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# Distinct clinico-genomic factors drive outcomes in patients with myelofibrosis and disease-related anemia

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**Background:** Disease related anemia in myelofibrosis (MF) is common and prognostically detrimental. Anemia in MF poses a therapeutic challenge as it contributes to poor quality of life and often interferes with JAK inhibitor therapy. Still, the causes for anemia in MF are varied raising the question as to whether all patients with MF-related anemia should be viewed through the same prognostic lens.

**Methods:** In this retrospective study, we analyzed clinical and genomic data of patients with MF-related anemia using an institutional MF database. Anemia was defined as the requirement of red blood cell transfusions or a hemoglobin level of <10 g/dL at presentation. Multivariable analysis performed using Cox regression formed the basis of a proposed prognostic scoring system for patients with anemic MF.

**Results:** Among 739 patients with MF, 365 (49.5%) were anemic at presentation. Anemic patients were older, had lower platelet count, lower serum albumin, and higher ferritin level than non-anemic patients. The presence of a *JAK2* mutation was less common, whereas mutations in *U2AF1* and *EZH2* were enriched in the anemic cohort. Blast phase transformation was more common in anemic patients. After a median follow up of 34.5 months, median overall survival (OS) was significantly shorter in anemic vs. non-anemic MF (30.2 vs. 73.9 months;  $p < 0.01$ ). Leukocytosis, thrombocytopenia, low serum albumin, and the presence of a mutation involving *SRSF2* or *TP53* were independent predictors of inferior OS in anemic MF on multivariable analysis. A proposed prognostic model including these factors stratified anemic MF cohort into low, intermediate, and high-risk categories, with median OS of 69, 37.7, and 11.6 months, respectively ( $p < 0.01$ ).

**Conclusions:** Our study highlights the heterogeneity of patients with MF and anemia and identifies key prognostic correlates in this subgroup. Our proposed model may help guide therapeutic decision-making in this high-risk cohort.

#### KEYWORDS

cytopenic myelofibrosis, prognostic model, overall survival, albumin, thrombocytopenia, SRSF2, TP53

## Introduction

Myelofibrosis (MF) is a chronic myeloid malignancy that can occur either *de-novo* (primary MF) or after a prior diagnosis of polycythemia vera or essential thrombocythemia (1). Patients with MF have heterogeneous clinical features and variable disease course (1, 2). Almost all patients with MF develop anemia during the course of their disease (3). The causes of anemia in MF vary amongst patients. Contributing factors include an impaired bone marrow niche, ineffective hematopoiesis due to somatically acquired gene mutations, intramedullary hemolysis, organomegaly, chronic inflammation, and an inappropriate erythropoietin response to anemia (4, 5). Across numerous prognostic scoring systems, disease-related anemia consistently demonstrates a detrimental prognostic impact in patients with MF (2, 6, 7).

On the other hand, anemia can also result after treatment with a Janus kinase (JAK) inhibitor such as ruxolitinib. Treatment-induced anemia often leads to suboptimal dosing and is a common reason for therapy discontinuation (8, 9). Studies have shown that treatment-related anemia does not impact clinical response to ruxolitinib (10); however, this data comes from clinical trial populations in which dose modification and treatment discontinuation due to anemia occur less frequently. Often, it can be hard to distinguish disease-related anemia from treatment-related anemia given the frequency of the former and ubiquitous nature of the latter. The recently proposed RR6 model showed that red blood cell transfusion dependence (whether driven by disease or treatment) within first 6 months of ruxolitinib treatment is predictive of worse OS (11). More recently approved JAK inhibitors like momelotinib and pacritinib have shown beneficial effect on MF-related anemia and may provide an alternative for some patients (12–14). Still, questions remain. Should anemia in MF be viewed as uniformly detrimental regardless of the underlying cause? Which patients would benefit from a change in therapy? Answering these questions requires a more nuanced understanding of what factors drive poor outcomes in anemic MF.

In the current study, we aimed to identify clinical and molecular factors that significantly influence outcomes in patients with MF and anemia. From this, we propose distinct prognostic clusters to

aid clinicians in better evaluating disease risk, thereby informing critical treatment decisions.

## Materials and methods

### Study population and datapoints

In this single-center retrospective cohort study, we included all adult patients who presented to Moffitt Cancer Center between 2001 and 2021 with a diagnosis of primary MF per World Health Organization (WHO) 2016 classification criteria, or post-polycythemia vera or post-essential thrombocythemia MF per International Working Group (IWG) criteria (15, 16). The study protocol was approved by the institutional review board at the University of South Florida. Clinical and genomic data on eligible patients were collected from a well-annotated institutional MF database.

Clinical and genomic variables were collected at time of first presentation to our center. Constitutional symptoms were largely recorded by treating physicians as part of routine clinical care. Anemia was defined as the requirement of red blood cell transfusions and/or a hemoglobin level of <10 g/dL at presentation. Patients who started ruxolitinib ≤ 6 months prior to presentation were excluded from this analysis to better isolate disease-related rather than JAK inhibitor-driven anemia.

Cytogenetic findings were used to categorize patients into ‘favorable’, ‘unfavorable’, and ‘very high risk’ categories (17). Data on relevant somatic mutations was obtained as part of routine clinical care using an in-house targeted DNA sequencing panel for myeloid malignancies. Although this panel has evolved over the years, it has always had coverage for *JAK2*, *MPL*, *CALR*, *TET2*, *DNMT3A*, *ASXL1*, *SRSF2*, *U2AF1*, *EZH2*, *IDH1*, *IDH2*, *KRAS*, *NRAS*, *RUNX1*, and *TP53*. A minimum variant allele frequency of 5% was used to call single nucleotide variants, and a cutoff of 10% was used for insertions or deletions. Pathogenicity of variants was determined by an internally relied on variant classification system, based on joint consensus recommendations of the Association for Molecular Pathology, American Society of Clinical Oncology, and

the College of American Pathologists, in conjunction with review of COSMIC and cBioPortal databases as needed.

## Statistical analysis

Categorical variables were summarized in the form of numbers and percentages. Standard descriptive statistics were used for the continuous variables. Categorical variables were compared using the Fisher's exact test or Chi-squared test, as applicable. Comparisons of continuous variables were performed with the Wilcoxon rank sum test or Kruskal–Wallis test, as appropriate. OS was estimated using the Kaplan–Meier method with survival estimates being compared using log rank test. OS was calculated from the time of presentation. A univariate Cox regression analysis identified variables that correlated with OS. For serum albumin, median level in study cohort was used as cut-off for regression analysis. All variables with a *p* value of <0.10 were included in the subsequent multivariate analyses. Due to missing genomic data in a significant proportion of patients, two multivariable analyses were performed. The first was performed on the entire cohort and only included clinical variables. The second was only performed on patients with available genomic information. From these findings, a

novel prognostic scoring system was proposed, incorporating the independent predictors for OS on multivariate analyses with scores assigned based on magnitude of hazard ratios (HR). All statistical analyses were conducted in IBM SPSS statistics (version 29).

## Results

### Clinical and genomic characteristics

Among total 737 patients with MF, 365 (49.5%) patients were anemic at presentation, including 205 patients who required transfusions. The median age at presentation was 68.5 (range, 18.9–91.4) years. Median time from MF diagnosis to presentation was 2.8 months. Clinical and genomic characteristics of study population are shown in **Table 1**. Patients with anemic MF were significantly older (median age, 69.8 vs. 67.2 years; *p* <0.01). Anemic patients were more likely to be diagnosed with primary MF (72% vs. 65%; *p* = 0.04). Patients with anemic MF had a lower median WBC ( $6.9 \times 10^9/L$  vs.  $11 \times 10^9/L$ ; *p* <0.01) and platelet count ( $146 \times 10^9/L$  vs.  $279 \times 10^9/L$ ; *p* <0.01) than non-anemic patients. Serum erythropoietin and ferritin levels were significantly higher in anemic MF (median, 91 vs. 18 mIU/ml, and 567 vs. 136 microgram/L, respectively; *p* <0.01). Median

TABLE 1 Clinical and genomic characteristics of study population.

Variables	No. of Evaluable Patients	Overall (n= 739)	Anemic at Presentation (n=365)	Non-anemic at Presentation (n= 374)	P Value
Age at Presentation [median (range), years]	737	68.5 (18.9-91.4)	69.8 (21-91.4)	67.2 (18.9-87.7)	<0.01
Male Sex (n, %)	739	431 (58%)	212 (58%)	219 (59%)	0.89
Time from Diagnosis to Presentation (median, months)	739	2.8	6	1.7	<0.01
Type of MF (n, %)	737				0.04
Primary MF		506 (69%)	262 (72%)	244 (65%)	
Secondary MF		231 (31%)	101 (28%)	130 (35%)	
Constitutional Symptoms (n, %)	735	262 (36%)	140 (39%)	122 (33%)	0.10
Splenomegaly (n, %)	723	528 (73%)	265 (75%)	263 (71%)	0.27
WBC Count (median, range, $\times 10^9/L$ )	736	9 (0.5-335)	6.9 (0.5-335)	11 (1.8-122.7)	<0.01
Platelet Count (median, range, $\times 10^9/L$ )	736	206 (1-2209)	146 (1-1834)	279 (8-2209)	<0.01
EPO Level (median, range, mIU/mL)	339	43 (0-2587)	91 (3-2587)	18 (0-1357)	<0.01
Ferritin (median, range, microgram/L)	679	249 (4-11167)	567 (12- 11167)	136 (4-9381)	<0.01
Albumin (median, range, gm/dL)	733	4.3 (2.2-5.2)	4.1 (2.2-5.2)	4.4 (2.5-5.2)	<0.01
Haptoglobin (median, range, mg/dL)	485	86 (5-563)	88.5 (5-563)	85 (8-412)	0.49
Elevated LDH (n, %)	715	628 (88%)	305 (88%)	323 (88%)	0.88
Positive DAT IgG (n, %)	739	31 (4%)	24 (7%)	7 (2%)	<0.01
Degree of Fibrosis (n, %)	722				<0.01
MF-0 or 1		87 (12%)	30 (8%)	57 (16%)	
MF-2 or 3		635 (88%)	326 (92%)	309 (84%)	
Unfavorable or Very High Risk Cytogenetics (n, %)	669	212 (32%)	114 (36%)	98 (28%)	0.03

(Continued)

TABLE 1 Continued

Variables	No. of Evaluable Patients	Overall (n= 739)	Anemic at Presentation (n=365)	Non-anemic at Presentation (n= 374)	P Value
<b>Somatic Mutations (n, %)</b>					
<i>JAK2</i>	697	427 (61%)	191 (57%)	236 (65%)	<b>0.02</b>
<i>ASXL1</i>	505	141 (28%)	71 (31%)	70 (25%)	0.15
<i>CALR</i>	510	118 (23%)	49 (22%)	69 (24%)	0.45
<i>SRSF2</i>	501	53 (11%)	19 (8%)	34 (12%)	0.15
<i>U2AF1</i>	501	52 (10%)	44 (20%)	8 (3%)	<b>&lt;0.01</b>
<i>MPL</i>	545	46 (8%)	25 (10%)	21 (7%)	0.20
<i>U2AF1</i> Q157	450	38 (8%)	32 (15%)	6 (3%)	<b>&lt;0.01</b>
<i>EZH2</i>	503	41 (8%)	26 (11%)	15 (6%)	<b>0.01</b>
<i>SF3B1</i>	501	32 (6%)	14 (6%)	18 (7%)	0.87
<i>TP53</i>	503	19 (4%)	10 (4%)	9 (3%)	0.51
<i>IDH1</i>	503	15 (3%)	10 (4%)	5 (2%)	0.08
<i>IDH2</i>	503	14 (3%)	5 (2%)	9 (3%)	0.47
Blast Phase Transformation (n, %)	738	75 (10%)	49 (14%)	26 (7%)	<b>&lt;0.01</b>
<b>Treatment Modalities- All Lines of Therapy (n, %)</b>					
ESA	739	234 (32%)	175 (48%)	59 (16%)	<b>&lt;0.01</b>
Hydroxyurea	739	251 (34%)	94 (26%)	157 (42%)	<b>&lt;0.01</b>
Interferon	739	38 (5%)	15 (4%)	23 (6%)	0.20
Thalidomide/Lenalidomide	739	176 (24%)	131 (36%)	45 (12%)	<b>&lt;0.01</b>
Ruxolitinib	739	277 (38%)	120 (33%)	157 (42%)	<b>0.01</b>
Androgen	739	60 (8%)	48 (13%)	12 (3%)	<b>&lt;0.01</b>
Allo-HSCT	737	103 (14%)	61 (17%)	42 (11%)	<b>0.03</b>

Allo-HSCT, Allogeneic Hematopoietic Stem Cell Transplant; DAT, Direct antiglobulin test; ESA, Erythropoiesis stimulating agents; MF, Myelofibrosis; n/no., Number; WBC, White blood cell. Significant p value of <0.05 was highlighted in bold font.

serum albumin was 4.1 gm/dL in anemic patients vs. 4.4 gm/dL in others ( $p < 0.01$ ). Patients with anemic MF had higher frequency of advanced (MF 2 or 3) marrow fibrosis.

MF patients with anemia more frequently harbored unfavorable or very high-risk cytogenetics than patients without disease-driven anemia (36% vs. 28%;  $p = 0.03$ ). Mutations in *JAK2* were less common in anemic than non-anemic subgroup (57% vs. 65%;  $p = 0.02$ ), whereas mutations involving *U2AF1* (20% vs. 3%;  $p < 0.01$ ) and *EZH2* (11% vs. 6) were more prevalent in patients with anemic MF.

## Treatment and outcomes

In our study population, the treatment modalities across all lines of therapy included erythropoiesis stimulating agents (ESA) (32%), hydroxyurea (34%), thalidomide or lenalidomide (24%), ruxolitinib (38%), androgen (8%), and interferon (5%). Use of ESA, thalidomide or lenalidomide, and androgen was more common, and treatment with hydroxyurea and ruxolitinib was

less common in the anemic MF subgroup (Table 1). Overall, 14% of patients underwent allogeneic hematopoietic stem cell transplant (allo-HSCT), with this intervention being more commonly used in anemic patients (17% vs. 11%;  $p = 0.03$ ).

Rate of blast phase transformation was significantly higher in patients with anemic MF than those without anemia (14% vs. 7%;  $p < 0.01$ ). After a median follow up duration of 34.5 months, median OS was 45.4 (95% confidence interval [CI]: 39.9-50.8) months for the entire study population. Anemia at presentation was associated with significantly worse OS in patients with MF (30.2 [95% CI: 24-36.4] vs. 73.9 [95% CI: 54.6-93.3] months;  $p < 0.01$ ).

## Prognostic factors for overall survival

In the univariate analysis (Table 2), variables associated with an inferior OS in the anemic MF cohort (n=365) included age > 65 years (HR 2.07;  $p < 0.01$ ), constitutional symptoms (HR 1.59;  $p < 0.01$ ), WBC count >25 x 10<sup>9</sup>/L (HR 3.23;  $p < 0.01$ ), platelet count <100 x 10<sup>9</sup>/L (HR

1.57;  $p < 0.01$ ), peripheral blasts  $\geq 1\%$  (HR 1.66;  $p < 0.01$ ), serum albumin  $< 4.1$  gm/dL (HR 1.86;  $p < 0.01$ ), and the presence of an *ASXL1* (HR 1.23;  $p < 0.01$ ), *EZH2* (HR 1.22;  $p < 0.01$ ), *SRSF2* (HR 1.21;  $p = 0.01$ ), or *TP53* (HR 1.22;  $p < 0.01$ ) mutation.

Two separate multivariate analyses were performed. In the first multivariate analysis, we used data from entire anemic MF cohort ( $n=365$ ) and included all clinical and laboratory variables with borderline significance ( $p < 0.10$ ) on univariate analysis. Age  $> 65$  years (HR 1.90;  $p < 0.01$ ), WBC count  $> 25 \times 10^9/L$  (HR 2.43;  $p < 0.01$ ), platelet count  $< 100 \times 10^9/L$  (HR 1.54;  $p < 0.01$ ), peripheral blasts  $\geq 1\%$  (HR 1.41;  $p = 0.02$ ), and serum albumin  $< 4.1$  gm/dL (HR 1.47;  $p = 0.01$ ) were the independent prognostic factors for OS.

The second multivariate analysis included only the anemic MF cases with available baseline molecular information ( $n=211$ ). In this analysis, clinical, laboratory and genomic variables were included. WBC count  $> 25 \times 10^9/L$  (HR 2.19, 95% CI: 1.16-4.14;  $p = 0.01$ ), platelet count  $< 100 \times 10^9/L$  (HR 2.15, 95% CI: 1.31-3.53;  $p < 0.01$ ), serum albumin  $< 4.1$  gm/dL (HR 1.71, 95% CI: 1.06-2.75;  $p = 0.02$ ), *SRSF2* mutation (HR 2.79, 95% CI: 1.47-5.28;  $p < 0.01$ ), and *TP53* mutation (HR 2.49, 95% CI: 1.10-5.61;  $p = 0.02$ ) were the independent predictors of OS in patients with MF and anemia (Table 2, Figure 1).

## Development of prognostic scoring system

Based upon these findings, we developed a prognostic scoring system comprised of the five significant variables in the multivariate analysis on molecularly annotated cohort of anemic MF. This score included serum albumin  $< 4.1$  gm/dL (1 point), WBC count  $> 25 \times 10^9/L$  (1.5 points), platelet count  $< 100 \times 10^9/L$  (1.5 points), *TP53* mutation (1.5 points), and *SRSF2* mutation (2 points). Patients in the low risk (no risk factor; aggregate score 0), intermediate risk (1 risk factor; aggregate score 1 to 2), and high risk ( $> 2$  risk factor; aggregate score  $> 2$ ) categories had distinct median OS estimates of 69, 37.7, and 11.6 months, respectively ( $p < 0.01$ ) (Figure 2).

## Discussion

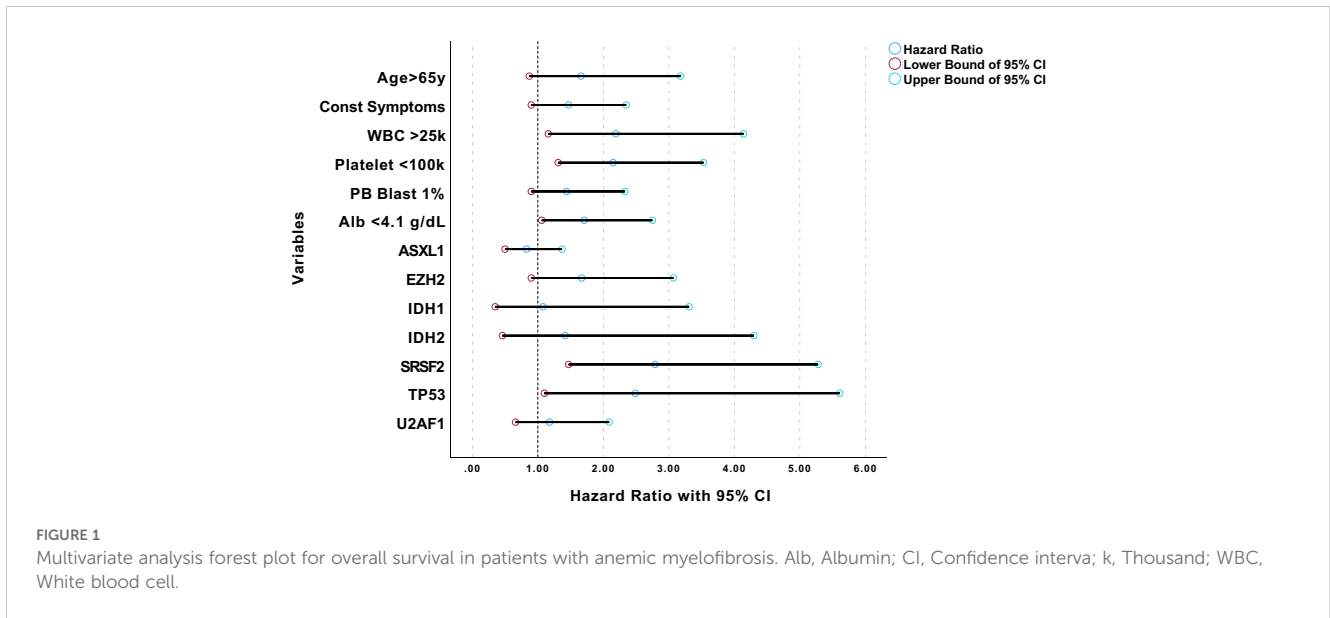
In this single center analysis, we validated several key characteristics of anemic MF which have been previously shown. Patients with disease-related anemia at presentation were significantly older and had higher proportion of primary MF compared to non-anemic cohort (3, 17, 18). In addition, patients with MF and anemia had lower WBC and platelet counts, lower

TABLE 2 Results of univariate and multivariate Cox-regression analysis in anemic myelofibrosis cohort.

Variables	Univariate Analysis ( $n=365$ )		Multivariate Analysis <sup>1</sup> ( $n=365$ )		Multivariate Analysis <sup>2</sup> ( $n=211$ )	
	HR (95% CI)	P Value	HR (95% CI)	P Value	HR (95% CI)	P Value
Age $> 65$ years	2.07 (1.48-2.90)	<b>&lt;0.01</b>	<b>1.90</b> (1.35-2.67)	<b>&lt;0.01</b>	1.66 (0.87-3.18)	0.12
Constitutional symptoms	1.59 (1.19-2.12)	<b>&lt;0.01</b>	1.23 (0.91-1.65)	0.16	1.47 (0.9-2.35)	0.10
Time from diagnosis to presentation	0.99 (0.99-1.002)	0.41				
WBC count $> 25 \times 10^9/L$	3.23 (2.15- 4.84)	<b>&lt;0.01</b>	<b>2.43</b> (1.57-3.76)	<b>&lt;0.01</b>	<b>2.19</b> (1.16-4.14)	<b>0.01</b>
Platelet count $< 100 \times 10^9/L$	1.57 (1.18- 2.09)	<b>&lt;0.01</b>	<b>1.54</b> (1.14-2.07)	<b>&lt;0.01</b>	<b>2.15</b> (1.31-3.53)	<b>&lt;0.01</b>
PB blast count $\geq 1\%$	1.66 (1.25- 2.21)	<b>&lt;0.01</b>	<b>1.41</b> (1.05-1.90)	<b>0.02</b>	1.44 (0.9-2.33)	0.12
Active ruxolitinib at presentation*	1.42 (0.8-2.52)	0.22				
Albumin $< 4.1$ gm/dL	1.86 (1.40-2.47)	<b>&lt;0.01</b>	<b>1.47</b> (1.09-1.98)	<b>0.01</b>	<b>1.71</b> (1.06-2.75)	<b>0.02</b>
Degree of bone marrow fibrosis (MF-2 or 3)	1.17 (0.66-2.06)	0.57				
Hypocellular marrow	0.99 (0.65-1.49)	0.96				
Unfavorable/very high risk cytogenetics	1.26 (0.93-1.72)	0.13				
ASXL1	1.23 (1.05-1.45)	<b>&lt;0.01</b>			0.83 (0.50-1.37)	0.48
EZH2	1.22 (1.05- 1.42)	<b>&lt;0.01</b>			1.67 (0.9-3.07)	0.09
IDH1	1.13 (0.98-1.31)	0.08			1.08 (0.35-3.31)	0.88
IDH2	1.15 (0.99-1.32)	0.05			1.42 (0.46-4.30)	0.53
SRSF2	1.21 (1.04- 1.39)	<b>0.01</b>			<b>2.79</b> (1.47-5.28)	<b>&lt;0.01</b>
TP53	1.22 (1.05-1.41)	<b>&lt;0.01</b>			<b>2.49</b> (1.10-5.61)	<b>0.02</b>
U2AF1	1.13 (0.97-1.32)	0.09			1.18 (0.66-2.09)	0.56

\*Ruxolitinib started  $> 6$  months before presentation and active at presentation.

<sup>1</sup>All patients with anemic MF; <sup>2</sup>Patients with anemic MF with molecular annotations. CI, Confidence interval; HR, Hazard ratio; PB, Peripheral blood; WBC, White blood cell. Significant  $p$  value of  $< 0.05$  was highlighted in bold font.

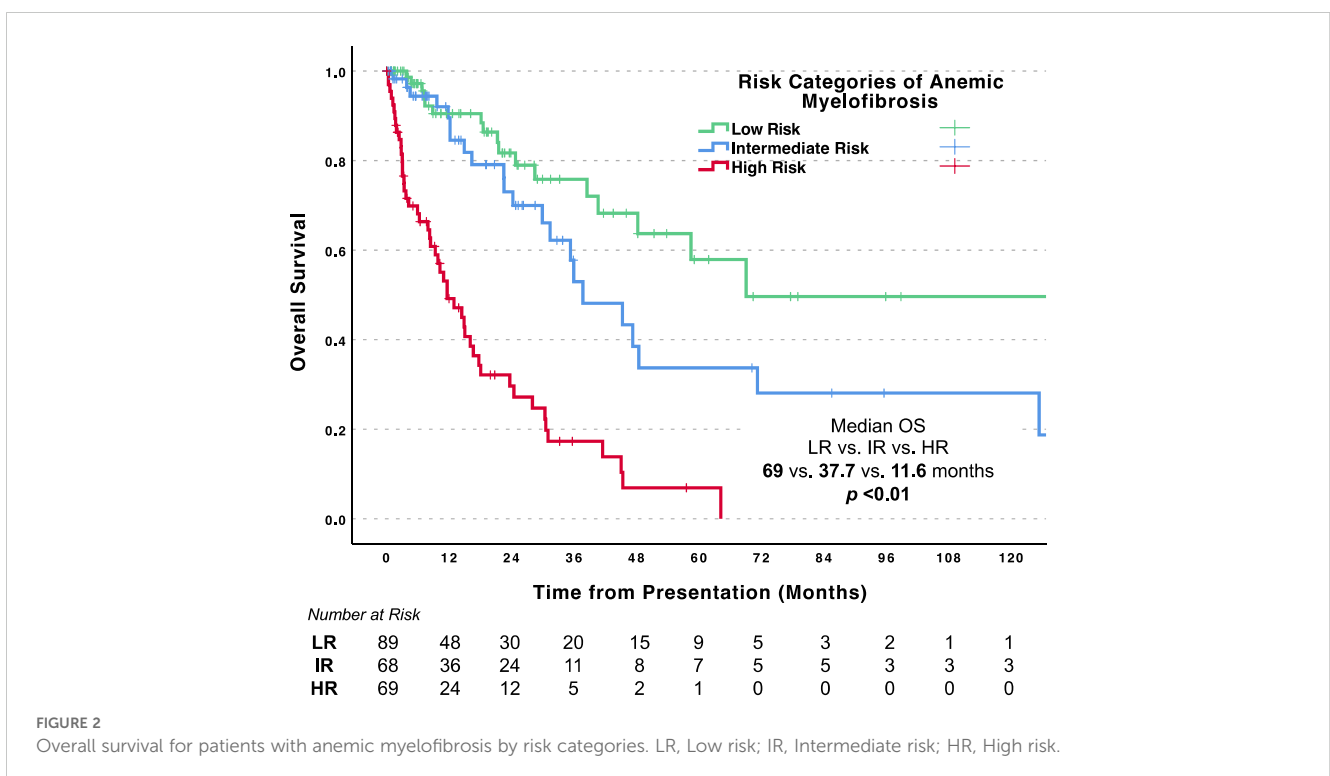


serum albumin levels, higher ferritin and were more likely to harbor high-risk genetic features (2, 3, 6). In line with these findings, blast phase transformation was more common and overall survival was worse in this subgroup (10).

Beyond this, we assessed whether all patients with MF and anemia should be viewed as high-risk. We identified clinical and genetic variables that are specifically prognostic in this anemic subgroup. Interestingly, we found that patients with MF and anemia have a unique set of variables that hold prognostic significance. Thrombocytopenia, leukocytosis, low serum albumin, and the presence of an *SRSF2* or *TP53* mutation hold prognostic significance in this cohort and MF at large (6); however, variables such as age,

peripheral blast percentage, presence of constitutional symptoms, and presence of *ASXL1* or *U2AF1* mutations did not demonstrate prognostic significance (2, 3, 6). Several studies have demonstrated the value of serum albumin in cachexia and overall prognosis in patients with cancers including MF (18, 19). We previously showed that serum albumin level could be prognostic in patients with MF and disease-related thrombocytopenia (20), and can serve as a dynamic marker for outcomes in patients with MF treated with ruxolitinib (21). Findings of our current study help to hone in on the variables that drive outcomes in patients with an anemic phenotype.

We leveraged these findings to build a prognostic system that could stratify patients by risk. Interestingly, we found a low-risk



subgroup that lacked leukocytosis, thrombocytopenia, low albumin, and high-risk mutations (*SRSF2* and *TP53*), which comprised more than a third of the anemic cohort. This subgroup ultimately demonstrated an expected survival that closely resembled that of non-anemic patients. Alternatively, patients who harbored at least two risk factors for high-risk disease exhibited a median OS of less than one year. These findings highlight the importance of appropriate risk stratification for clinical management in patients with anemic MF, where traditional MF prognostic models may be of limited value (2, 6). Clinicians can use this model to identify patients with anemic MF (intermediate and high risk), who could potentially benefit from immediate evaluation for allo-HSCT.

Our study is strengthened by the use of a large molecularly annotated cohort of patients with MF with long follow-up and well documented outcomes. However, this single center retrospective study has some clear limitations. Differentiating between disease-related and treatment-induced anemia in MF can be challenging. We tried to limit the influence of ruxolitinib-induced anemia by excluding those who had been on ruxolitinib for less than 6 months at the time of baseline assessment as treatment-induced anemia is most common in this window (9). Still, we likely included some patients whose anemia was driven, at least in part, by treatment. Additionally, this analysis was observational, and we were not able to evaluate the responses to specific therapies in patients with anemic MF or assess how these therapies may impact outcome. Since we did not routinely use structured symptom assessment form, the data on constitutional symptoms was largely dependent on the subjective assessment by treating physicians.

In conclusion, our study highlights the unique clinico-molecular prognostic correlates for MF with disease-related anemia and proposes a novel prognostic model for effective risk stratification in these patients. Anemic MF often defines a high-risk disease state; however, an evolving treatment landscape that includes less myelosuppressive JAK inhibitors with ACVR1 inhibitor activity (13, 14), improved feasibility of allo-HSCT, and promising agents in development offers new hope (22). We hope that our findings will support individualized, risk-adapted treatment decisions for patients with anemic MF.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The studies involving humans were approved by University of South Florida Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

## Author contributions

SB: Data curation, Formal Analysis, Project administration, Writing – original draft, Writing – review & editing. NA: Data curation, Writing – review & editing. AJ: Data curation, Writing – review & editing. LA: Data curation, Writing – review & editing. SY: Writing – review & editing. OC: Writing – review & editing. ZX: Writing – review & editing. DS: Writing – review & editing. JL: Writing – review & editing. EP: Writing – review & editing. RK: Writing – review & editing. AK: Conceptualization, Methodology, Supervision, Writing – review & editing.

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