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Bonemarrow Fibrosis Grade; A Useful Prognostic Marker in Myeloproliferative Neoplasms

Muhammad Bilal Asghar, Hamid Saeed Malik, Nabila Rafique, Manzar Bozdar, Rafia Mahmood, Intzar Ali

Department of Hematology, Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS) Rawalpindi Pakistan

ABSTRACT

***Objective*:** To determine the prognostic significance of bone marrow fibrosis grade in predicting the outcome of myeloproliferative neoplasms.

***Study Design*:** Prospective longitudinal study.

***Duration and Place of Study*:** Armed Forces Institute of Pathology, Rawalpindi Pakistan, from Jun 2021 to May 2022.

***Methodology*:** A total of 114 patients with myeloproliferative neoplasms were included. Under aseptic conditions, a bone marrow aspiration and a Trephine biopsy were obtained. Following processing, the samples underwent staining with Hemotoxylin and Eosin and Reticulin. The WHO bone marrow fibrosis grading system was used to grade the fibrosis. Clinical findings and haematological parameters documented at initial diagnosis were compared with one-year interval follow-up counts.

***Results*:** Out of a total 114, 72(63.2%) were male and 42(36.8%) were female. Generalised weakness and pallor were documented in 51(44.7%) and 27(23.7%), respectively. While splenomegaly and/or hepatomegaly were detected in 61(53.5%) and 27(23.7%), respectively, 16(14.9%) transformed into other MPNs and 3(2.6%) into acute leukemia. People who had higher levels of MF-2 and MF-3 reticulin fibrosis had the worst prognosis when it came to peripheral blood cytopenias, disease progression, and transformation.

Conclusion: Myeloproliferative neoplasms are very different from one another in terms of how they look and behave. As the grade of fibrosis rises, there is a high chance that the disease will progress to myelofibrosis or change into acute leukaemia, both of which are very bad for overall survival.

Keywords: Myeloproliferative neoplasm, Marrow fibrosis, Prognostic factor.

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INTRODUCTION

Myeloproliferative neoplasms (MPNs) show heterogeneous features of clinical and pathological significance, ranging from benign disorders to malignant systemic diseases.[1] Therefore, the World Health Organisation (WHO) 2022 version has classified MPNs in a separate category. Chronic myeloid leukaemia (CML) is the most common disorder. However, key BCR-ABL1 Negative MPNs are primary myelofibrosis (PMF), polycythemia vera (PV), and essential thrombocythemia (ET).[2,3] These disorders present with varying degrees of fibrous bone marrow. Researchers have shown that higher levels of bone marrow fibrosis in MPNs cause lower levels of peripheral blood cell counts, which show up as different levels of cytopenia and include a leucoerythroblastic blood picture and tear-drop red blood cells on peripheral film. On a clinical examination, splenomegaly may be present.[4,5]

Correspondence: Dr Muhammad Bilal Asghar, Department of Hematology, Armed Forces Institute of Pathology, Rawalpindi Pakistan
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Bone marrow fibrosis is caused by a number of things, including megakaryocytes that don't work properly, JAK-STAT signalling that doesn't work right, more inflammatory cytokines, and transforming growth factor-β. The most effective stimulator for fibroblasts is platelet-derived growth factor (PDGF), but its effect on reticulin fibrosis is limited.[6] The role of transforming growth factor beta (TGF-beta) has been emphasised in newer studies in stimulating collagen synthesis by fibroblasts. Interleukins (IL), particularly IL-11 and IL-3, also enhance reticulin fibrosis.[7] Additionally, serum procollagen III peptide (PIIINP) has been found to be a significant biochemical marker that is closely linked to reticulin fibrosis. WHO has adopted semi-quantitative bone marrow fibrosis grading system with modifications .[8,9]

Our objective was to determine the prognostic value of bone marrow fibrosis grade and predict the outcome of myeloproliferative neoplasms. Through this study, we wanted to document the correlation between different marrow fibrosis grades in MPNs and determine whether higher fibrosis is associated with disease severity, transformation, and the worst

prognostic outcome, so we could manage patients through targeted therapies right at the time of diagnosis.

METHODOLOGY

The prospective longitudinal study was carried out at the Armed Forces Institute of Pathology (AFIP), Rawalpindi Pakistan, from June 2021 to May 2022. The WHO calculator was used to determine the sample size, with a prevalence of 57% in previous study.[10]

Inclusion Criteria: Patients aged 19 and 70, of either gender, with myeloproliferative neoplasms including cases of PV, ET, and MF receiving standard treatment protocols, were included. People with polycythemia vera, essential thrombocythemia, and primary myelofibrosis who came to the Bone Marrow Department were also included.

Exclusion Criteria: Patients who had known co-morbidities, reactive bone marrow fibrosis due to autoimmune disorders or acute infections, and those who were no longer being followed up on were not included.

After receiving informed written consent and observing all possible aseptic measures, a bone marrow aspiration and a trephine biopsy sample were collected from the patient's posterior superior iliac spine. Following fixation in Zenker's solution, decalcification using 5% formic acid, tissue processing, and cutting using a semi-automated rotary microtome, we obtained tissue sections that were approximately 05 micrometers thin. It was followed by mounting on slides, de-waxing, rehydration, and staining with hematoxylin, eosin, and reticulin stain. Under light microscopy, reticulin fibres stain black, collagen fibres stain pink, and nuclei and cytoplasm stain shades of grey. After dehydration with increasing concentrations of alcohol and clearance in xylene, the slides were mounted with DPX. These were then observed under the microscope.[11]

Grading was performed in accordance with the WHO fibrosis scoring system. Grade MF-0 displays scattered linear fibres with no intersections. The MF-1 has a loose reticulin network with many intersections. MF-2 shows a dense and widespread rise in reticulin with lots of intersections and focal bundles of thick collagen fibres. Grade MF-3, on the other hand, shows dense reticulin fibres with bundles of thick collagen fibres linked to osteosclerosis and a pinkish look under a microscope.[12,13]

For quantitative variables, data was recorded as Mean±SD, while percentages and frequencies were calculated for qualitative variables. The Pearson Chi-square and ANOVA were applied for inferential statistics. The *p*-value of ≤0.05 was considered significant.

RESULTS

A total of 114 patients were included in the research. The mean age was 51.11±16 years, with 63.2%(n=72) males and 36.8%(n=42) female patients. Three age groups were devised, i.e., <30, 30–50, and >50 years, to see the impact of increasing age in our study group. In our study cohort, the majority of the patients (63) were sorted into a >50-year-old age group with male predominance. Higher grades of fibrosis were observed in this group, i.e., 10 patients with MF-3. The 30-50-year-old age group showed a relative better gender distribution, with 21 males and 18 females, and the majority (15) having MF-0 fibrosis grade.

Among presenting complaints, generalised weakness, abdominal discomfort, bleeding, bruising, and fever were the most common, accounting for 51(44.7%) 40(35.1%), 9(7.9%), 6(5.3%), and 9(7.9%), respectively, as illustrated in Figure-1.

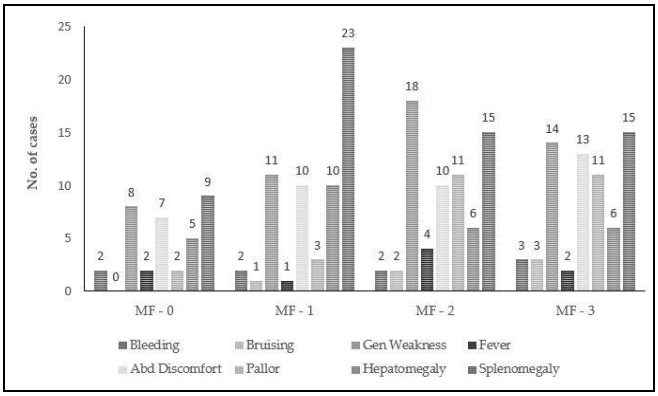

Figure-1: Presenting Complaints and Physical Examination findings with respect to Marrow Fibrosis Grade (n=114)

The diagnosis was finalized based on findings from bone marrow aspiration, a trephine biopsy, and molecular genetics. Our study included cases of primary myelofibrosis (PMF), polycythemia vera (PV), and essential thrombocythemia (ET). Respective percentages as compared to fibrosis grade are shown in Table-I. Upon molecular analysis, JAK2 V617F was the most common mutation detected (69.3%). CALR was positive in 2.6%, while 26.3% were negative for all three mutation analyses.

Table-I: Diagnosis with Respect to Marrow Fibrosis (n=114)

			Diagnosis				Total
			PV	ET	PMF-PF	PMF-OF	
Fibrosis Grade	MF-0	n	18	10	4	0	32
		%	37.5%	43.5%	36.4%	0.0%	28.1%
	MF-1	n	21	6	7	0	34
		%	43.8%	26.1%	63.6%	0.0%	29.8%
	MF-2	n	9	6	0	16	31
		%	18.8%	26.1%	0.0%	50.0%	27.2%
	MF-3	n	0	1	0	16	17
		%	0.0%	4.3%	0.0%	50.0%	14.9%
Total		n	48	23	11	32	114
		%	100.0%	100.0%	100.0%	100.0%	100.0%

Peripheral blood counts were also documented at the time of diagnosis. The mean values recorded for white blood cells/total leucocyte count (WBC/TLC), hemoglobin levels (Hb), hematocrit (HCT), and platelets (PLT) were 14.8±13.4 x109/L, 13.6±4.7 g/dL, 41.2±12.4x109/L, and 655±425x109/L, respectively. As shown in Table-II, the mean blood counts were analyzed in correlation with marrow fibrosis grades. The results were significant for haemoglobin, hematocrit, and platelets.

Table-II: Comparison Between Mean of Blood Counts and Marrow Fibrosis Grades (n=114)

		n	Mean	ANOVA (Significance Between Groups)	Bonferroni (Significance Between Groups)
TLC(x109/L)	MF-0	32	11.2488	0.321	MF-0–MF-2: 0.56
	MF-1	34	16.3974		
	MF-2	31	16.9681		
	MF-3	17	14.9088		
Hb(g/dL)	MF-0	32	14.8938	<0.001	MF-0–MF-3: <0.001
	MF-1	34	15.9735		MF-1–MF-2: 0.004
	MF-2	31	12.2452		MF-1–MF-3: <0.001
	MF-3	17	9.3588		
HCT(%)	MF-0	32	45.2125	<0.001	MF-0–MF-2: <0.012
	MF-1	34	47.6676		MF-0–MF-3: <0.001
	MF-2	30	36.6767		MF-1–MF-2: <0.001
	MF-3	17	28.8941		MF-1–MF-3: <0.001
PLT(x109/L)	MF-0	32	723.34	0.035	MF-0–MF-3: <0.041
	MF-1	34	694.94		MF-1–MF-3: <0.071
	MF-2	31	694.71		
	MF-3	17	380.65		

Similarly, a correlation between the underlying diagnosis and the mean blood count at the time of diagnosis was also identified. Data revealed significant differences in haemoglobin, hematocrit, and platelets between different MPNs. The results are presented in Table-III. Patients were again evaluated at a one-year interval for assessment of blood counts and to see whether they had responded to management with normalisation in blood counts. Furthermore, their clinical history and physical findings were compared with those of the previous ones to determine whether they responded to the prescribed treatment or not.

Table-III: Comparison Between Mean of Blood Counts and Diagnosis (n=114)

		n	Mean	ANOVA (Significance Between Groups)	Bonferroni (Significance Between Groups)
TLC(x109/L)	PV	48	17.3656	0.261	ET–PV: 0.556
	ET	23	11.6117		
	PMF-PF	11	16.9491		
	PMF-OF	32	12.8084		
Hb(g/dL)	PV	48	16.5021	<0.001	PV-ET: 0.026
	ET	23	13.5087		PV-PF-PMF: 0.001
	PMF-PF	11	11.2545		PV- OF-PMF: <0.001
	PMF-OF	32	10.3688		PF-PMF-ET: 0.03
Hct(%)	PV	47	51.2766	<0.001	PV-ET: <0.001
	ET	23	36.9435		PV-PF-PMF: <0.001
	PMF-PF	11	36.8909		PV- OF-PMF: <0.001
	PMF-OF	32	31.0469		
PLT(x109/L)	PV	48	589.81	<0.001	PV-ET: 0.002
	ET	23	962.57		ET-OF-PMF: <0.001
	PMF-PF	11	823.45		
	PMF-OF	32	477.31		

Peripheral blood counts were documented at a 1-year interval. The mean values recorded for WBC, Hb, and platelets were 12.5±9.0(x109/L), 11.8±2.5(g/dL), and 562±436(x109/L), respectively. It was observed that there was a decline in response to treatment with increasing fibrosis grade, with a significant p value (p<0.001). Therefore, a higher bone marrow fibrosis grade leads to a poor treatment response.

In terms of disease transformation, the prognosis was documented. 3(2.6%) of cases transformed to

acute leukaemia, while 16(14%) showed progression to myelofibrosis. The prognostic importance of grade of fibrosis is depicted in Figure-2, which highlights the number of cases transformed from each group. We can appreciate that most cases from MF-0 did not transform. In the MF-MF-1 group, a total of 8 patients underwent transformation to myelofibrosis, of which 4 had ET. 4 had a diagnosis of PV, while 4 had ET. In the MF MF-2 group, 6 patients developed overt myelofibrosis, of which 4 had PV and 2 had ET. Meanwhile, 1 patient, initially diagnosed with ET, underwent a transformation from MF to acute leukemia. Similarly, in the MF-MF-3 group, two patients developed acute leukemia, one from ET and the other from overt myelofibrosis.

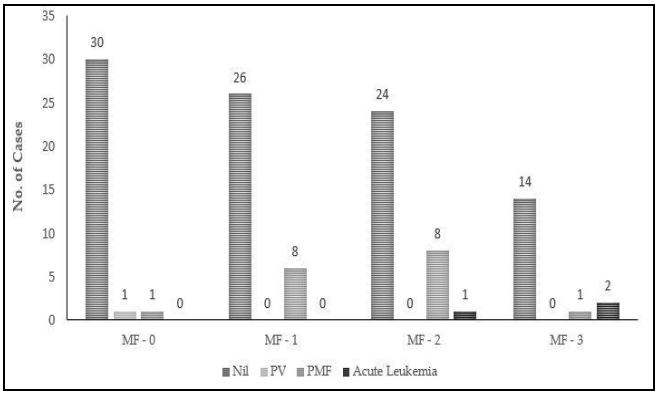

Figure-2: Disease Transformation in each Corresponding Marrow Fibrosis Grade (n=114)

DISCUSSION

Myeloproliferative neoplasms are clonal disorders originating from somatic mutations emerging in hematopoietic stem cells, with driving gene mutations in the JAK2, MPL, and CALR genes. However, about 9% of patients display none of them. Other mutations include ASXL1, IDH1/2, EZH2, and the SRSF2 gene, which are linked with poor overall survival (OS) and/or leukaemia-free survival (LFS).[14,15]

The International Working Group for Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) looked at the prognostic significance of grade ⩾1 BM fibrosis in 526 patients with PV. They found that higher BM fibrosis has an effect on thrombosis-free survival and myelofibrosis-free survival (MFFS) but not on OS or LFS.[16] In this study, we aimed to confirm these earlier findings and pinpoint fibrosis as an additional risk factor for MFFS and LFS. Our study aligns with the observations that fibrosis negatively impacts MFFS and LFS. Boveri *et al*. studied the effects of marrow fibrosis in MPN patients who had not been treated before using clinicopathological parameters and molecular markers. They found a link between the degree of marrow fibrosis and the size of the spleen. Furthermore, it has already been shown that splenomegaly in PMF correlates with the severity of BM fibrosis, which is consistent with our findings.[17] In 2016, Guglielmelli *et al*. conducted a study and reported that patients with higher grades of fibrosis were associated with adverse cytogenetics and an abnormal karyotype. It also stated that a higher grade of marrow fibrosis is an independent unfavourable factor, prognostically, for survival. In addition, it provides extra details on the IPSS score for lower-risk groups. However, this finding was not significant in the higher IPSS risk categories it was.[18]

Our study supports the previous literature because the follow-up analysis showed that patients with lower bone marrow fibrosis grade had milder symptoms when they were first diagnosed and a better response to treatment. On the other hand, as reticulin fibrosis grade rises, so does the risk of myelofibrotic progression and change to acute leukaemia. This additional information will be pertinent to prognostic assessment in younger patients of lower risk groups associated with higher marrow fibrosis grade, still under evaluation for clear indications for management.

In a formal statistical analysis, we recommend including bone marrow fibrosis grade as an individual risk variable in addition to the standard IPSS score, allowing for stratification of diverse patient groups with statistically significant variable survival.

LIMITATIONS OF STUDY

Although the current study has the advantage of being in a "real-life" setting, inter-observer variability can affect the reproducibility of the quantitative assessment of bone marrow fibrosis grade.

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We would like to express our sincere gratitude to the research participants.

CONCLUSION

We concluded that myeloproliferative neoplasms are very different from one another in terms of how they look and how likely they are to progress to myelofibrosis or acute leukaemia, both of which are very bad for overall survival. Bone marrow fibrosis is a useful prognostic marker in predicting the outcome of myeloproliferative neoplasms. Early diagnosis and timely management can limit the

disease and benefit the patient's rehabilitation to a healthy life.

Conflict of Interest: None.

Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

MBA & HSM: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

NR & MB: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

RM & IA: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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