

Research Article

Clinical and Prognostic Correlates of the Reticulin-Collagen-Osteosclerosis Score in Primary Myelofibrosis

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ABSTRACT

Primary myelofibrosis (PMF) is hallmarked by stromal bone marrow changes, whose accurate evaluation holds diagnostic and prognostic implications. The reticulin fibrosis (MF), collagen (Co), osteosclerosis (Ost) score (RCO-S) was developed in 2017 and proved to be accurate and prognostically informative. In this retrospective, single-center study, we validated the RCO-S and explored its clinical, genetic, and prognostic correlates in a large cohort of patients with PMF, including 121 prefibrotic (pre-PMF) and 101 overt PMF (oPMF). Overall, stromal changes evolved harmonically in the 2 PMF subtypes, with pre-PMF showing milder alterations. A higher RCO-S was associated with distinct high-risk clinical and molecular features and shortened overall survival, particularly in oPMF. Receiver operator characteristic analysis confirmed 5 as the most accurate RCO-S cutoff value (RCO-S⁵) to differentiate low-grade (<5) and high-grade (≥5) PMF. High-grade disease was associated with adverse clinical and molecular profiles, worse overall survival, and higher cumulative incidence of leukemic transformation. Notably, integrating RCO-S into the mutation-enhanced international prognostic score system prognostic model improved risk stratification and accuracy compared with the standard scoring system. Despite study limitations, our findings showed that (1) the RCO-S provides a comprehensive assessment of PMF stromal changes; (2) it holds clinical, genetic, and prognostic relevance; and (3) its integration into existing prognostic models has the potential to improve prognostic stratification.

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Introduction

Among Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs), primary myelofibrosis (PMF) is the most clinically aggressive variant.¹ Histologically, PMF is characterized by a

range of distinctive changes within the bone marrow (BM), including hyperplastic granulopoiesis, reduced erythroid proliferation and maturation, atypical megakaryocytes organized in dense clusters, and distinct stromal alterations including reticulin fibrosis, collagen deposition, osteosclerosis, and microvessel proliferation.^{2,3} These changes are an epiphenomena resulting from complex cytokine-induced responses related to clonal

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myeloproliferation.^{4,5} Stromal changes are particularly pronounced during the overt fibrotic phase (oPMF) as opposed to the prefibrotic stage (pre-PMF), with the latter accounting for up to 50% of all PMF cases.³ Pre-PMF is defined by absent or mild reticulin fibrosis (G1) although exhibiting distinct morphological, clinical, and molecular characteristics that align with oPMF. Precise evaluation of BM fibrotic changes is of paramount importance in PMF diagnosis on account of their negative prognostic implications and association with high-risk clinical and molecular features.⁶⁻⁹ Consequently, the BM reticulin fibrosis has been included in the mutation-enhanced international prognostic score system (MIPSS70) model.¹⁰

Although the integration of artificial intelligence-assisted technologies is expected to improve assessment of BM stroma changes in MPNs,¹¹ the current definition of myelofibrosis still relies on visual estimation of reticulin fibers on silver impregnation, according to the 4-tiered World Health Organization (WHO) grading system (ranging from MF-0 to MF-3), adapted from the 2005 European Consensus on Grading of Bone Marrow Fibrosis.^{2,12} In cases with at least MF-2 reticulin fibrosis, the current WHO classification also recommends evaluation and grading of collagen fibrosis, using Masson trichrome staining, and osteosclerosis.² Nevertheless, it is acknowledged that reticulin fibrosis is only one facet of the stromal remodeling induced by clonal myeloproliferation. Given its prognostic significance, it is reasonable to speculate that integration of reticulin fibrosis with other stromal changes, such as collagen fibrosis and osteosclerosis, may enrich PMF diagnosis and possibly contribute to refining prognostic evaluation.

To overcome the limitations inherent to a stromal evaluation primarily reliant on reticulin fibrosis, in 2017, Gianelli et al¹³ proposed a comprehensive scoring system based on the reticulin (R), collagen (Co), osteosclerosis (Ost) score (RCO-S). This score includes all 3 parameters of the European Consensus on grading of bone marrow fibrosis (EGCGMF)/WHO, namely reticulin, collagen fibrosis, and osteosclerosis grading, initially proposed by Kvasnicka et al¹⁴ and subsequently adopted by the 2016 WHO classification of hematolymphoid tumors.¹⁵ To simplify the clinical interpretation of the RCO-S, the patients were dichotomized into 2 groups: low-grade (RCO-S <5) and high-grade (RCO-S ≥5). Patients in the RCO-S high-grade group displayed laboratory and clinical findings consistent with advanced disease, higher international prognostic score system (IPSS) risk categories, and poorer overall survival (OS). Moreover, the combined evaluation of IPSS and RCO-S proved to be more prognostically accurate than the IPSS alone, whereas IPSS and RCO-S taken together were more accurate than the combination of IPSS and European Consensus on Grading of Bone Marrow Fibrosis/WHO reticulin fibrosis grading in identifying high-risk PMF patients.

Given these compelling findings, the present study aimed to provide a validation of the RCO-S and explore its clinical, genetic, and prognostic associations within a large cohort of clinically and molecularly annotated PMF patients meeting the 2022 WHO and international consensus conference criteria.^{2,16}

Materials and Methods

Patients

After obtaining approval from the Institutional Review Board of the University of Florence, patients with a diagnosis of pre-MF and oPMF referring to CRIMM (Center for Research and Innovation of Myeloproliferative Neoplasms) were included in the current study. For all patients, an adequate BM trephine biopsy for

length (≥1 cm) and quality (core specimen taken at a right angle from the cortical bone, without fragmentation) was available at PMF diagnosis or first referral. The vast majority of cases included in the study were diagnosed shortly after the appearance of the first symptoms, clinical signs, or laboratory abnormalities, thereby reducing the variability introduced by the timing of biopsy relative to disease onset. All patients well fully annotated for clinical, molecular, and follow-up data, and most underwent targeted NGS for 29 myeloid-relevant genes on DNA obtained from peripheral blood collected at the time of diagnosis or first referral, by previously described methods.¹⁰ No patient had been exposed to cytoreductive therapy.

Bone Marrow Evaluation

Formalin-fixed, paraffin-embedded BM trephine biopsies were cut at 3-μm intervals, and the resulting sections were stained with hematoxylin and eosin, Giemsa, Gomori's silver impregnation, and Masson's trichrome. All samples were reviewed, and the diagnosis was confirmed by 4 experienced hematopathologists (R.S., U.G., G.D.S., and C.P.), following the current WHO fifth edition of the classification of hematolymphoid tumors and the international consensus conference criteria.^{2,3,16} Specifically, each case was independently evaluated by 2 pathologists (G.D.S. and C.P.) before being reviewed in a panel meeting with both reviewers, senior researcher U.G., who first described the RCO scoring system, and R.S. A final diagnosis was established only upon full consensus among panel members. Discrepancies were limited in number and were observed in cases showing heterogeneous or partial stromal alterations.

BM reticulin fibrosis, collagen fibrosis, and osteosclerosis were assessed and graded for each case, according to the current WHO-proposed criteria.² We did not include the microvessel density in the evaluation, for which a well-defined scoring system has not been developed yet.^{17,18} Internal quality controls included the assessment of perivascular reticulin and collagen fibers, whereas the evaluation of fiber density was limited to lacunar hematopoietic regions. As per WHO recommendations, in case of heterogeneous patterns, the final grade was considered the highest in at least 30% of BM cellulated areas. Reticulin fibrosis was evaluated on Gomori's silver impregnation-stained slides and scored as: MF-0, scattered linear reticulin with no intersections (cross-overs), corresponding to normal BM; MF-1, loose network of reticulin with many intersections, especially in perivascular areas; MF-2, diffuse and dense increase in reticulin with extensive intersections, occasionally with focal bundles of thick fibers mainly consistent with collagen and/or focal osteosclerosis; and MF-3, diffuse and dense increase in reticulin with extensive intersections and coarse bundles of thick fibers mainly consistent with collagen, usually associated with osteosclerosis.² Collagen deposition was evaluated on Masson's trichrome stained slides and scored as: Co-0, perivascular collagen only (normal); Co-1, focal paratrabecular or central collagen deposition with no connecting meshwork; Co-2, paratrabecular or central deposition of collagen with focally connecting meshwork or generalized paratrabecular apposition of collagen; and Co-3, diffuse (complete) connecting meshwork of collagen.² Finally, osteosclerosis was evaluated on hematoxylin and eosin-stained slides and scored as: Ost-0, regular bone trabeculae (distinct paratrabecular borders); Ost-1, focal budding, hooks, spikes, or paratrabecular apposition of new bone; Ost-2, diffuse paratrabecular new bone formation with thickening of trabeculae, occasionally with focal

interconnections; and Ost-3, an extensive interconnecting meshwork of new bone with overall effacement of marrow spaces.²

Based on a previous publication by Gianelli et al,¹³ we summed reticulin, collagen, and osteosclerosis grading to obtain a RCO cumulative score for each case, ranging from 0 to 9.

Statistical Analysis

Statistical analyses considered clinical and laboratory parameters obtained at diagnosis or first referral. Continuous variables were presented as median (range), and categorical variables as frequency (percentage). Differences in the distribution of continuous variables between categories were compared by the Mann-Whitney test. Categorical variables were compared using the χ^2 test. OS analysis was computed from the date of PMF diagnosis to the date of death (uncensored) or last contact (censored). Date of blast phase (BP) transformation replaced the date of death, as the uncensored variable, for estimating leukemia-free survival. Limited to the pre-PMF cohort, date of progression to oPMF replaced date of death, as the uncensored variable, for estimating fibrotic progression-free survival. For this purpose, progression to oPMF was established by the clinical features consistent with advanced PMF, including worsening of anemia, progressive splenomegaly, overt leukoerythroblastosis, and/or histologic documentation of BM fibrosis grades 2-3. The Kaplan-Meier method was used to prepare OS and leukemia-free survival curves, which were compared by the log-rank test. Cumulative incidence (Cul) of BP and oPMF progression was calculated after competing risk analysis and compared between groups using the Gray's test. Cox proportional hazards analysis followed by backward stepwise selection was used for univariate and multivariable analyses. *P* values less than .05 were considered significant. The prognostic performance of conventional and integrated clinical-molecular score systems was evaluated by computing the Harrell's C-index, Brier score, and area under the curve (AUC) of the time-dependent receiver operator characteristic (ROC) curve, using the R packages *timeROC* and *pec*.^{19,20} The JMP Pro 16.1.0 software application from SAS Institute and R software version 4.1.1 from R Core Team were used for all calculations.

Results

Patient Population

The study population included 222 PMF patients, of whom 121 (55%) were pre-PMF, and 101 (45%) were oPMF. Table 1 summarizes the main clinical, laboratory, and molecular characteristics of the study cohort. The median age at diagnosis was 66 years (range: 18-91), and 127 (57%) patients were men. The median follow-up period was 57 months (95% confidence interval [CI], 45-71).

Bone Marrow Evaluation and Reticulin (R), Collagen (Co), Osteosclerosis (Ost) Score Calculation

The results of the BM histological analysis according to the RCO algorithm are listed in Table 2, and 4 representative cases displaying different RCO-S are reported in Figure 1. The reticulin fibrosis analysis revealed MF-0 in 9% (20/222), MF-1 in 45% (101/222), MF-2 in 22% (48/222), and MF-3 in 24% (53/222) of cases.

Trichrome staining highlighted a prevalence of cases with Co-0 (59%, 130/222), followed by Co-1 (15%, 33/222), Co-2 (14%, 31/222), and Co-3 cases (13%, 28/222). Finally, osteosclerosis evaluation revealed a predominance of Ost-0 cases (66%, 146/222), followed by Ost-1 (20%, 45/222), Ost-2 (8%, 18/222), and Ost-3 cases (6%, 13/222). According to the RCO scale, PMF cases were distributed as: RCO-S 0 *n* = 20 (9%), RCO-S 1 *n* = 93 (42%), RCO-S 2 *n* = 16 (7%), RCO-S 3 *n* = 19 (9%), RCO-S 4 *n* = 9 (4%), RCO-S 5 *n* = 16 (7%), RCO-S 6 *n* = 13 (6%), RCO-S 7 *n* = 17 (8%), RCO-S 8 *n* = 11 (5%), and RCO-S 9 *n* = 8 (4%; Table 2).

We evaluated the RCO-S according to PMF diagnosis (Table 2) and the distribution of the Co and Ost variables according to the grading of reticulin fibrosis (Supplementary Table S1). Overall, all morphological features evolved harmonically, with pre-PMF showing the least severe stromal changes, and higher reticulin fibrosis being associated with the highest scores for collagen deposition and osteosclerosis. Among patients with pre-PMF, the grading was consistently <2 for all the 3 histological features; 101 (83%) cases were assessed as having MF-1, whereas only 1 (1%) and 7 (6%) patients presented Co-1 and Ost-1, respectively. Predictably, the RCO-S for patients with pre- and oPMF was significantly different, with median values of 1 (range, 0-2) and 5 (range, 2-9), respectively (*P* < .0001).

Phenotypic and Prognostic Correlation of the Reticulin (R), Collagen (Co), Osteosclerosis (Ost) Score in Overt Primary Myelofibrosis

Next, we investigated the correlations of the RCO-S with distinct clinical and molecular characteristics, separately in the oPMF and pre-PMF cohorts. Among oPMF patients, a higher RCO-S was associated with lower hemoglobin (Spearman's ρ , -0.29; *P* = .0023), lower platelet count (Spearman's ρ , -0.27; *P* = .0058), higher blasts (Spearman's ρ , 0.28; *P* = .0053), and higher prevalence of splenomegaly (median, 6 vs 4; *P* = .0250) and constitutional symptoms (median, 5 vs 3; *P* = .0352). With regard to molecular variables, a higher RCO-S was more likely to be correlated with *ASXL1* mutation (median, 7 vs 5; *P* = .0064), *TET2* mutation (median, 7 vs 5; *P* = .0269), *KRAS* mutation (median, 8 vs 3; *P* = .0247), and high molecular risk (HMR) mutations (ie, at least 1 mutation in *ASXL1*, *EZH2*, *IDH1*, *IDH2*, *SRSF2*, and *U2AF1*; median, 6 vs 5; *P* = .0080), and conversely with a *TP53* wild-type status (median, 6 vs 3; *P* = .0470).

Subsequently, we focused on the prognostic implications of the RCO-S. Median OS of the oPMF cohort was 81 months (95% CI, 54-103). In regard to risk stratification, we did not observe any significant correlation with main prognostic models, including IPSS, dynamic IPSS (DIPSS), and MIPSS70. On univariate Cox regression analysis, there was no correlation of the RCO-S with the risk of death. However, by analyzing the single components of the RCO-S, we found that reticulin fibrosis and collagen deposition correlated with OS (respectively: hazard ratio [HR], 2.3; 95% CI, 1.3-4.3; *P* = .0066; HR, 2.7; 95% CI, 1.1-7.0; *P* = .0310), unlike osteosclerosis. Similarly, the RCO-S was directly correlated with the risk of leukemic transformation (HR, 7.8; 95% CI, 1.1-72.1; *P* = .0473), with no significant association with the individual RCO-S components.

Phenotypic and Prognostic Correlation of the Reticulin (R), Collagen (Co), Osteosclerosis (Ost) Score in Prefibrotic Primary Myelofibrosis

As expected, the phenotypic and prognostic implications of the RCO-S were less remarkable in the pre-PMF cohort. The only

Table 1

Characteristics of the whole patient population and separately in low-grade (reticulin-collagen-osteosclerosis score <5) versus high-grade (reticulin-collagen-osteosclerosis score ≥5) primary myelofibrosis

Variable	All patients <i>n</i> = 222	Low-grade PMF <i>n</i> = 157 (71%)	High-grade PMF <i>n</i> = 65 (29%)	<i>P</i>
Clinical and demographics				
WHO 2016 diagnosis; <i>n</i> (%)				
Prefibrotic PMF; <i>n</i> (%)	121 (55)	121 (77)	0	<.0001 ^b
Overt PMF; <i>n</i> (%)	101 (45)	36 (23)	65 (100)	
Male gender; <i>n</i> (%)	127 (57)	82 (52)	45 (69)	.0198 ^b
Age, y; median (range)	66 (18-91)	61 (18-91)	69 (36-80)	.1
Age >65 y; <i>n</i> (%)	122 (55)	81 (52)	41 (63)	.1
Leukocytes, ×10 ⁹ /L; median (range)	8.8 (1.5-250)	7.7 (1.5-56.3)	9.1 (3.2-250)	.0280 ^b
Leukocytes >25 × 10 ⁹ /L; <i>n</i> (%)	16 (7)	9 (6)	7 (11)	.2
Hemoglobin, g/dL; median (range)	13.1 (4.2-17.5)	13.7 (4.7-17.5)	10.7 (4.2-15.3)	<.0001 ^b
Hemoglobin <10 g/dL; <i>n</i> (%)	37 (17)	9 (6)	28 (43)	<.0001 ^b
Platelets, ×10 ⁹ /L; median (range)	557 (19-1800)	652 (60-1800)	264 (19-1378)	<.0001 ^b
Platelets <100 × 10 ⁹ /L; median (range)	15 (7)	4 (3)	11 (17)	.0001 ^b
PB blasts, %; median (range)	0 (0-10)	0 (0-6)	0 (0-10)	<.0001 ^b
PB blasts ≥1%; <i>n</i> (%)	35 (16)	8 (5)	27 (42)	<.0001 ^b
PB blasts ≥2%; <i>n</i> (%)	23 (10)	5 (3)	18 (28)	<.0001 ^b
Splenomegaly; <i>n</i> (%)	114 (53)	65 (42)	49 (80)	<.0001 ^b
Constitutional symptoms; <i>n</i> (%)	48 (22)	22 (14)	26 (40)	<.0001 ^b
Prognostic stratification				
IPSS risk stratification				
Low-risk; <i>n</i> (%)	75 (34)	65 (41)	10 (15)	<.0001 ^b
Intermediate-1 risk; <i>n</i> (%)	80 (36)	67 (43)	13 (20)	
Intermediate-2 risk; <i>n</i> (%)	34 (15)	15 (10)	19 (29)	
High-risk; <i>n</i> (%)	33 (15)	10 (6)	23 (35)	
DIPSS risk stratification				
Low-risk; <i>n</i> (%)	75 (34)	65 (41)	10 (15)	<.0001 ^b
Intermediate-1 risk; <i>n</i> (%)	99 (45)	77 (49)	22 (34)	
Intermediate-2 risk; <i>n</i> (%)	39 (18)	14 (9)	25 (38)	
High-risk; <i>n</i> (%)	9 (4)	1 (1)	8 (12)	
MIPSS70 risk stratification				
Low-risk; <i>n</i> (%)	66 (37)	65 (54)	1 (2)	<.0001 ^b
Intermediate risk; <i>n</i> (%)	79 (45)	46 (38)	33 (58)	
High-risk; <i>n</i> (%)	32 (18)	9 (8)	23 (40)	
MPN drivers				
<i>JAK2</i> mutated; <i>n</i> (%)	161 (73)	120 (77)	41 (64)	.1
<i>JAK2</i> ^{V617F} AB; median (range); evaluable = 159				
<i>CALR</i> mutated; <i>n</i> (%)	37 (18)	26 (18)	11 (18)	.9
<i>MPL</i> mutated; <i>n</i> (%)	14 (7)	11 (8)	3 (5)	.5
Triple negative; <i>n</i> (%)	13 (6)	4 (3)	9 (14)	.0010 ^b
Myeloid neoplasm-associated genes				
<i>ASXL1</i> mutated; <i>n</i> (%); evaluable = 177	44 (25)	17 (14)	27 (47)	<.0001 ^b
<i>CBL</i> mutated; <i>n</i> (%); evaluable = 166	7 (4)	7 (6)	0 (0)	0.1
<i>CSF3R</i> mutated; <i>n</i> (%); evaluable = 130	0 (0)	0 (0)	0 (0)	-
<i>DNMT3A</i> mutated; <i>n</i> (%); evaluable = 99	17 (10)	10 (9)	7 (13)	.5
<i>EZH2</i> mutated; <i>n</i> (%); evaluable = 177	15 (8)	3 (3)	12 (21)	<.0001 ^b
<i>IDH1/2</i> mutated; <i>n</i> (%); evaluable = 177	4 (2)	2 (2)	2 (4)	.4
<i>KIT</i> mutated; <i>n</i> (%); evaluable = 148	3 (2)	3 (3)	0 (0)	.2
<i>KRAS</i> mutated; <i>n</i> (%); evaluable = 148	3 (2)	0 (0)	3 (6)	.0129 ^b
<i>NF-E2</i> mutated; <i>n</i> (%); evaluable = 126	8 (6)	4 (5)	4 (9)	.4
<i>NRAS</i> mutated; <i>n</i> (%); evaluable = 148	3 (2)	1 (1)	2 (4)	.2
<i>PTPN1</i> mutated; <i>n</i> (%); evaluable = 130	1 (1)	1 (1)	0 (0)	.5
<i>RUNX1</i> mutated; <i>n</i> (%); evaluable = 148	5 (3)	1 (1)	4 (8)	.0234 ^b
<i>SETBP1</i> mutated; <i>n</i> (%); evaluable = 130	3 (2)	2 (2)	1 (3)	.9
<i>SF3B1</i> mutated; <i>n</i> (%); evaluable = 148	7 (5)	6 (6)	1 (2)	.3
<i>SH2B3/LNK</i> mutated; <i>n</i> (%); evaluable = 148	5 (3)	3 (3)	2 (4)	0.7
<i>SRSF2</i> mutated; <i>n</i> (%); evaluable = 177	63 (36)	25 (21)	38 (67)	<.0001 ^b
<i>TET2</i> mutated; <i>n</i> (%); evaluable = 164	39 (24)	24 (22)	15 (28)	.4
<i>TP53</i> mutated; <i>n</i> (%); evaluable = 148	5 (3)	4 (4)	1 (2)	.5
<i>U2AF1</i> mutated; <i>n</i> (%); evaluable = 148	7 (5)	2 (2)	5 (10)	.0273 ^b
<i>ZRSR2</i> mutated; <i>n</i> (%); evaluable = 130	9 (7)	7 (8)	2 (5)	.6
HMR mutations ^a ; <i>n</i> (%); evaluable = 159	65 (41)	26 (25)	39 (72)	<.0001 ^b
≥2 HMR mutations; <i>n</i> (%); evaluable = 159	20 (14)	7 (7)	13 (27)	.0011 ^b

Table 1 (continued)

Variable	All patients <i>n</i> = 222	Low-grade PMF <i>n</i> = 157 (71%)	High-grade PMF <i>n</i> = 65 (29%)	<i>P</i>
Cytogenetics				
Abnormal karyotype; evaluable = 92	45 (24)	26 (19)	19 (42)	.0013 ^b
Revised 3-tiered cytogenetic; evaluable = 92				
Favorable karyotype; <i>n</i> (%)	161 (87)	130 (93)	31 (69)	.0002 ^b
Unfavorable karyotype; <i>n</i> (%)	19 (10)	8 (6)	11 (24)	
Very high-risk karyotype; <i>n</i> (%)	5 (3)	2 (1)	3 (7)	
Outcomes				
Leukemic transformation	17 (8)	6 (4)	11 (17)	.0008 ^b
Death	74 (33)	39 (25)	35 (54)	<.0001 ^b

AB, allele burden; DIPSS, Dynamic International Prognostic Score System; HMR, high molecular risk mutation; IPSS, International Prognostic Score System; MIPSS70, mutation-enhanced international prognostic score system; PB, peripheral blood; PMF, primary myelofibrosis; RCO-S, reticulin-collagen-osteosclerosis score; WHO, World Health Organization.

^a HMR mutations include pathogenic variants in any of the following genes: *ASXL1*, *EZH2*, *IDH1*, *IDH2*, *SRSF2*, or *U2AF1*; ≥ 2 HMR mutations indicates the presence of 2 or more mutations (2 or more mutations in the same gene are counted as 1).

^b *P* < .05.

meaningful correlations of a higher RCO-S were with higher blood cell count (Spearman's ρ , 0.23; *P* = .0117) and lactate dehydrogenase (Spearman's ρ , 0.21; *P* = .0300), and with *TET2* mutation (median, 2 vs 1; *P* = .0373).

Median OS of the pre-PMF cohort was 134 months (95% CI, 86-not reached [NR]). In regard to risk stratification, no significant correlation between the RCO-S and risk stratification according to IPSS, DIPSS, and MIPSS70 was observed. Univariate Cox regression analysis showed no significant association between the risk of death or leukemic transformation and either the RCO-S or its individual components. Finally, we aimed to assess whether the RCO-S was correlated with the risk of progression to oPMF. A total of 109 (90%) pre-PMF patients were informative, based on the availability of clinical and/or histologic data defining the progression to oPMF; of these, 8 (8%) progressed to the overtly fibrotic phase. We found no significant association between the risk of oPMF progression and either the RCO-S or its individual components.

Prognostic Validation of the Reticulin (R), Collagen (Co), Osteosclerosis (Ost) Score

In the whole PMF cohort, ROC analysis with death as endpoint identified 5 as the optimal cutoff value for RCO-S (RCO-S⁵). Accordingly, patients were classified as having low-grade PMF (RCO-S <5; *n* = 157 [71%], including 121 [77%] pre-PMF and 36

[23%] oPMF) and high-grade PMF (RCO-S ≥ 5 ; *n* = 65 [29%], all oPMF). We performed the same ROC analysis considering only patients with oPMF, still confirming 5 as the more accurate cutoff RCO-S value.

Clinical and molecular correlations of high-grade versus low-grade PMF are reported in Table 1. High-grade PMF was associated with male gender (*P* = .0198), higher leukocytes (*P* = .0280), lower hemoglobin (*P* < .0001), lower platelet count (*P* < .0001), higher blasts (*P* < .0001), higher prevalence of splenomegaly (*P* < .0001), and constitutional symptoms (*P* = .0004). Moreover, the high-grade cohort was enriched in abnormal cytogenetics (*P* = .0013) and higher risk karyotype according to the revised cytogenetic risk stratification²¹ (*P* = .0002), triple negative status (ie, absence of *JAK2*, *MPL* or *CALR* driver mutations; *P* = .0010), and mutations in *ASXL1* (*P* < .0001), *EZH2* (*P* < .0001), *KRAS* (*P* = .0129), *RUNX1* (*P* = .0234), *SRSF2* (*P* < .0001), and *U2AF1* (*P* = .0273) and HMR mutations (*P* < .0001).

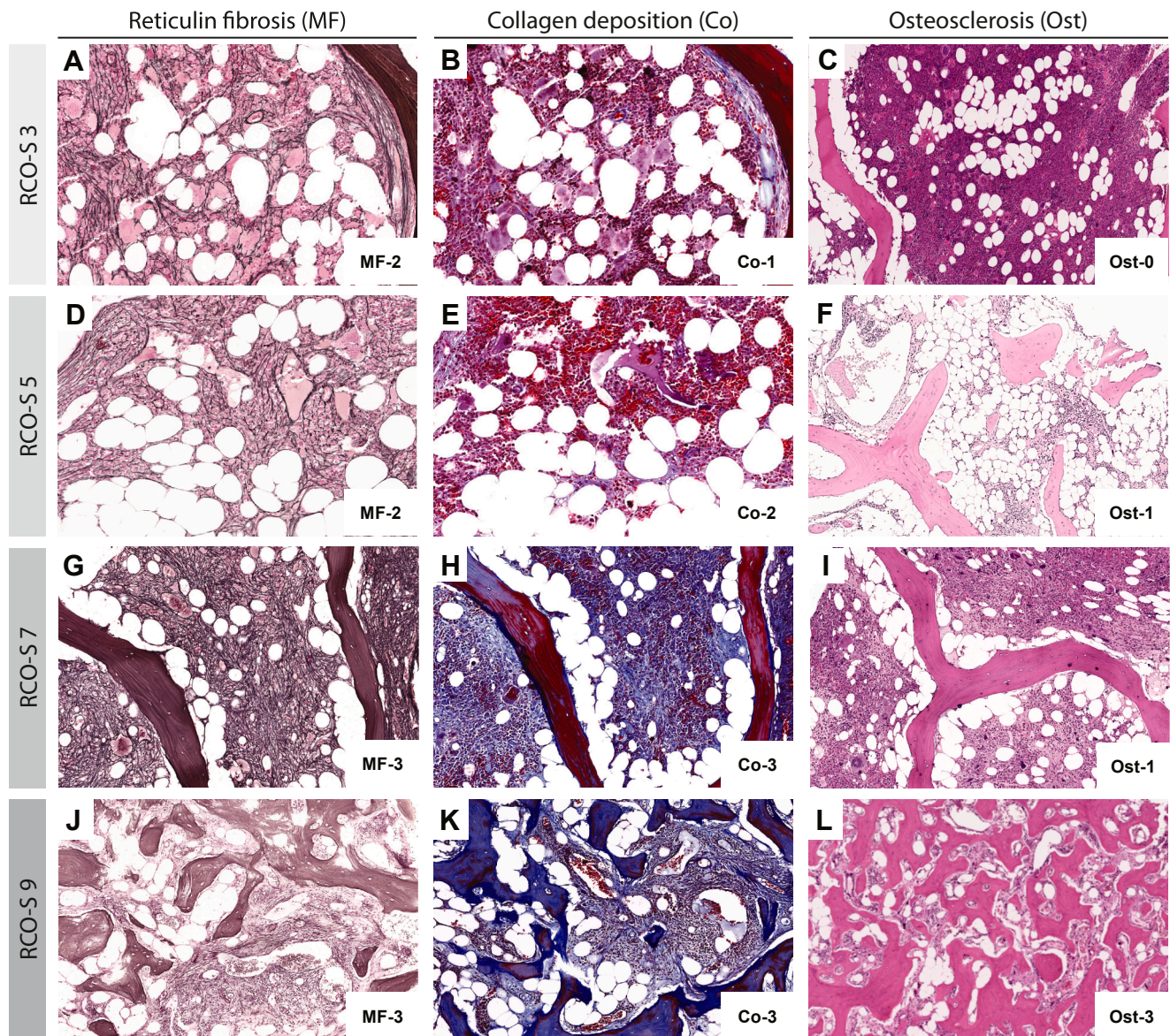
In regard to disease outcomes, the patients with high-grade PMF exhibited significantly worse OS than patients with low-grade PMF, with median values of 64 (range: 47-93) versus 134 months (range: 91-not reached; HR, 2.2; 95% CI, 1.4-3.5; *P* = .0007; Fig. 2A). In regard to leukemic progression, after competing risk analysis, the 5-year Cul of BP transformation was significantly higher in patients with high-grade PMF compared with the low-grade counterpart (4%, 95% CI, 2-9 and 15%, 95% CI, 7-26, respectively; Gray test *P* = .0046; Fig. 2B).

Table 2

Distribution of reticulin fibrosis, collagen deposition, and osteosclerosis grading and primary myelofibrosis subtypes among reticulin-collagen-osteosclerosis score categories

RCO-S	Reticulin fibrosis (MF)				Collagen deposition (Co)				Osteosclerosis (Ost)				PMF diagnosis	
	MF-0	MF-1	MF-2	MF-3	Co-0	Co-1	Co-2	Co-3	Ost-0	Ost-1	Ost-2	Ost-3	Prefibrotic PMF	Overt PMF
RCO-S 0 <i>n</i> = 20 (9%)	20	0	0	0	20	0	0	0	20	0	0	0	20	0
RCO-S 1 <i>n</i> = 93 (42%)	0	93	0	0	93	0	0	0	93	0	0	0	93	0
RCO-S 2 <i>n</i> = 16 (7%)	0	8	8	0	15	1	0	0	9	7	0	0	8	8
RCO-S 3 <i>n</i> = 19 (9%)	0	0	19	0	2	17	0	0	17	2	0	0	0	19
RCO-S 4 <i>n</i> = 9 (4%)	0	0	7	2	0	8	1	0	3	6	0	0	0	9
RCO-S 5 <i>n</i> = 16 (7%)	0	0	10	6	0	5	11	0	4	9	3	0	0	16
RCO-S 6 <i>n</i> = 13 (6%)	0	0	2	11	0	2	11	0	0	9	4	0	0	13
RCO-S 7 <i>n</i> = 17 (7%)	0	0	1	16	0	0	5	12	0	12	4	1	0	17
RCO-S 8 <i>n</i> = 11 (5%)	0	0	1	10	0	0	3	8	0	0	7	4	0	11
RCO-S 9 <i>n</i> = 8 (4%)	0	0	0	8	0	0	0	8	0	0	0	8	0	8
All patients <i>n</i> = 222	20	101	48	53	130	33	31	28	146	45	18	13	121	101

RCO-S, reticulin-collagen-osteosclerosis score; PMF, primary myelofibrosis.

**Figure 1.**

Four representative cases of the RCO-S. (A-C) Overt PMF case with a final RCO-S of 3 resulting from MF-2, Co-1, and Ost-0. (D-F) Overt PMF with an RCO-S of 5 resulting from MF-2, Co-2, and Ost-1. (G-I) Overt PMF with an RCO-S of 7 resulting from MF-3, Co-2, and Ost-1. (J-L) Overt PMF with an RCO-S of 9 resulting from MF-3, Co-3, and Ost-2. Co, collagen deposition; MF, reticulin fibrosis; PMF, primary myelofibrosis; RCO-S, reticulin-collagen-osteosclerosis score; Ost, osteosclerosis.

To evaluate the independent prognostic value of the RCO-S, we conducted both univariate and multivariable Cox regression analyses incorporating the RCO-S alongside established risk factors ([Supplementary Table S2](#)). In a one-to-one comparison, the RCO-S emerged as an independent predictor of inferior OS, together with most clinical and molecular covariates, except for peripheral blood blasts >2% and HMR status. In the final multivariate model including all categorical variables, the RCO-S did not retain its independent prognostic value for OS. This finding may be attributed to the limited molecular data available, with only 177 of 222 patients (80%) having complete molecular information. To address this, we conducted a secondary multivariable analysis including only clinical covariates. In this subanalysis, the RCO-S regained its independent prognostic significance for inferior OS, alongside older age and leukocytosis.

Integration of Reticulin (R), Collagen (Co), Osteosclerosis (Ost) Score in the Mutation-Enhanced International Prognostic Score System Model

Finally, we aimed to evaluate the prognostic contribution of the validated RCO-S to risk stratification of PMF patients. Comparative analysis showed that the RCO-S alone was less accurate than conventional prognostic models (ie, IPSS and DIPSS) for OS prediction ([Supplementary Fig. S1](#)). As expected, similar results were obtained when comparing the RCO-S with the MIPSS70, which considers different clinical and molecular parameters alongside BM MF grading (9), among the 177 of 222 patients (80%) for whom complete molecular information was available ([Supplementary Fig. S1](#)). We then sought to determine whether the RCO-S could add prognostic value within the MIPSS70 framework. To this end, we computed the MIPSS70 by

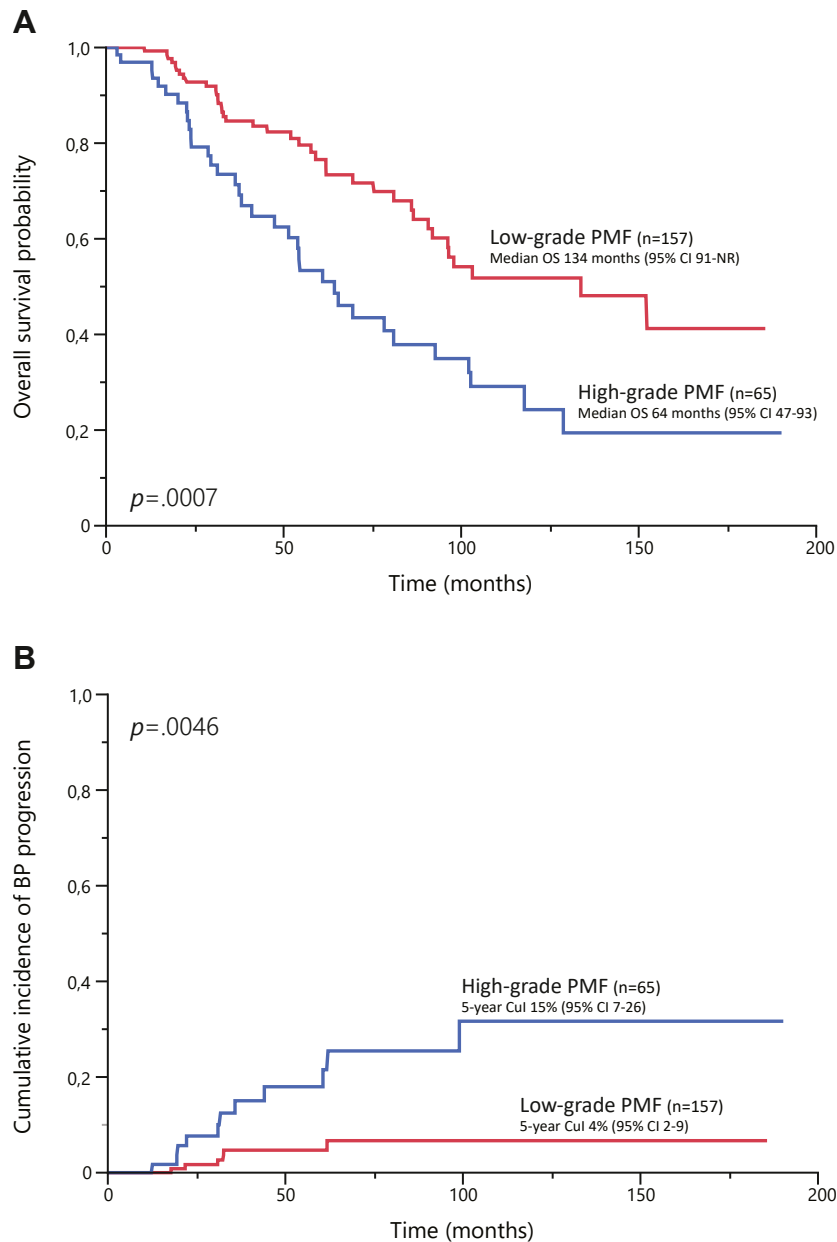


Figure 2.

(A) Kaplan-Meier estimates of overall survival in PMF patients with high-grade (RCO-S ≥ 5) versus low-grade (RCO-S < 5) disease. (B) Competing risks-adjusted estimates of cumulative incidence of leukemic transformation in PMF patients with high-grade (RCO-S ≥ 5) versus low-grade (RCO-S < 5) disease. BP, blast phase; CI, confidence interval; Cul, cumulative incidence; OS, overall survival; PMF, primary myelofibrosis; RCO-S, reticulin-collagen-osteosclerosis score.

replacing the original MF grade ≥ 2 variable with the dichotomous distinction of low and high-grade based on the RCO-S (< 5 vs ≥ 5); we defined this adjusted MIPSS70 as “RCO-S⁵-integrated MIPSS70.” This approach resulted in some redistribution of MF patients across the 3 risk classes, with 11 (14%) and 3 (9%) of standard MIPSS intermediate and high-risk patients being reclassified in the RCO-S⁵-integrated MIPSS70 as low- and intermediate-risk groups, respectively (Supplementary Fig. S2A). Actuarial survival curves according to standard and RCO-S⁵-integrated MIPSS70 are reported in Supplementary Figure S2B.

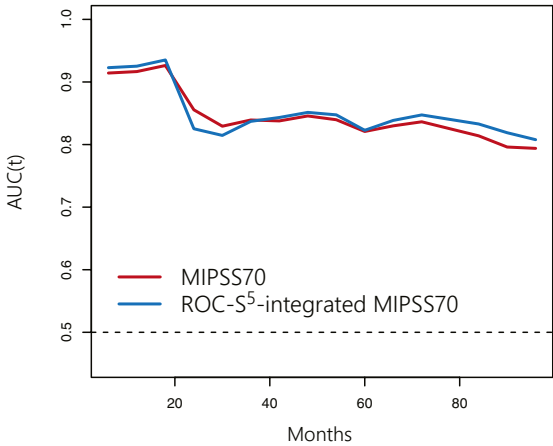
Next, we aimed to compare the 2 models by calculating the respective C-index, Brier score, and time-dependent AUC (Fig. 3). Overall, we found that the integration of the RCO-S⁵ into the

MIPSS70 significantly improved the prognostic power of the original score, with accuracy values (Brier score) consistently higher for the RCO-S⁵-integrated MIPSS70 at all-time points considered (24, 48, 72, and 96 months). Similarly, the highest values for performance (C-index and, almost invariably, AUC) were displayed by the integrated score. Ultimately, in order to counter the dichotomous subdivision of the RCO-S -which can actually range from 0 to 9, we recomputed the MIPSS70 assigning a value of 0.2 to each RCO unit increment; low-, intermediate-, and high-risk categories were defined by a final score of < 2 , ≥ 2 to < 5 , and ≥ 5 , respectively. This attempt (named RCO-S¹⁻⁹-integrated MIPSS70) produced the same risk distribution as the RCO-S⁵-integrated MIPSS70, and consequently displayed identical

A

Comparison of the prognostic performance of the standard and RCO-S-integrated MIPSS70									
	C-index	Events at 12 months		Events at 24 months		Events at 36 months		Events at 48 months	
		Brier score	AUC	Brier score	AUC	Brier score	AUC	Brier score	AUC
MIPSS70	72.9	0.008	91.3	0.022	84.5*	0.043	83.9	0.060	82.7
RCO-S ⁵ -integrated MIPSS70	76.5*	0.008	92.2*	0.022	84.3	0.042*	85.1*	0.059*	84.6*
RCO-S19-integrated MIPSS70	76.5*	0.008	92.2*	0.022	84.3	0.042*	85.1*	0.059*	84.6*

B



C

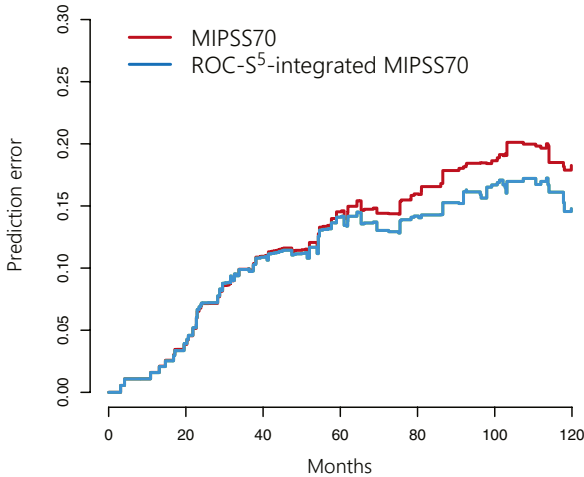


Figure 3. (A) Prognostic performance of standard and RCO-S-integrated MIPSS70. (B) Time-dependent area under the curve (AUC) for prediction of death for standard and RCO-S⁵-integrated MIPSS70. (C) Brier score for prediction of death measured over time for standard and RCO-S⁵-integrated MIPSS70. Notes: Asterisk and bold indicate the best values. AUC, area under the curve; MIPSS70, mutation-enhanced international prognostic score system; RCO-S, reticulin-collagen-osteosclerosis score.

values for performance and accuracy. Overall, these findings confirm the prognostic relevance of the RCO-S-based distinction between low- and high-grade PMF.

Discussion

Primary myelofibrosis imposes significant clinical challenges due to its aggressive nature and marked biological heterogeneity, distinguishing it among Philadelphia chromosome-negative MPNs. Key histological features of PMF include

reticulin fibrosis, collagen deposition, osteosclerosis, and microvessel proliferation. Recognition of these stromal changes as integral components of PMF pathobiology is underscored by the detrimental prognostic implications associated with fibrotic progression. The current study proposes a paradigm shift from conventional stromal evaluation, almost exclusively reliant on reticulin fibrosis, to a more comprehensive approach based on the RCO-S, previously introduced by Gianelli et al.¹³ Overall, our results confirm its clinical, molecular, and prognostic relevance within a cohort of extensively annotated PMF patients.

The RCO comprehensive scoring system, derived from the ECGMF/WHO reticulin, collagen, and osteosclerosis grading as first described by Kvasnicka et al,¹⁴ enhances the morphological assessment of PMF, eventually providing a valuable tool for refining the diagnostic and prognostic workup. In this retrospective, single-center study, we were able to validate the RCO-S in a large cohort of patients, including 121 prefibrotic and 101 overt PMF. The independent grading of the 3 stromal alterations offered a more nuanced perspective on stromal modifications. As expected, the 3 morphological features tended to progress harmonically, with pre-PMF showing the least severe stromal changes, and higher reticulin fibrosis being associated with the highest values of collagen deposition and osteosclerosis. In pre-PMF, the most relevant stromal alteration was reticulin fibrosis (MF-1 in 101 [83%] patients), whereas a minority had Co-1 (1 [1%]) or Ost-1 (7 [6%]); notably, no patients were graded >1 for collagen deposition or osteosclerosis.

In line with the well-known 4-tier WHO grading system for reticulin fibrosis, separate grading systems for reticulin fibrosis, collagen deposition, and osteosclerosis may serve as simple and reliable diagnostic and prognostic tools that are easy to apply in routine practice. In a previous multicentric study by Kvasnicka et al,¹⁴ involving 11 hematopathologists who assessed each component of the fibrous matrix in a variable-wise manner, the overall interrater reliability ranged between 0.898 and 0.926 for the 3 parameters analyzed. Moreover, though the 3 variables included in the RCO-S are not entirely independent, our approach was guided by established grading frameworks for reticulin fibrosis, collagen deposition, and osteosclerosis,¹⁴ and it effectively captures the overall extent and severity of BM structural remodeling.

Besides the validation of the RCO-S, we aimed to explore its clinical, genetic, and prognostic correlates. In oPMF, a higher RCO-S was associated with distinct clinical and molecular features that define higher risk disease, including lower hemoglobin, lower platelet count, higher blast count, and higher prevalence of mutations in *ASXL1*, *KRAS*, and *HMR* status. Moreover, we observed that although reticulin fibrosis and collagen deposition correlated with OS, the final RCO-S did not. In contrast, the risk of BP progression showed a direct correlation with the RCO-S but was not significantly associated with its individual components. In the pre-PMF cohort, the impact of the RCO-S on both phenotypic and prognostic characteristics was less pronounced, as anticipated, likely due to the smaller patient population and the narrower scoring range.

By the use of ROC analysis, we validated 5 as the optimal cutoff value of the RCO-S, distinguishing low-grade (RCO-S <5) and high-grade (RCO-S ≥5) patients, both in the whole PMF and in the oPMF cohorts. Although high-grade group included only patients with oPMF, the composition of the low-grade category was more heterogeneous, including 77% of pre-PMF and 23% of oPMF. As expected, high-grade PMF was associated with high-risk clinical and molecular features. These included higher leukocytes, lower hemoglobin, lower platelet count, higher blasts, higher prevalence of splenomegaly, and constitutional symptoms. These results are in line with previous studies, and recent findings by our and other groups reported a higher BM fibrosis grade among PMF patients with a cytopenic phenotype.^{7,22,23}

Moreover, we found that high-grade disease was correlated with prognostically negative molecular profiles, that is, abnormal cytogenetics and higher risk karyotype, triple negativity, mutations in *ASXL1*, *EZH2*, *KRAS*, *SRSF2*, *U2AF1*, and *HMR* genes. The role of molecular factors in the development and progression of PMF-related stromal changes is currently poorly characterized.

Although abnormal karyotype and *JAK2* allele burden have been previously correlated to the extension of BM fibrosis,^{6-9,24} no evidence has been reported for triple negative disease (ie, negative for the 3 driver mutations in of the *JAK2*, *CALR*, and *MPL* genes). There is also a growing body of evidence linking high-risk mutations to the degree of BM fibrosis.⁷ In particular, a recent study by Shi et al²⁵ reported on a critical role of *ASXL1* mutations in driving BM fibrosis via *EGR1*-*TNFA* axis-mediated neoplastic fibrocyte generation.

Survival analysis showed that patients with high-grade PMF had a worse OS and increased Cul of BP compared with the low-grade counterpart. The prognostic impact of BM fibrosis in PMF has been previously described. In a study of the AIRC Gruppo Italiano Malattie Mieloproliferative (AGIMM) group on 490 patients (37% per-PMF, 63% oPMF), higher grades of fibrosis were an independent predictor of inferior OS regardless of IPSS and mutational status, with an adverse impact of fibrosis that was especially noticeable in the IPSS lower risk population.⁷ More recently, reticulin fibrosis grade was included in the MIPSS70 model as an independent predictor of survival, thus confirming the prognostic significance of BM stroma change evaluation.¹⁰ It should be emphasized that the RCO-S allows for the differentiation patients with a high-risk disease from those associated with low-risk RCO-S and a more favorable prognosis within the oPMF categories.

Univariate and multivariate Cox regression analyses incorporating the RCO-S and established risk factors indicated that the prognostic contribution of the RCO-S is largely complementary, rather than redundant, to existing predictors. Although the RCO-S alone demonstrated moderate prognostic performance, it was outperformed by IPSS, DIPSS, and MIPSS70 across all-time points analyzed. This aligns with expectations, as IPSS and DIPSS incorporate a wide range of clinical variables, and MIPSS70 further integrates clinical, molecular, and histological (MF ≥2) risk factors. Finally, we aimed to ascertain whether the integration of the RCO-S might improve the predictive performance of current prognostic models such as the MIPSS70. The MIPSS70 provides prognostic information in PMF patients and encompasses different clinical parameters, 1 histopathological parameter (ie, BM fibrosis ≥2), and molecular alterations.¹⁰ Replacement of the original BM fibrosis grade variable with the dichotomous RCO-S⁵ (≥/ <5) produced some modification in the composition of the 3 risk categories, mainly pertaining to the standard MIPSS70 intermediate and high groups. More importantly, the new RCO-S⁵-integrated MIPSS70 enabled us to achieve a more accurate prognostic assessment in terms of both accuracy and performance than the standard score, thus emphasizing the potential clinical utility of a comprehensive stromal evaluation. Of note, identical results were obtained when integrating the RCO-S as an ordinal variable.

We acknowledge potential limitations in the interpretation of current findings, including the retrospective nature of the analysis, harboring intrinsic selection biases, and the small sample size. Prospective studies with larger cohorts are mandatory to determine RCO-S implications in guiding PMF patient management both at diagnosis and following therapy. Furthermore, broadening the RCO-S to include microvessel density, a crucial BM architectural alteration in PMF, could be highly valuable. Future areas of research will also include the evaluation of the RCO-S performance in secondary MF and during disease-directed treatment.

In conclusion, the RCO-S offers a more comprehensive and nuanced analysis of stromal changes in PMF together with a novel categorization into low-grade and high-grade disease that proved to enhance the prognostic performance of the conventional MIPSS70 model.

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Author Contributions

A.M.V., U.G., P.G., R.S., and G.C. designed the research, interpreted the results and wrote/edited the final manuscript; R.S., U.G., G.D.S., and C.P. performed the histopathological analysis; G.G.L., A.A., and P.C. collected clinical and molecular data; G.C. performed statistical analysis. All authors read and approved the final draft of the manuscript.

Data Availability

The data sets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Declaration of Competing Interests

The authors have nothing to declare in relation to this work.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki and European and Italian regulations, and was approved by the ethical committees of each institution prior to the start of the study.

Supplementary Material

The online version contains supplementary material available at <https://doi.org/10.1016/j.modpat.2025.100854>.

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