# Associations and prognostic interactions between circulating levels of hepcidin, ferritin and inflammatory cytokines in primary myelofibrosis

Animesh Pardanani,[1,2]* Christy Finke,[1,2] Ramy A. Abdelrahman,[1,2] Terra L. Lasho,[1,2] and Ayalew Tefferi[1,2]

**Iron homeostasis is dysregulated in primary myelofibrosis (PMF), given the high prevalence of anemia, need for red blood cell (RBC) transfusions, and disease-associated inflammatory state. We measured plasma hepcidin levels in 203 consecutive PMF patients at the time of first referral; hepcidin levels were significantly higher as compared to healthy controls ($P < 0.0001$), and were correlated with hemoglobin of <10 g/dL, RBC transfusion requirement, serum ferritin of >500 μg/L, higher dynamic international prognostic scoring system (DIPSS)-plus risk category, the presence of circulating blasts, age of >65 years, and leukocyte count of <4 × $10^9$/L. Increased hepcidin levels predicted for inferior survival independent of six out of the eight DIPSS-plus prognostic parameters (hazard ratio [HR] = 1.8; $P = 0.02$), but not when RBC transfusion requirement, hemoglobin of <10 g/dL, or increased serum ferritin were included in the Cox model. Multivariable analysis that considered the four overlapping prognostic variables revealed that increased hepcidin (HR = 1.9; $P = 0.03$) and increased ferritin (HR = 2.3; $P = 0.04$), but not hemoglobin of <10 g/dL or RBC transfusion requirement, independently retained their significance for predicting survival. Accordingly, increased levels of both hepcidin and serum ferritin (seen in 29% of patients) predicted inferior survival independent of DIPSS-plus or increased inflammatory cytokine levels (HR = 2.4; $P = 0.002$), and could be considered in future prognostic models for PMF. Am. J. Hematol. 88:312–316, 2013.** © 2013 Wiley Periodicals, Inc.

## Introduction

There is little information with regard to iron homeostasis in primary myelofibrosis (PMF). Iron homeostasis can be reasonably predicted to be disturbed in this population, given the high prevalence of severe anemia, a surrogate for ineffective erythropoiesis; 38% of patients have a hemoglobin level of <10 g/dL and 24% have required red blood cell (RBC) transfusions at initial diagnosis, with increasing prevalence of anemia manifest during the disease course [1]. Additional inputs into disturbed iron homeostasis in PMF include dysregulated circulating inflammatory cytokine expression [2,3] and iron overload [4,5]; the latter is associated chiefly with increased infusional iron in the form of RBC transfusions, but also possibly abnormal gut iron absorption.

Anemia is a well-established prognostic factor in PMF (hemoglobin of <10 g/dL and RBC transfusion requirement confer independent prognostic information in the dynamic international prognostic scoring system [DIPSS]-plus model) [6]. Prognosis in PMF is also influenced by increased levels of circulating inflammatory cytokines (e.g., interleukin [IL]-2 receptor [IL-2R], and/or IL-8), within specific DIPSS-plus risk categories [2]. These data suggest that markers of abnormal iron homeostasis, which are known to be affected by both iron overload and inflammation, may also have prognostic value in PMF.

Hepcidin is a key regulator of iron homeostasis; binding of hepcidin to its receptor, the iron channel ferroportin, leads to internalization and degradation of the latter in tissues that play a key role in iron handling such as duodenal enterocytes and macrophages [7,8]. This effect blocks dietary iron absorption, recycling of iron from senescent erythrocytes as well as release of iron from its stores. The regulation of hepcidin expression is complex with opposing inputs coming from systemic iron availability, erythropoietic activity, hypoxia, and inflammatory signals [9,10]. Convergence of ineffective erythropoiesis, inflammation, and iron overload in PMF suggests dysregulation and potential prognostic impact of circulating hepcidin levels.



## Methods

### Patients and samples

This study was approved by the Mayo Clinic institutional review board. All patients provided written informed consent for plasma sample collection as well as participation in the research study. Inclusion to this study required availability of archived plasma, bone marrow biopsy, and cytogenetic information at the time of first referral to our institution. The diagnosis of PMF was according to the World Health Organization criteria [11]. Unfavorable karyotype designation and DIPSS-plus risk categorization were as described previously [6,12]. Patient information was updated through review of patient histories and correspondence, social security death index, or a telephone call to the patient or their local physician.

### Plasma cytokine and molecular profiling

Peripheral blood was collected under a Mayo Clinic protocol for patients with myeloid malignancies and standard procedures were followed to isolate plasma and store aliquots at −80°C. Plasma total hepcidin levels were measured in duplicate by ELISA (USCN Life Sciences, Wuhan, China) as per the manufacturer's instructions [13,14]. Samples were initially diluted 50- to 100-fold and measurements were performed on a SpectraMax 190 plate reader (Molecular Devices, Sunnyvale, CA); the resulting data were evaluated using SoftMax Pro version 5 (Molecular Devices) software. The observed intensities of duplicate samples were averaged and mapped to a fitted curve derived from a serial dilution series of known hepcidin standards (0–4,000 pg/mL); observed intensities above the standard range were re analyzed after further sample dilution. Serum ferritin was measured as part of routine clinical assessment (normal range: males: 24–336 μg/L; females: 11–307 μg/L). Concentrations of 30 plasma cytokines

[1]Division of Hematology, Mayo Clinic, Rochester, Minnesota; [2]Department of Medicine, Mayo Clinic, Rochester, Minnesota

Conflict of interest: Nothing to report.

*Correspondence to: Animesh Pardanani, MBBS, PhD, Mayo Clinic, 200 First Street SW, Rochester, MN 55905. E-mail: Pardanani.animesh@mayo.edu

Contract grant sponsor: The Predolin Foundation for Leukemia Research.

Received for publication 10 January 2013; Revised 23 January 2013; Accepted 25 January 2013

Am. J. Hematol. 88:312–316, 2013.

Published online 6 February 2013 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.23406

**TABLE I. Clinical Characteristics of 203 Patients with PMF with Total Hepcidin Measurement at the Time of First Referral to Our Institution**

| Characteristic | No. of patients (%) | Median (range) |
|---|---|---|
| Total | 203 | |
| Age (years) | | 63 (17–83) |
| >65 Years | 82 (40%) | |
| Males | 136 (67%) | |
| Hemoglobin (g/dL) | | 10 (5.8–15.3) |
| Leukocyte count ($\times 10^9$/L) | | 8.9 (1–132) |
| Platelet count ($\times 10^9$/L) | | 212 (11–1,007) |
| DIPSS-plus risk group | | |
| Low | 22 (11%) | |
| Intermediate-1 | 26 (13%) | |
| Intermediate-2 | 85 (42%) | |
| High | 70 (34%) | |
| Constitutional symptoms | 74 (36%) | |
| Circulating blasts ≥1% | 73 (36%) | |
| Hemoglobin <10 g/dL | 119 (59%) | |
| Required RBC transfusion | 77 (38%) | |
| Leukocytes >25 × $10^9$/L | 25 (12%) | |
| Platelets <100 × $10^9$/L | 39 (19%) | |
| Leukocytes <4 × $10^9$/L | 38 (19%) | |
| Ferritin; no. tested (% ≤500 μg/L) | 146 (30%) | |
| Patients with unfavorable karyotype | 25 (12%) | |
| JAK2V617F status tested (% positive) | 200 (61%) | |

Abbreviations: No., number; g, grams; dL, deciliter; L, liter; μg, micrograms; cm, centimeters; DIPSS, dynamic international prognostic scoring system; and %, percentage.

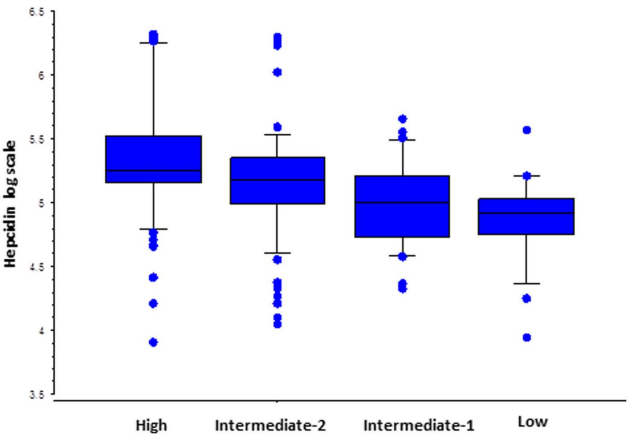


Figure 1. Distribution of circulating hepcidin levels in PMF patients stratified by DIPSS-plus risk category. Box-plot representation shows hepcidin levels (pg/mL, log scale) are correlated with DIPSS-plus risk category: Low-risk (median, 81,704; range, 8,870–372,009), intermediate-1 (median, 100,182; range, 214,530–456,312), intermediate-2 (median, 151,600; range, 11,060–1,981,142), and high-risk (median, 178,576; range, 8,082–2,088,002) ($P < 0.0001$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

were analyzed in duplicate using Multiplex Bead-based Luminex Technology (Invitrogen, Carlsbad, CA), as described previously [2]. Archived DNA was available in 200 out of the 203 patients for use in determining *JAK2*V617F mutational status [15].

### Statistical analysis

All statistical analyses considered clinical and laboratory parameters obtained at time of first referral to the Mayo Clinic, which coincided in all instances with time of plasma collection for hepcidin/cytokine analysis. Differences in the distribution of continuous variables between categories were analyzed by either the Mann–Whitney test (for comparison of two groups) or the Kruskal–Wallis test (comparison of three or more groups). Patient groups with nominal variables were compared by $\chi^2$-test. Overall survival analysis was considered from the date of first referral to the Mayo Clinic (i.e., date of plasma collection) to the date of death (uncensored) or last contact (censored). Overall survival curves were prepared by the Kaplan–Meier method and compared by the log-rank test. A Cox proportional hazards regression model was used for multivariable analysis. *P*-values of <0.05 were considered significant. The Stat View statistical package (SAS Institute, Cary, NC) was used for all calculations.

## Results

Two hundred and three patients with PMF (median age, 63 years; range, 17–83 years; male, 67%) were eligible for this study based on the availability of plasma samples; Their DIPSS-plus risk categorization was: low-risk 22 (11%), intermediate-1 26 (13%), intermediate-2 85 (42%), and high-risk 70 (34%); other demographic, clinical, and laboratory features are summarized in Table I.

### Phenotype–hepcidin level associations

Plasma hepcidin levels were measured in 203 PMF patients and compared to 29 normal controls. Hepcidin levels were significantly higher in PMF patients (median, 156,279 pg/mL; range, 8,082–2,088,002) as compared to normal controls (median, 13,449 pg/mL; range, 0–80,203) ($P < 0.0001$).

Increased hepcidin levels in PMF were associated with hemoglobin of <10 g/dL, RBC transfusion requirement, serum ferritin of >500 μg/L, higher DIPSS-plus risk category (Fig. 1), the presence of circulating blasts, age of >65 years, and leukocyte count of <4 × $10^9$/L ($P < 0.05$). No correlations were observed with leukocyte count of >25 × $10^9$/L, platelet count of <100 × $10^9$/L, the presence of constitutional symptoms, unfavorable karyotype, or *JAK2*V617F mutational status ($P > 0.05$).

Hepcidin levels were strongly correlated with serum ferritin levels ($r^2 = 0.44$, $P < 0.001$; Fig. 2A); this association was found independent of DIPSS-plus risk category (low or intermediate-1 risk vs. intermediate-2 or high risk; $P < 0.0001$), or RBC transfusion requirement ($P < 0.0001$). Correlations with hemoglobin level ($r^2 = 0.07$, $P = 0.0002$; Fig. 2B) and age ($r^2 = 0.05$, $P = 0.001$; Fig. 2C) were weaker. There was no correlation between hepcidin and any of the 30 cytokines studied, including IL-6 ($r^2 = 0.0002$, $P = 0.8$; Fig. 2D), IL-2R ($r^2 = 0.003$, $P = 0.5$; Fig. 2E), or IL-8 ($r^2 = 0.0003$, $P = 0.8$; Fig. 2F) levels. All of the aforementioned hepcidin associations, with the exception of age >65 years, were sustained when analyses considered hepcidin as a categorical variable using 3 standard deviations (3SD) from the normal mean (>91,604 pg/mL) as the cutoff level (Table II).

### Prognostic relevance of hepcidin

Median follow-up of the study cohort from the time of plasma collection was 35 months; during this period, 99 (49%) deaths and 18 (9%) leukemic transformations were recorded. On univariate analysis, increased hepcidin levels were associated with shortened survival (hazard ratio [HR] = 2.3; $P = 0.0008$). On multivariable analysis, increased hepcidin levels retained their significance for predicting survival when six out of the eight DIPSS-plus prognostic parameters were included in the Cox model: age of >65 years, the presence of constitutional symptoms, circulating blasts, unfavorable karyotype, leukocyte count of >25 × $10^9$/L, and platelet count of <100 × $10^9$/L (HR = 1.8; $P = 0.02$). The prognostic significance of increased hepcidin was lost, however, when the remaining two DIPSS-plus variables were added to the above model: RBC transfusion requirement or hemoglobin <10 g/dL. Prognostic significance from hepcidin was also lost when serum ferritin was included in the multivariable model. In other words, the four related parameters including hepcidin, serum ferritin, hemoglobin of <10 g/dL, and RBC transfusion requirement displayed overlapping prognostic value, whereas they were significant when considered individually in the Cox model that included all other prognostic

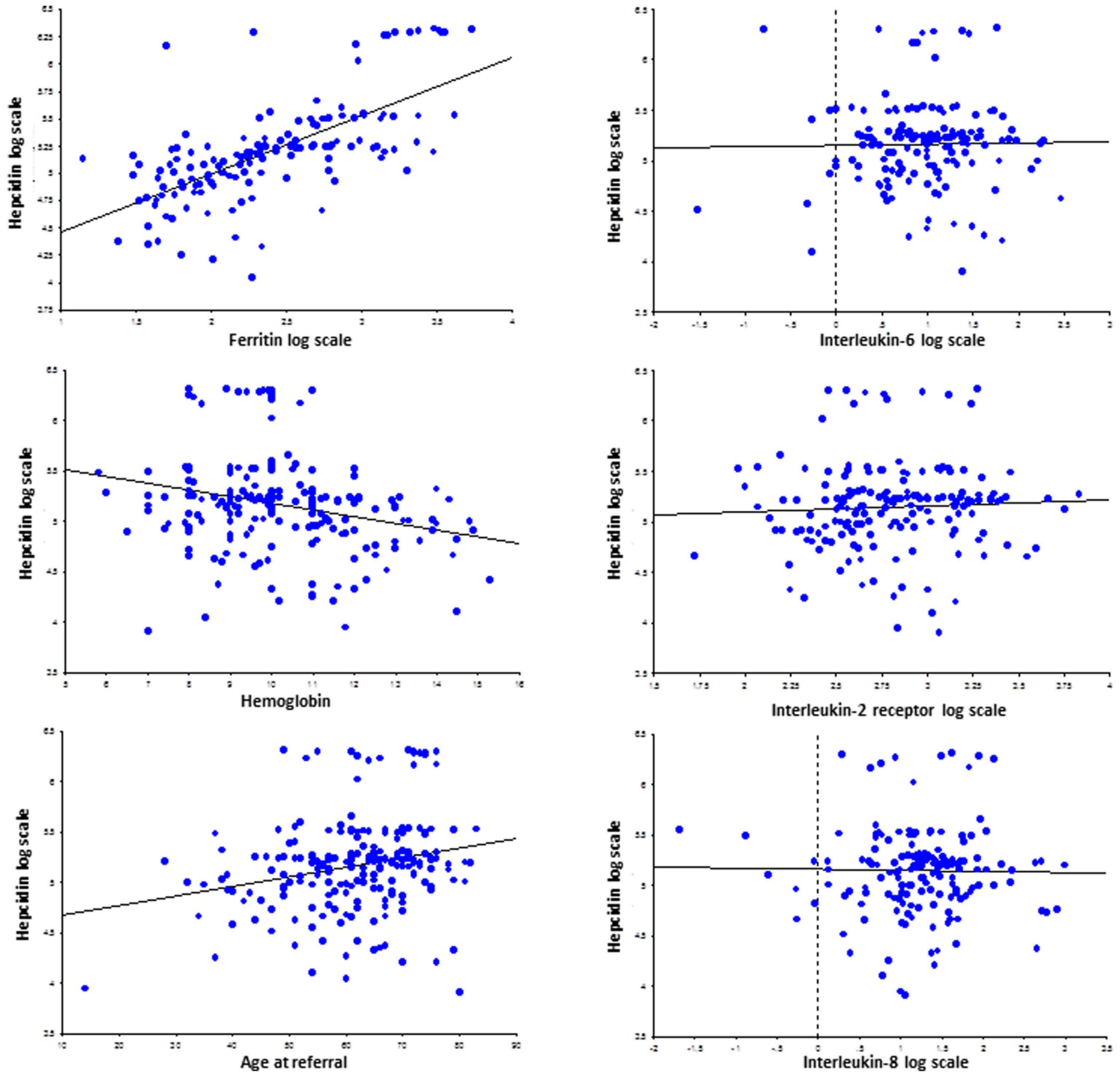


Figure 2. Correlation of circulating hepcidin (pg/mL, log scale) levels with (A) serum ferritin (μg/L, log scale), (B) hemoglobin level (g/dL), (C) age at referral (years), (D) IL-6 (pg/mL, log scale), (E) IL-2R (pg/mL, log scale), and (F) IL-8 (pg/mL, log scale). Linear regression plots are shown. For coefficient of determination ($r^2$) and *P*-value for each correlation, refer the text. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

variables, their significance was lost when considered in any combination(s).

To further clarify the prognostic interdependence among serum hepcidin, serum ferritin, hemoglobin of <10 g/dL, and RBC transfusion requirement, a multivariable analysis that included only these four parameters was performed and revealed that increased hepcidin (HR = 1.9; $P = 0.03$) and increased ferritin (HR = 2.3; $P = 0.04$) retained their significance, independent of each other, for predicting survival. Increased levels of both hepcidin (>3SD above mean) and serum ferritin (>500 μg/L) were found in 29% of the study cohort and predicted inferior survival (HR = 3.0; $P < 0.0001$) that was not accounted for by either the DIPSS-plus model or the increased levels of inflammatory cytokines (IL-2R and/or IL-8) (HR = 2.4; $P = 0.002$). This combination was able to effectively risk stratify patients for overall survival in the overall cohort (Fig. 3A; $P < 0.0001$) and within DIPSS-plus intermediate-2 or high-risk categories (Fig. 3B; $P = 0.01$). A similar analysis was not possible in DIPSS-plus intermediate-1 or low-risk patients, given the limited number of patients with elevated levels of both hepcidin and serum ferritin.

## Discussion

Our data provide a description of abnormalities in iron homeostasis in PMF primarily through analysis of circulating hepcidin levels. We describe the interaction between key determinants of iron homeostasis including hepcidin levels, ferritin levels, the presence of significant anemia or need for RBC transfusions, and circulating cytokine levels. We also describe the phenotypic associations and prognostic implications of increased hepcidin levels in PMF.

**TABLE II. Comparison of Clinical Characteristics of Patients with PMF With Increased Levels of Total Hepcidin at the Time of First Referral to Our Institution**

| Characteristic | Total hepcidin | | *P*-value |
|---|---|---|---|
| | ≤3SD | >3SD | |
| Total | 57 | 146 | |
| Age (years) | | | |
| Median | 60 | 64 | 0.006 |
| Range | | | |
| >65 | 18 (32%) | 65 (45%) | 0.1 |
| Males | 33 (58%) | 103 (71%) | 0.1 |
| Hemoglobin (g/dL) | | | |
| Median | 11.0 | 9.9 | 0.005 |
| Range | 6.5–15.3 | 5.8–14.8 | |
| Leukocyte count ($\times 10^9$/L) | | | |
| Median | 9.0 | 8.5 | 0.8 |
| Range | 2–92 | 1–132 | |
| Platelet count ($\times 10^9$/L) | | | |
| Median | 224 | 209 | 0.3 |
| Range | 15–1,007 | 11–796 | |
| DIPSS-plus risk group | | | <0.0001 |
| Low | 14 (25%) | 8 (5%) | |
| Intermediate-1 | 12 (21%) | 14 (10%) | |
| Intermediate-2 | 19 (33%) | 66 (45%) | |
| High | 12 (21%) | 58 (40%) | |
| Constitutional symptoms | 17 (30%) | 57 (39%) | 0.3 |
| Circulating blasts ≥1% | 14 (25%) | 59 (40%) | 0.04 |
| Hemoglobin <10 g/dL | 22 (39%) | 97 (66%) | 0.0004 |
| RBC transfusion need | 7 (12%) | 70 (48%) | <0.0001 |
| Leukocytes >25 × $10^9$/L | 6 (11%) | 19 (13%) | 0.8 |
| Platelets <100 × $10^9$/L | 8 (14%) | 31 (21%) | 0.3 |
| Leukocytes <4 × $10^9$/L | 5 (9%) | 33 (23%) | 0.03 |
| Ferritin; no. tested (% >500 μg/L) | 40 (5%) | 106 (40%) | <0.0001 |
| Patients with unfavorable karyotype | 6 (11%) | 19 (13%) | 0.8 |
| JAK2V617F status tested (% positive) | 55 (58%) | 145 (61%) | 0.8 |

Abbreviations: No., number; SD, one standard deviation above the normal mean value; g, grams; dL, deciliter; L, liter; μg, micrograms; cm, centimeters; DIPSS, dynamic international prognostic scoring system; and %, percentage.

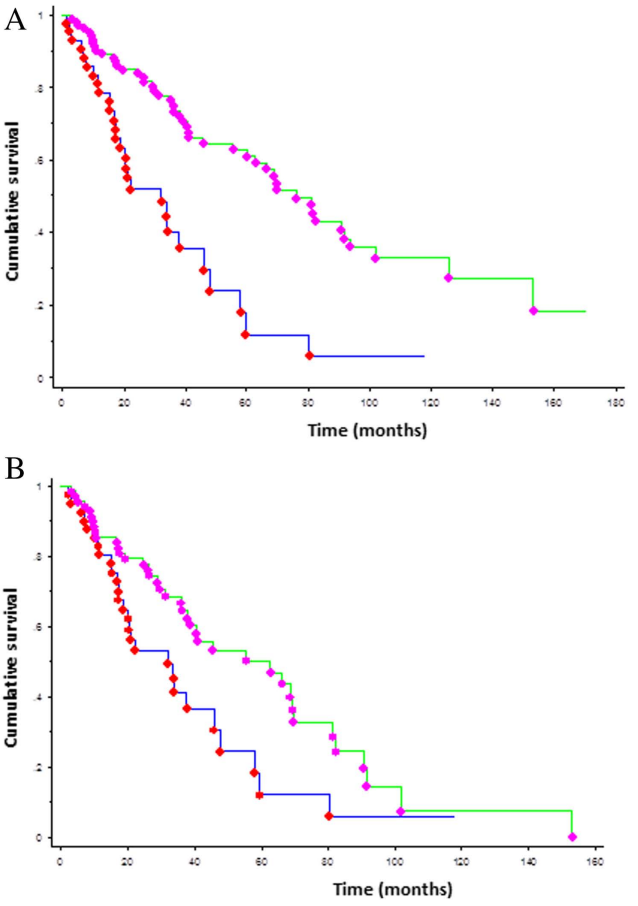


Figure 3. Kaplan–Meier survival curves of PMF patients stratified by increased circulating hepcidin (>3SD; >91,604 pg/mL) and ferritin (>500 μg/L) levels. (A) PMF patients with increased circulating levels of both hepcidin and ferritin had a significantly inferior survival (bottom curve; $n = 42$; median survival = 30 months) as compared to those who did not have these characteristics (top curve; $n = 104$; median survival = 76 months) (log-rank $P < 0.0001$). (B) PMF patients with DIPSS-plus intermediate-2 or high-risk disease stratified by increased circulating levels of both hepcidin and ferritin. Patients with increased circulating levels of both hepcidin and ferritin had a significantly inferior survival (bottom curve; $n = 41$; median survival = 32 months) as compared to those who did not have these characteristics (top curve; $n = 70$; median survival = 57 months) (log-rank $P = 0.01$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Our observations reveal that hepcidin levels are significantly dysregulated in PMF; despite this, there was evidence of relatively preserved homeostatic control of hepcidin by iron, as reflected by the strongly positive correlation between hepcidin and ferritin levels. This correlation was independent of DIPSS-plus risk stratification, unlike what has been observed in MDS patients stratified by WHO subtype [16,17]; the latter analysis suggested relatively preserved homeostatic control in some MDS subtypes such as refractory anemia, refractory anemia with ringed sideroblasts, and 5q-syndrome but near complete loss of regulation in others such as refractory anemia with excess blasts and chronic myelomonocytic leukemia. The positive correlation between hepcidin and ferritin levels in PMF was found regardless of whether patients were RBC transfusion requiring at the time of sample collection. Only 5% of transfusion-independent patients had iron overload (ferritin, >500 μg/L) at sample collection as compared to 72% of transfusion-dependent patients; this suggests that homeostatic control of hepcidin by iron is operative over a wide range of ferritin values, but at the same time, does not exclude an independent positive feedback on hepcidin levels by other high-risk disease features (as transfusion dependency also upstages PMF disease risk). In contrast, the inverse correlation between hemoglobin and hepcidin levels is reminiscent of the suppressive effect of increased erythropoietic drive on hepcidin levels that has been described in thalassemic patients, particularly those who are transfusion naïve [18], possibly acting through growth differentiation factor 15 [19]. This correlation was relatively weak, however, and is confounded in that the effect of proximate transfusion on hemoglobin levels cannot always be controlled for in a retrospective analysis. Somewhat unexpectedly, our data set showed no correlation between hepcidin and circulating cytokine levels, including those implicated in the host inflammatory response. Overall, these data suggest that, in PMF, iron loading and higher-risk disease by DIPSS-plus have a dominant stimulatory role on hepcidin levels as compared to the effects from inflammatory signals or increased erythropoietic drive.

Our data revealed that increased hepcidin levels were predictive of inferior survival in PMF, independent of most of the adverse prognostic variables that comprise the DIPSS-plus model. Further, our analysis showed that out of the four correlated variables, namely increased hepcidin, increased ferritin, hemoglobin of <10 g/dL, and RBC transfusion requirement, only increased hepcidin and ferritin levels had independent prognostic value for survival. This observation suggests the possibility of introducing these variables in future prognostic models. Overall, this observation is hardly surprising as both hepcidin and ferritin are complex biomarkers with inputs from iron loading (both intrinsic and iatrogenic), inflammation, and ineffective erythropoietic activity, all of which are operative in PMF patients. A disadvantage inherent to retrospective analyses,

however, is that the cause of death frequently cannot be determined with certainty; consequently, it is not clear whether the basis for excess mortality in PMF patients with increased hepcidin and ferritin levels relates to increased risk of disease progression/transformation, infections, cardiovascular events, or other causes. Another potential weakness is the lack of harmonization of hepcidin levels as determined by various methodologies [20].

Although factors related to the disease clone(s) such as cytogenetic abnormalities, cytopenias, and leukocytosis remain the cornerstones for determining prognosis in PMF, our data suggest that dysregulated iron homeostasis has also an important contribution in this regard. Although it is unlikely that the detrimental effect of the latter is primarily manifest through an increased risk of cardiac siderosis, other potential mechanisms such as a heightened pro-oxidative state that promotes genomic instability and disease transformation, increased risk of infections, or increased therapy-related adverse effects including those associated with hematopoietic stem cell transplantation may be operative in this setting [21].

## Author Contributions

AP and AT contributed patients, abstracted clinical data, analyzed the data, and wrote the initial draft of the manuscript. RAA abstracted clinical data from the medical record. CF and TLL performed the laboratory experiments including hepcidin measurements. All authors approved the final version of the paper for submission.

## References


1. Tefferi A, Lasho TL, Jimma T, et al. One thousand patients with primary myelofibrosis: The mayo clinic experience. Mayo Clin Proc 2012;87:25–33.
2. Tefferi A, Vaidya R, Caramazza D, et al. Circulating interleukin (IL)−8, IL-2R, IL-12, and IL-15 levels are independently prognostic in primary myelofibrosis: A comprehensive cytokine profiling study. J Clin Oncol 2011;29:1356–1363.
3. Verstovsek S, Kantarjian H, Mesa RA, et al. Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis. N Engl J Med 2010;363:1117–1127.
4. Leitch HA, Chase JM, Goodman TA, et al. Improved survival in red blood cell transfusion dependent patients with primary myelofibrosis (PMF) receiving iron chelation therapy. Hematol Oncol 2010;28:40–48.
5. Winder A, Lefkowitz R, Ghoti H, et al. Urinary hepcidin excretion in patients with myelodysplastic syndrome and myelofibrosis. Br J Haematol 2008;142:669–671.
6. Gangat N, Caramazza D, Vaidya R, et al. DIPSS plus: A refined Dynamic International Prognostic Scoring System for primary myelofibrosis that incorporates prognostic information from karyotype, platelet count, and transfusion status. J Clin Oncol 2011;29:392–397.
7. Ganz T. Hepcidin and iron regulation, 10 years later. Blood 2011;117:4425–4433.
8. Nemeth E, Tuttle MS, Powelson J, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 2004;306: 2090–2093.
9. Hentze MW, Muckenthaler MU, Galy B, et al. Two to tango: Regulation of Mammalian iron metabolism. Cell 2010;142:24–38.
10. Sun CC, Vaja V, Babitt JL, et al. Targeting the hepcidin-ferroportin axis to develop new treatment strategies for anemia of chronic disease and anemia of inflammation. Am J Hematol 2012;87:392–400.
11. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: Rationale and important changes. Blood 2009;114:937–951.
12. Caramazza D, Begna KH, Gangat N, et al. Refined cytogenetic-risk categorization for overall and leukemia-free survival in primary myelofibrosis: A single center study of 433 patients. Leukemia 2011;25:82–88.
13. Cheng PP, Jiao XY, Wang XH, et al. Hepcidin expression in anemia of chronic disease and concomitant iron-deficiency anemia. Clin Exp Med 2011;11:33–42.
14. Cheng PP, Sun ZZ, Jiang F, et al. Hepcidin expression in patients with acute leukaemia. Eur J Clin Invest 2012;42:517–525.
15. Kittur J, Knudson RA, Lasho TL, et al. Clinical correlates of JAK2V617F allele burden in essential thrombocythemia. Cancer 2007;109:2279–2284.
16. Santini V, Girelli D, Sanna A, et al. Hepcidin levels and their determinants in different types of myelodysplastic syndromes. PLoS One 2011;6:e23109.
17. Alessandrino EP, Angelucci E, Cazzola M, et al. Iron overload and iron chelation therapy in patients with myelodysplastic syndrome treated by allogeneic stem-cell transplantation: Report from the working conference on iron chelation of the Gruppo Italiano Trapianto di Midollo Osseo. Am J Hematol 2011;86:897–902.
18. Tanno T, Bhanu NV, Oneal PA, et al. High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin. Nat Med 2007;13:1096–1101.
19. Origa R, Galanello R, Ganz T, et al. Liver iron concentrations and urinary hepcidin in beta-thalassemia. Haematologica 2007;92:583–588.
20. Kroot JJ, van Herwaarden AE, Tjalsma H, et al. Second round robin for plasma hepcidin methods: First steps toward harmonization. Am J Hematol 2012;87:977–983.
21. Pullarkat V. Objectives of iron chelation therapy in myelodysplastic syndromes: More than meets the eye? Blood 2009;114:5251–5255.