). Adhesion molecule CXCR4 (CD184) expression in circulating blasts was similar between the two groups.

In our cohort, PV and ET patients developed MF at a median of 185 (range 31 – 359) and 130 (range 60 – 233) months, respectively (Table 2). We found LDH levels in the post-PV/ET MF group to be equally high (median 853, range 249–1709) as the PMF group (median 935, range 63–1741), with no statistically significant difference between the 2 groups (Table 2).

Follow-up data (timed from progression to MF) in post-PV MF ranged from 1 – 96 (median = 31) months, and for post-ET MF patients ranged from 1 – 72 (median = 14) months. PMF patients had follow-up data ranging over a period of 3 – 252 (median = 27) months from time of diagnosis (Table 2). Occurrence of clinical events, in particular transformations to acute leukemia, death, vascular thrombosis, and other similar complications were considered. In the post-PV MF group, one patient developed AML after 71 months of progression to MF, and another patient died due to an unrelated reason. In the post-ET MF cohort, one patient developed AML at the onset of MF, and another had splenic thrombosis; three patients in this group had expired (due to unrelated reasons) at the time of last follow-up. In the PMF group, one patient expired following transformation to acute leukemia, and another died of unrelated reasons. Use of statistical methods in evaluating EFS was limited due to the small sample size and infrequency of events.

The *MPL* W515K/L mutation was detected in a single post-PV/ET MF individual and was not detected in any of PMF individuals.

The only parameter to show a statistical difference between the 2 groups was *JAK2* V617 mutation allelic burden (Table 2; $p=0.011$). This was significantly higher in the post-PV/ET MF patients, when compared to the PMF patients. Subgroup analysis separately comparing post-PV/ET MF individuals with *JAK2* mutated PMF and *JAK2* nonmutated PMF individuals revealed no statistical differences for any of the above parameters.

DISCUSSION

Myeloproliferative neoplasms (MPN) are a heterogeneous group of clonal disorders affecting one or several myeloid cell lineages. Accurate categorization of MPN requires a combination of clinical, histomorphologic and molecular data, and bone marrow histology is essential.[6] It is not uncommon for MPNs, such as ET or PV to develop fibrosis in the terminal stages, rendering them difficult to distinguish from PMF in the absence of clinical information or sequential biopsies.[2]

Previous studies have not addressed if post-PV/ET MF and PMF differ in biologic, clinical, or prognostic features. Post PV/ET fibrosis and PMF share overlapping features which, in some cases, makes distinguishing between these entities difficult to impossible without appropriate clinical history or sequential bone marrow studies. Specifically, neither the score of MF using quantitative image analysis, bone marrow histology, nor megakaryocyte morphology/histotopography were sufficiently different in our study to reliably distinguish between PMF and post-PV/ET MF.

The only biologic parameter which differed significantly between the 2 study groups was *JAK2* V617 mutation allele burden ($p=0.011$), which was higher in the post-PV/ET MF individuals compared to PMF individuals. This is consistent with previous studies reporting a direct relationship between high *JAK2* mutant allele burden and the risk of developing myelofibrosis in PV.[1011] Approximately 5% of PV and about 50% of ET and PMF patients are negative for *JAK2* V617F mutation. Mutations of the thrombopoietin receptor gene (*MPL*) at codon 515 (W515K/L) have been reported in up to 10% of *JAK2* V617F

mutation negative ET and PMF patients.[12] In our series, only one post-PV/ET MF patient had a *MPL* mutation.

Increased serum LDH levels are part of the WHO minor criteria for diagnosis of PMF; however, in our experience LDH levels in the post-PV/ET MF individuals are equally high.

In PMF, increased CD34+ progenitors are often increased in peripheral blood possibly due to reduced CXCR4 adhesion protein expression.[13] ET and PV (without MF) are not associated with significant increase in circulating CD34+ progenitors.[14] We found that CD34+ percentages were increased in all three groups, but coexpression of the adhesion molecule CXCR4 (CD184) on circulating blasts and maturational patterns in post-PV/ET MF and PMF groups showed no significant differences.

CONCLUSION

Our findings indicate that, with the exception of a higher *JAK2* mutant allelic burden observed in the post-PV/ET MF group, there are otherwise no morphologic, phenotypic or prognostic differences between patients with post-PV/ET MF or PMF. Despite the small sample size, our study indicates that these two groups are prognostically similar and biologically indistinguishable. Until further validation in larger studies, our findings suggest that these entities be considered as equivalent for diagnostic and management purposes.

Acknowledgments

MS was the principal investigator and takes primary responsibility for the paper. JP recruited the patients. DB, JP, MS, SP, and CT performed the laboratory work for this study. AW participated in the statistical analysis. KH coordinated the research. NS and JC wrote the paper.

REFERENCES

1. Thiele J, Kvasnicka HM, Orazi A, et al. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Swerdlow, SH.; Campo, E.; Harris, NL., et al., editors. Lyon, France: International Agency for Research on Cancer; 2008.
2. Thiele J, Kvasnicka HM. Myelofibrosis--what's in a name? Consensus on definition and EUMNET grading. *Pathobiology*. 2007; 74:89–96. [PubMed: 17587880]
3. Teman CJ, Wilson AR, Perkins SL, et al. Quantification of fibrosis and osteosclerosis in myeloproliferative neoplasms: a computer-assisted image study. *Leuk Res*. 2010; 34:871–876. [PubMed: 20122729]
4. Thiele J, Kvasnicka HM, Facchetti F, et al. European consensus on grading bone marrow fibrosis and assessment of cellularity. *Haematologica*. 2005; 90:1128–1132. [PubMed: 16079113]
5. Brousseau M, Parot-Schinkel E, Moles MP, et al. Practical application and clinical impact of the WHO histopathological criteria on bone marrow biopsy for the diagnosis of essential thrombocythemia versus prefibrotic primary myelofibrosis. *Histopathology*. 2010; 56:758–767. [PubMed: 20546341]
6. Barbui T, Thiele J, Passamonti F, et al. Survival and disease progression in essential thrombocythemia are significantly influenced by accurate morphologic diagnosis: an international study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2011; 29:3179–3184. [PubMed: 21747083]
7. Barosi G, Mesa RA, Thiele J, et al. Proposed criteria for the diagnosis of post-polycythemia vera and post-essential thrombocythemia myelofibrosis: a consensus statement from the International

Working Group for Myelofibrosis Research and Treatment. *Leukemia*. 2008; 22:437–438. [PubMed: 17728787]

8. Nussenzweig RH, Swierczek SI, Jelinek J, et al. Polycythemia vera is not initiated by JAK2V617F mutation. *Exp Hematol*. 2007; 35:32–38. [PubMed: 17198871]
9. Manz MG, Miyamoto T, Akashi K, Weissman IL. Prospective isolation of human clonogenic common myeloid progenitors. *Proc Natl Acad Sci U S A*. 2002; 99:11872–11877. [PubMed: 12193648]
10. Passamonti F, Rumi E, Pietra D, et al. A prospective study of 338 patients with polycythemia vera: the impact of JAK2 (V617F) allele burden and leukocytosis on fibrotic or leukemic disease transformation and vascular complications. *Leukemia*. 2010; 24:1574–1579. [PubMed: 20631743]
11. Silver RT, Vandris K, Wang YL, et al. JAK2(V617F) allele burden in polycythemia vera correlates with grade of myelofibrosis, but is not substantially affected by therapy. *Leuk Res*. 2010; 35(2): 177–182. [PubMed: 20650526]
12. Kilpivaara O, Levine RL. JAK2 and MPL mutations in myeloproliferative neoplasms: discovery and science. *Leukemia*. 2008; 22:1813–1817. [PubMed: 18754026]
13. Rosti V, Massa M, Vannucchi AM, et al. The expression of CXCR4 is down-regulated on the CD34+ cells of patients with myelofibrosis with myeloid metaplasia. *Blood Cells Mol Dis*. 2007 May-Jun;38(3):280–286. [PubMed: 17350297]
14. Michiels JJ. Bone marrow histopathology and biological markers as specific clues to the differential diagnosis of essential thrombocythemia, polycythemia vera and prefibrotic or fibrotic agnogenic myeloid metaplasia. *Hematol J*. 2004; 5:93–102. [PubMed: 15048058]

Figure 1.
Objectively assessed myelofibrosis using digital image analysis.

Figure 2.

Bone marrow biopsy of a representative patient with essential thrombocythemia (ET) that progressed to myelofibrosis (MF) (A) ET showing panmyelosis with numerous megakaryocytes with eosinophilic background indicative of fibrosis. The megakaryocytes primarily showed cloud like morphology with hyperchromatic nuclei (H&E); however, residual hyperbolate/stag horn forms are also present. (B) Reticulin stain shows a tight cluster of megakaryocytes with increased reticulin fibrosis from the same patient. (C) Flow cytometry scatter plots demonstrating the gate on the CD34/CD38 positive cell population. (D) The relative distribution of common myeloid (CM), Myeloid-erythroid (ME) or the granulocytic (GM) clonogenic progenitors based on CD123 and CD45RA plots.

Figure 3.

(A) Bone marrow core (H&E) from a patient with polycythemia vera (PV) progressed to myelofibrosis (MF) showing panmyelosis, and megakaryocytes of different sizes forming loose clusters – maintaining the histomorphologic features of PV. (B) Higher magnification showing loose clustering of megakaryocytes with normally folded or deeply lobulated nuclei. (C) Reticulin stain on the same case demonstrating myelofibrosis in the form of diffuse and dense increase in reticulin (MF-2).

Table 1

	Cytogenetic Findings	N
Post PV-MF	Normal karyotype	4
	46,XY,+1,der(1;7)(q10;p10)	1
Post ET-MF	Normal karyotype	1
	46,XY,del(13)(q14q22)[18]/46,XY[2]	1
	45,XX,add(2)(q32),add(4)(q27),del(5)(q13q31),-7,del(20)(q11q13)[3]/45,sl,del(3)(q21)[17]	1
PMF	Normal karyotype	9
	46,XX,add(4)(q21),del(12)(q12q14)[9]/46,XX[11]	1
	46,XY,del(3)(q21q23),t(2;13)(q31;q32)[1 9]/46,XY[1]	1
	48,XX,dup(1)(q24q21),+8,+9[15]/47,sl,der(6)inv(6)(p25q16)ins(6;?)(p12;?),-8[3]/46,XX[2]	1
	38-44,XX,del(3)(p13),add(5)(q13),-7,-12[cp4]	1

Table 2

	Age (years)	Sex	JAK2 (%)	LDH (U/L) ¹	Megakaryocytes			Time for progression to MF (months)	Peripheral blood CD34 %	Progenitor cell type representing over 30%			
					Clustering loose/tight	Stag horn morphology ²	Cloud like morphology ²			CM	CM/ ME	CM/ GM	GM
Post PV-MF	Range (median) 66–76 (71)	M/F 4/6	Range (median) 57–99 (83)	Range (median) 549–1139 (770)	5/3	3	6	31–359 (185)	Range (median) 0.06–0.64 (0.128)	1	2		
Post ET-MF	Range (median) 39–87 (59)	M/F 2/7	Range (median) 0–90 (6.4)	Range (median) 249–1709 (1006)	4/4	4	4	60–233 (130)	Range (median) 0.16–11.1 (1.18)	4	3	1	1
PMF	Range (median) 23–79 (64)	M/F 10/8	Range (median) 0–88 (0)	Range (median) 63–1791 (935)	1/17	0	18	-	Range (median) 0.06–7.15 (0.88)	10	7	1	

¹ Normal reference range for lactate dehydrogenase (LDH) = 140 – 280 U/L

² One post PV-MF case showed both staghorn and cloud-like megakaryocyte morphology Post polycythemia vera myelofibrosis (Post PV-MF), post essential thrombocythemia myelofibrosis (Post ET-MF), primary myelofibrosis (PMF), common myeloid (CM), Myeloid erythroid (ME), granulocytic (GM)