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Original Article

Pathology Section

Role of Angiogenesis and Endothelial-mesenchymal Transition in Bone Marrow Fibrosis Associated with Haematopoietic Neoplasms: A Cross-sectional Immunohistochemical Analysis

SHWETA AGARWAL[1], SHRUTI SHARMA[2], MONAL TRISAL[3], ZEEBA S JAIRAJPURI[4], SUJALA KAPUR[5], SUMITA SALUJA[6], PURNIMA PALIWAL[7]

ABSTRACT

Introduction: The bone marrow examination is an essential investigation in the diagnosis and management of many haematological disorders. The integration of all investigations, including peripheral blood analysis, bone marrow aspirate, and trephine biopsy findings, along with supplementary tests such as immunophenotyping, cytogenetic analysis, and molecular genetic studies, is crucial for arriving at a final diagnosis.

Aim: To assess the presence of reticulin fibres in bone marrow biopsy sections in haematological malignancies, to evaluate the grade of BMF associated with haematological malignancies and to assess the role of angiogenesis using IHC markers in various haematological malignancies.

Materials and Methods: A cross-sectional study was conducted in the Department of Pathology at the National Institute of Pathology, Safdarjung Hospital, New Delhi, India in 2009 for a duration of 18 months. Thirty-eight patients with a diagnosis of Acute Myeloid Leukaemia (AML), Acute Lymphoblastic Leukaemia (ALL), Chronic Myeloid Leukaemia (CML), and Chronic Lymphoproliferative Disorder (CLPD) were studied. Bone marrow biopsies were taken, fixed in 10% formalin, and decalcified in 10% Ethylene Diamine Tetraacetic Acid (EDTA). Routine paraffin embedding was performed, and serial sections of 4 µm were obtained on poly-L-lysine-coated slides for Immunohistochemistry (IHC) {Vimentin, Vascular Endothelial Growth Factor (VEGF), CD-34, Smooth Muscle Actin (SMA)}. The presence of reticulin fibres in the bone marrow biopsy sections was assessed using two special stains: Gomori's Silver Impregnation and Masson's Trichrome. Fibrosis was quantified according to the Baurmeister 0-4 grading system of Bone Marrow Fibrosis (BMF).

Results: The results of the present study suggest that Endothelial to Mesenchymal Transition (EndMT) may play a role in the pathogenesis of BMF. Various grades of fibrosis were observed, with 15 cases (39.47%) in Grade 3, followed by 11 cases (28.95%) in Grade 2, 8 cases (21.05%) in Grade 1, and 4 cases (10.53%) in Grade 4.

Conclusion: BMF was a significant finding even in the early stages of the majority of the lesions studied and was closely linked with angiogenesis. This study showed that angiogenesis plays an important role in the pathogenesis of haematological neoplasms and that VEGF is a prominent stimulus in the majority of these disorders. Additionally, this study suggests that EndMT has a possible role in the pathogenesis of BMF.

Keywords: Acute lymphoblastic leukaemia, Acute myeloid leukaemia, Chronic lymphoproliferative disorder, Chronic myeloid leukaemia, Epithelial

INTRODUCTION

Bone marrow examination is an essential investigation in the diagnosis and management of many haematological disorders. The integration of all investigations, like peripheral blood analysis, bone marrow aspirate, and trephine biopsy findings, along with supplementary tests like immunophenotyping, cytogenetic analysis, and molecular genetic studies, is essential for the final diagnosis. BMF refers to an increase in reticulin or increase in both reticulin and collagen, serving as a useful non specific indicator that the marrow is abnormal. Reticulin is a normal component of bone marrow that may be increased in a wide variety of neoplastic and non neoplastic conditions. The relationship between increase in bone marrow stromal fibres and disease pathology is not well understood but has been the subject of clinical investigations [1,2]. Angiogenesis, the formation of new blood vessels from existing vasculature, has been associated with the growth, dissemination, and metastasis of solid tumours [3]. Although it is suggested that angiogenesis plays a role in the development of fibrosis, only a few studies have correlated it with the extent of fibrosis in the marrow. However, some authors have demonstrated a correlation between angiogenesis and the degree of reticulum fibrosis in cases of Chronic Idiopathic Myelofibrosis (CIMF) [3].

The proliferation of myofibroblasts is responsible for exaggerated and unchecked extracellular matrix production during the development as well as progression of pathological fibrosis. Myofibroblasts in densely fibrotic tissues arise from the activation of resident fibroblasts, the transformation of epithelial cells into mesenchymal cells (Epithelial-Mesenchymal Transition, or EMT), and the tissue migration of circulating bone marrow-derived fibrocytes [4,5]. Recently, EndMT has emerged as a possible origin of tissue myofibroblasts. EndMT is often categorised as a specialised form of EMT. It is a process in which resident endothelial cells delaminate from an organised cell layer and acquire a mesenchymal phenotype, characterised by the loss of cell-cell junctions, loss of endothelial markers, gain of mesenchymal markers, and acquisition of invasive and migratory properties. These processes are associated with the emergence of mesenchymal markers such as Fibroblast-Specific Protein (FSP1), alpha SMA,

and vimentin, along with the downregulation of CD31/PECAM and β-catenin [6].

The present study aimed to assess the presence of reticulin fibres in bone marrow biopsy sections in haematological malignancies, to evaluate the grade of BMF associated with haematological malignancies and to assess the role of angiogenesis using IHC markers in various haematological malignancies.

MATERIALS AND METHODS

A cross-sectional study was conducted in the Department of Pathology at the National Institute of Pathology, Safdarjung Hospital, New Delhi, India, from January 2009 to July 2010. SPPS 18 version was used.

Inclusion criteria: A total of 38 patients with a diagnosis of AML, ALL, Chronic Myeloproliferative Diseases (CMPD), and Chronic Lymphocytic Leukaemia (CLL) were included in this study.

Exclusion criteria: All inadequate biopsies were excluded from the study group.

Bone marrow biopsies performed as part of the investigation for anaemia, which were found to be adequate and normal on histology, were taken as control samples.

Study Procedure

Biopsies were taken and fixed in 10% formalin, then decalcified in 10% EDTA. Processing was performed according to routine paraffin embedding, and serial sections of 4 μm were obtained on poly-L-lysine coated slides for IHC. The presence of reticulin fibres in the bone marrow biopsy sections was assessed using two special stains: Gomori's Silver Impregnation and Masson's Trichrome. Fibrosis was quantified according to the Baurmeister's 0-4 grading system of BMF [7].

- Grade 0-No reticulin fibres demonstrable
- Grade 1-Occasional fine individual fibres and foci of a fine fibre network
- Grade 2-Fine fibre network throughout most of the section; no coarse fibres
- Grade 3-Diffuse fibre network with scattered, thick, coarse fibres but no mature collagen (negative trichrome staining)
- Grade 4-Diffuse, often coarse fibre network with areas of collagenisation (positive trichrome staining)

Immunohistochemistry: All blood vessels were highlighted by staining endothelial cells with an anti-CD34 antibody using the Avidin-Biotin complex method (Diagnostic Bioscience, dilution of 1:50). Antibodies against Vimentin (Biogenix, undiluted) and SMA (Novocastra, dilution of 1:50) were used as mesenchymal markers. Vimentin expression was quantified as the percentage of cells showing vimentin positivity in high-power fields.

To measure bone marrow Microvessel Density (MVD), microvessels stained by CD34 were counted in three hotspots, and the average was reported as MVD. The hotspots were defined as areas of maximum vessel density observed through a 10× power ocular lens. Large vessels and vessels located beneath the periosteum were excluded from the analysis. The method used for counting the microvessels involved selecting areas of highest MVD (hotspots) at 40× magnification, followed by image capture and counting the microvessels in 10 selected fields of each slide at 200× magnification. The countable microvessels were defined according to the criteria established by Weidner N et al., [8].

STATISTICAL ANALYSIS

The mean of MVD was obtained for various subsets of samples and compared with those of the control group using Statistical Package for the Social Sciences (SPSS). Box plots were used to compare the distribution of the different sample sets, and statistical significance was determined using Student's t-test. To correlate MVD with the grade of fibrosis, linear least squares regression analysis was employed.

RESULTS

Thirty-eight samples from patients with haematological disorders with BMF were included in the study. The diagnosis of these patients ranged from acute leukaemia to chronic myeloproliferative disorders, Chronic Lymphoproliferative Disorders (CLPD), and Myelofibrosis (MF). The distribution of the cases according to histological diagnosis is provided in [Table/Fig-1].

Histopathological diagnosis	n (%)
Acute Myeloid Leukaemia, AML	8 (21.05)
Acute Lymphoid Leukaemia, ALL	7 (18.42)
Chronic Myeloid Leukaemia (CML)	6 (15.79)
Chronic Lymphoproliferative Disorders (CLPD)	9 (23.69)
Myelofibrosis (MF)	8 (21.05)

[Table/Fig-1]: Distribution of cases according to histological diagnosis.

The majority of the patients in the sample set were male, constituting 71.05% of the cases (27 out of 38), while female patients numbered 11, comprising 28.95% of the cases.

Grading of fibrosis was performed in all cases according to a 0-4 grading system. Gomori's silver impregnation was useful in identifying reticulin fibrosis of grades 1-3, while Masson's Trichrome stain showed collagen fibrosis (Grade-4). All four grades of fibrosis were observed in the sample set (n=38), with the majority belonging to Grade-3. The distribution of samples across the various grades of fibrosis was as follows: 15 cases (39.47%) in Grade-3, 11 cases (28.95%) in Grade-2, 8 cases (21.05%) in Grade-1, and 4 cases (10.53%) in Grade-4.

Upon further analysis, the distribution of cases in each histological category across the various grades of fibrosis revealed that cases of AML showed an equal distribution of Grade-2 and Grade-3 severity, i.e., three each out of eight cases, whereas in ALL, Grade-1 and Grade-3 showed an equal distribution of three cases each out of a total of seven cases. In CML three out of six cases (50%) exhibited Grade-2 fibrosis, while in CLPDs, four out of nine cases (45%) showed Grade-2 fibrosis. Additionally, in CLPDs, two cases demonstrated focal increases in reticulin (Grade-1), which were primarily concentrated around the nodular infiltrates. In MF, an equal distribution of Grade-3 and Grade-4 fibrosis was observed. Collagen fibrosis was primarily seen in samples of idiopathic MF, as depicted for analysis in [Table/Fig-2].

	Grade of fibrosis			
Histological type	1	2	3	4
AML (n=8)	2 (25.0%)	3 (37.5%)	3 (37.5%)	-
ALL (n=7)	3 (43%)	1 (14%)	3 (43%)	-
CML (n=6)	1 (17%)	3 (50%)	2 (33%)	-
CLPD (n=9)	2 (22%)	4 (45%)	3 (33%)	-
MF (n=8)	-	-	4 (50%)	4 (50%)
Total (n=38)	8	11	15	4

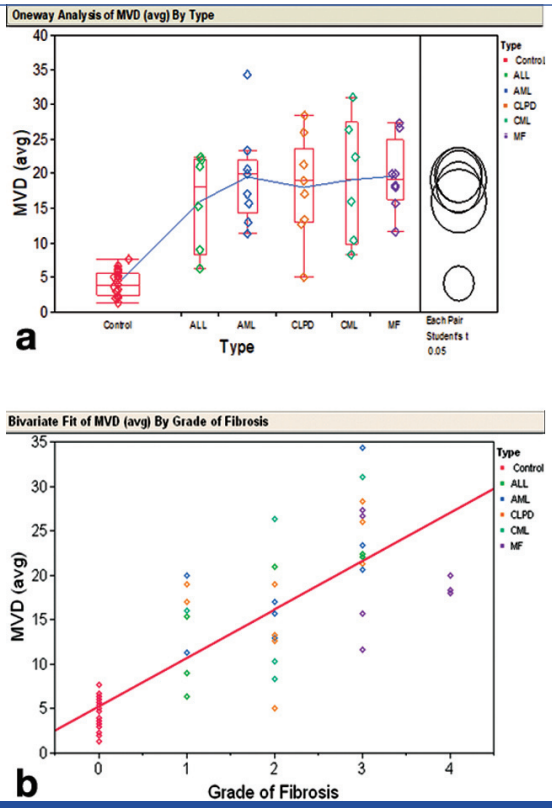
[Table/Fig-2]: Percentage distribution of samples in each category across various grades of fibrosis.

The vascularity showed a significant increase (p-value <0.001) in all the bone marrow biopsies included in the study compared to the control samples [Table/Fig-3,4a]. The majority of the newly formed vessels were found to be tortuous with abnormal branching, and some were composed of only 1-2 endothelial cells.

However, in the individual case analysis, there was a positive correlation in ALL (R^2=0.75), CML (R^2=0.30), AML (R^2=0.40), and CLPD (R^2=0.24), but not in MF. In CLPD, the two nodular samples showed a high MVD, but the vessels were primarily concentrated around the nodular infiltrates. Excluding these two samples from the analysis revealed a strong fit of MVD with grade of reticulin (R^2=0.69). This observation can be explained by the use of a semiquantitative method for estimation of MVD.

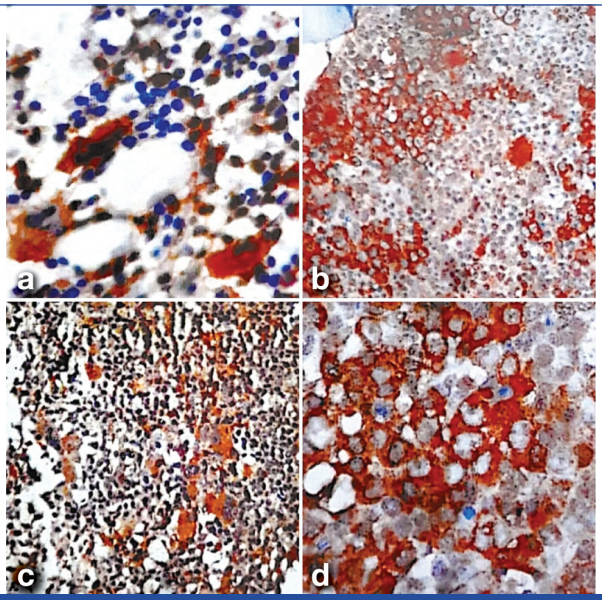
Histological type	MVD count (Avg)	p-value w.r.t. control
ALL (n=7)	16.0	<0.0001
AML (n=8)	19.6	<0.0001
CLPD (n=9)	18.0	<0.0001
CML (n=6)	19.1	<0.0001
MF (n=8)	19.7	<0.0001
Control	4.1	

[Table/Fig-3]: Comparison for MVD (student's t-test) based on histological type.



[Table/Fig-4]: a) One-way Analysis of Variance of MVD (Avg) by type of lesion. Bivariate analysis showed a strong correlation between MVD and the grade of fibrosis (R^2=0.64) for all the samples included in the study; b) Bivariate study of MVD (avg.) by grade of fibrosis.

Immunohistochemical studies for endothelial markers (VEGF and CD34) and mesenchymal markers (SMA and Vimentin) were conducted for analysis. VEGF showed crisp granular staining in normal bone marrow biopsies for megakaryocytes and myeloid progenitor cells, whereas erythroid and stromal cells did not express the cytokine. However, VEGF expression was markedly upregulated and was expressed by the leukaemic blasts and stromal cells as well [Table/Fig-5]. The findings were expressed as a VEGF score by counting the cells positive for VEGF in three high-power fields and calculating the average [Table/Fig-6]. The average VEGF score in bone marrow biopsies of patients with AML, CML, CLPD, and MF was found to be significantly higher compared to the biopsies from control samples. In ALL, however, there was no statistically significant difference in the VEGF score when compared to the control samples. In AML, a high score of 37.5/hpf was observed, with VEGF expressed by the majority of myeloblasts, megakaryocytes, and myeloid progenitor cells [Table/Fig-5b,d]. Similarly, in CML and MF, high scores of 33 and 47.6 per hpf, respectively, were noted, with markedly upregulated expression of VEGF in both myeloid as well as stromal cells [Table/Fig-5c]. In CLPDs, a highly variable pattern of VEGF expression was noted. In samples with diffuse infiltration of the bone marrow, the malignant cells showed strong positivity for VEGF, whereas samples with nodular infiltrates showed scant positivity to completely negative malignant nodules. The overall score obtained for all CLPD samples was 29.9/hpf. Regression analysis between VEGF and the grade of fibrosis in all samples showed a positive correlation (R^2=0.40). When analysing individual samples, a positive correlation between VEGF and the grade of fibrosis was found in ALL and CML, but not in ALL and CLPD. In MF, VEGF was found to be independent of the grade of fibrosis.



[Table/Fig-5]: a) Megakaryocytes and myeloid cells showing crisp granular staining for VEGF in control sample (100x); b) Myeloblasts along with few megakaryocytes strongly positive for VEGF (40x); c) VEGF upregulation in MF (40x); d) Leukaemic blasts showing crisp cytoplasmic positivity for VEGF (100x).

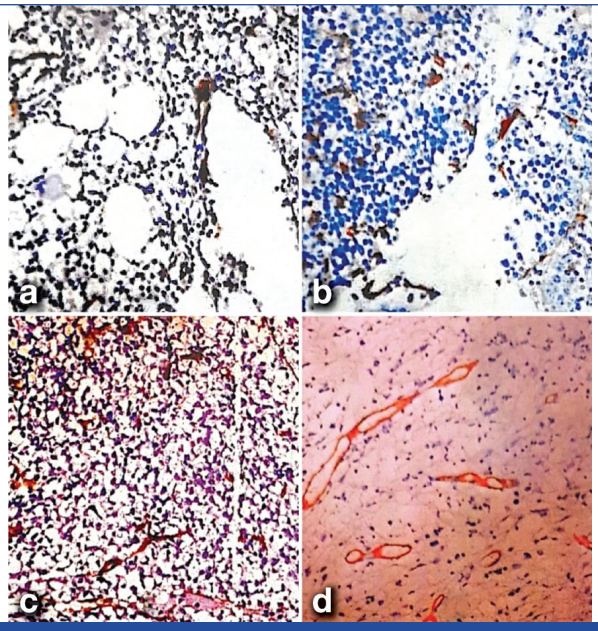
Histological type	Number of samples (n)	VEGF score (Avg)/hpf	p-value w.r.t control (Student's t-test)
ALL	7	21.5	0.5404
AML	8	37.5	0.0001
CLPD	9	29.9	0.0156
CML	6	33.0	0.009
MF	8	47.6	<0.0001
Control	20	18.1	

[Table/Fig-6]: Distribution of VEGF expression in various lesions.

The expression of CD34 was found in the endothelial cells of large vessels as well as in the micro-vessels in normal bone marrow biopsies. Micro-vessels stained by CD34 were counted in three hotspots, with the average reported as MVD [Table/Fig-7a]. The hotspots were defined as areas of maximum vessel density, and large vessels as well as vessels located beneath the periosteum were excluded from this count. A significant increase in vascularity was noted in all cases of this study compared to the control group [Table/Fig-7b-d,8]. The majority of the newly formed vessels were found to be tortuous with abnormal branching, and some were composed of only 1-2 endothelial cells. It was also observed that in the two nodular samples of CLPD, the hotspots were primarily concentrated around the nodular infiltrates.

Regression analysis between MVD and the grade of fibrosis in all samples showed a strong correlation (R^2=0.64). However, in individual case analysis, a positive correlation was seen in ALL, CML, AML, and CLPD, but not in MF. Furthermore, a correlation (R^2=0.48) was found between VEGF and MVD for all histological types included in this study. The analysis of individual histological types showed a positive correlation between VEGF and MVD in AML and CML, whereas no correlation was noted in ALL and MF. In CLPDs, there was a weak correlation, but an upward trend was observed.

The expression of the mesenchymal marker SMA in the control bone marrow biopsies was limited to the smooth muscle cells of the media of the large bone marrow vessels [Table/Fig-9a]. It was not expressed by the haematopoietic cells or the stromal cells of the marrow. In the study samples, α-SMA was expressed in the pericytes of large vessels and microvessels. Endothelial-to-Mesenchymal Transition (EndMT) has been hypothesised as one of

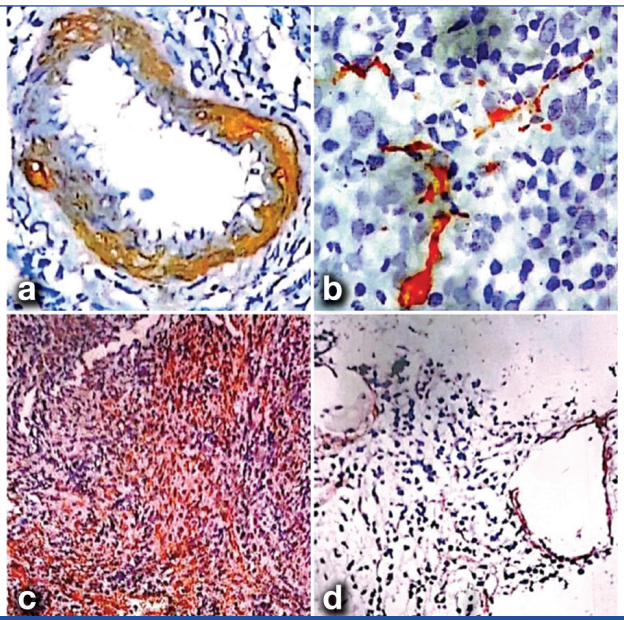


[Table/Fig-7]: a) Vessel labelled by CD34 in a control sample (40x); b) Microvessels in AML (40x); c) Microvessels in ALL (40x); d) Microvessels in CLL (40x).

Histological type	Number of samples (n)	MVD count (Avg)	p-value w.r.t control (Student's t-test)
ALL	7	16.0	<0.0001
AML	8	19.6	<0.0001
CLPD	9	18.0	<0.0001
CML	6	19.1	<0.0001
MF	8	19.7	<0.0001
Control	20	4.1	

[Table/Fig-8]: Distribution of MVD in various cases.

the origins of pericytes in microvessels formed during angiogenesis. Two discrete layers composed of inner endothelial cells and outer pericytes were observed in 10 out of 38 samples across all histological types except CLPDs [Table/Fig-9b-d,10]. In addition, three out of 38 samples showed upregulation of α-SMA in stromal cells as well as myeloid cells. Furthermore, microvessels in MF as well as CML showed α-SMA expression in endothelial cells in two samples each.



[Table/Fig-9]: a) α-SMA expressed by the myocyte layer in a large bone marrow vessel (100x), Control; b) Abnormal α-SMA expression by stromal cells in AML (100x); c) Upregulation of α-SMA in stromal cells in ALL (20x); d) Few microvessels and stromal cells positive for α-SMA in MF (40x).

Expression of Vimentin in normal bone marrow biopsies was seen in the stromal cells of normal marrow. The current study found upregulated

Histological type	α-SMA			
	Number of positive samples	Stromal cells	Endothelial cells	Pericytes
AML (n=8)	2	-	-	+
ALL (n=7)	1	+	-	+
CML (n=6)	2	+	+	+
MF (n=8)	5	+	+	+
CLPD (n=9)	-	-	-	-

[Table/Fig-10]: Expression of α-SMA in different histological samples.

expression of Vimentin in 12 out of 38 cases. Compared to normal bone marrow biopsies that showed positive Vimentin expression in 10-30% of cells per high-power field (hpf), the biopsies with fibrosis showed positive expression of 50-90% per hpf [Table/Fig-11].

Histological type	Vimentin expression	
	Number of positive samples	Percentage of positive cells/hpf
AML (n=8)	2	60
ALL (n=7)	1	60
CML (n=6)	2	50-80
MF (n=8)	3	90
CLPD (n=9)	4	80-90

[Table/Fig-11]: Vimentin expression in different histological types.

Among all the myeloid cells, stromal cells, and megakaryocytes, strong positivity for Vimentin was observed in 12 out of the 38 cases studied. This finding was noted in ALL (1 case), AML (2 cases), CML (2 cases), CLL (4 cases), and MF (3 cases). The samples with increased Vimentin expression showed grades of fibrosis ranging from Grade 1 to Grade 4.

DISCUSSION

BMF is the progressive replacement of haematopoietic cells by reticulin fibres, caused by the acquisition of somatic mutations in the haematopoietic stem cells. The various cellular and molecular mechanisms that drive the progression of BMF remain unclear. BMF is generally observed as a non specific indicator of an abnormal marrow. It results from a variety of aetiologies, including myeloproliferative disorders such as CML, polycythemia vera, and essential thrombocythemia, as well as acute leukaemias (both myeloid and lymphoid), lymphomas, and various other inflammatory causes [7].

The most frequently used grading systems applied to assess MF are essentially based on the Baumeister scale [7], which was modified by Manoharan [9]. The European Consensus on grading BMF and the assessment of cellularity [10] simplifies all previous descriptions of fibre scoring [8,9] by reducing them to four grades, including normal reticulin density. This approach aims to avoid excessive overlap and achieve a higher degree of reproducibility in routine diagnosis, which was adopted in this study. The confusion created in former systems, where normal reticulin was classified as Grade 1, was addressed by classifying normal reticulin as N-normal or Grade 0 [10].

Bone marrow biopsy is seldom performed in the work-up of acute CML, usually only when a dry tap is obtained on bone marrow aspiration. As is evident, data on reticulin fibrosis in these neoplasms is limited, despite the fact that BMF per se has poor prognostic value. The current study documents fibrosis in acute leukaemias (both myeloid as well as lymphoid) at the time of initial diagnosis, ranging from a focal increase in reticulin fibres (Grade 1) to marked fibrosis with scattered coarse fibres (Grade 3). The pathogenesis of AML with BMF remains unclear. It has been suggested that the abnormal proliferation of BMF is a secondary reaction to the clonal proliferation of haematopoietic cells [11] and is characterised by increased deposition of reticulin or collagen fibres. However, BMF,

which can be observed in any type of AML, is more frequent in Acute Megakaryocytic Leukaemia (AML-M7) [12]. Earlier studies have reported increased reticulin in 35% of AML patients and 57% of ALL patients at presentation [13,14].

CML, minimal reticulin to advanced collagen fibrosis has been reported earlier in 10-35% of cases at presentation [15]. It is now a well known factor that contributes to morbidity and mortality in these patients [15,16]. In the present study, CML samples with fibrosis ranged from Grade-1 to Grade-3. A single case of CML progressing to MF was also seen in this study; however, it has been categorised within the MF group for the uniformity of statistical analysis. The authors suggest the use of bone marrow biopsy as an important tool in the work-up of patients with suspected CML. Bone marrow core biopsy is valuable in the initial staging of CML in approximately 25% of patients suspected of having the disease, as it facilitates the identification of the disease phase or the presence of MF [17]. Of the eight samples included in the MF category, seven were in the primary idiopathic category (CIMF). The remaining case was secondary MF progressing from CML after a duration of four years and showed Grade-3 fibrosis. Among the remaining seven cases of CIMF, four cases showed Grade-4 fibrosis, while three cases showed Grade-3 fibrosis.

CLL has been associated with several other malignancies, including transformation to diffuse large B-cell lymphoma and various solid organ neoplasms, with bone marrow involvement in CLL always being secondary. CLL is not frequently associated with primary or secondary bone marrow fibrosis. The association between CLL and Primary Myelofibrosis (PMF) and other Myeloproliferative Neoplasms (MPN) is unusual [18]. Fibrosis in CLL is a rare finding and has been well documented only in Hairy Cell Leukaemia (HCL) amongst all the CLPDs [13]. There was a predominance of Grade-2 fibrosis in CLL samples (45%). A single case of HCL included in the study showed fibrosis of Grade-3, characterised by a delicate pattern of reticulin fibres closely associated with the tumour cells. The exact pathogenesis of fibrosis in HCL remains debatable, as some studies implicate fibrogenic cytokines as the source of fibrosis, while others suggest the role of fibronectin in the fibrotic process [19,20]. This data reveals that reticulin fibrosis is a prominent finding in both the initial and late stages of the majority of haematological neoplasms, thereby further validating the need to include bone marrow biopsy as well as reticulin staining in the investigations conducted for all leukaemias.

It has been proposed that angiogenesis, which is the formation of new blood vessels from existing vasculature, plays a role in the development of fibrosis in the bone marrow. The majority of the newly formed vessels were found to be tortuous and branched, while some were composed of only one or two endothelial cells. Steurer M et al., demonstrated a correlation between angiogenesis and the degree of reticulin fibrosis in cases of CIMF [3]. Present study aimed to evaluate angiogenesis and bone marrow fibrosis across a range of haematological malignancies. A significant increase in MVD was observed in acute leukaemias (both myeloid and lymphoid), chronic leukaemias, and MF was seen (p-value <0.0001). The average MVD for all neoplasms was four to five times that of control samples, and the results were comparable to those of previous studies. Although increased vascularity has been demonstrated in individual studies of these neoplasms, there have been conflicting results in patients with CLL [21-23]. The present study showed markedly increased vascularity in both diffuse as well as nodular types of CLL samples. In addition, the study was able to demonstrate a strong correlation between MVD and the grade of reticulin fibrosis, independent of aetiology (R^2=0.68).

Tumour-associated angiogenic factors are produced by tumour cells as well as inflammatory cells, such as macrophages. This angiogenic process is regulated by a close interplay of regulatory molecules, among which VEGF and β-FGF are among the most important. As reported earlier, VEGF expression has been seen in megakaryocytes, myeloid progenitor cells, plasma cells, stromal cells, as well as leukaemic blasts, exhibiting crisp granular positivity [23,24].

A significantly increased expression of VEGF was observed in AML, CML, CLL, and MF, but not in ALL. These findings strongly indicate the role of VEGF as a prominent angiogenic stimulus in the majority of haematological neoplasms studied, with marked upregulation in most cases, except for ALL [25]. Similar trends have been reported in the literature using IHC and/or serum levels of this angiogenic cytokine [26].

The association of MVD with the grade of fibrosis opens the discussion on the possible role of EndMT in the pathogenesis of fibrosis. EndMT is a complex process in which endothelial cells delaminate from an organised cell layer and transdifferentiate into mesenchymal cells [27] (such as pericytes or fibroblasts in this particular setting). During this process, some cells lose endothelial markers and begin expressing mesenchymal markers, such as α-SMA and/or vimentin [27,28]. In addition, a few cells exhibiting bi-phenotypic expression-characterised by the presence of both endothelial and mesenchymal markers-can also be found [28]. It has been suggested that fibroblasts may arise from endothelial cells via EndMT as one possible source of origin [22]. The current study focused on the use of IHC to evaluate such transitional cells in bone marrow fibrosis [29].

EndMT in bone marrow is not widely reported in the literature. It has been suggested that fibroblasts can arise from endothelial cells via EndMT, as well as from pericytes, which can also undergo EndMT as one of the possible source of origin [30]. During this process, it is possible to observe a few transitional cells that co-express endothelial and mesenchymal markers, as demonstrated in in-vitro studies. In-vitro, EndMT can be duplicated by incubating cultured endothelial progenitor cells or spleen-derived endothelial cells with inflammatory cytokines. Megakaryocytes may also be implicated in this process, as EndMT occurs predominantly in the microvessels closest to these cells, and megakaryocyte-derived supernatant fluid can generate the EndMT switch in-vitro [30].

In the current study, normal marrow showed α-SMA marking the myocyte layer around the endothelial cells, whereas Vimentin was positive in the stromal cells and fat cells. This study noted an upregulation of α-SMA in 10 out of 38 samples (26.3%). It was also seen that the expression of α-SMA was independent of the degree of fibrosis and aetiology. Furthermore, transitional cells with co-expression of endothelial and mesenchymal markers were seen in cases of CIMF exhibiting Grade 4 fibrosis. A study reported by Suetterlin R et al., used immunofluorescence along with confocal laser scanning microscopy to demonstrate upregulation of α-SMA in bone marrow samples with fibrosis [31]. In contrast to the study by Suetterlin R et al., which showed an increase in the expression of α-SMA with increasing degrees of fibrosis [32], the expression of α-SMA in the present study was found to be independent of the degree of fibrosis and aetiology. Furthermore, the, transitional cells with co-expression of endothelial and mesenchymal markers were seen in samples of CIMF exhibiting Grade 4 fibrosis.

Vimentin is a type III intermediate filament that maintains cell integrity and is involved in cell migration, motility, and adhesion. When overexpressed in solid cancers, vimentin drives EMT and ultimately metastasis. As an organiser of several crucial proteins, vimentin plays a role in attachment, migration, and cell signalling [32-34]. The present study documents increased expression of vimentin in 12 out of 38 samples (31.6%), which included all histological types and almost all grades of fibrosis. Almost all bone marrow cells, including myeloid cells, lymphoid cells, megakaryocytes, as well as stromal cells, were strongly positive for this mesenchymal marker. Although there are studies in the literature that have reported the expression of vimentin in haematopoietic cells, data supporting its role in EndMT in the bone marrow is limited [32].

The results of the present study suggest that EndMT has a possible role in the pathogenesis of bone marrow fibrosis. Although the data is limited, evidence of EndMT was observed in almost all types of histological samples with Grade 1 to Grade 4 fibrosis. The observations from this study open an avenue for exploring EndMT in bone marrow fibrosis using a wider panel of antibodies and potentially incorporating other ancillary techniques.

Limitation(s)

The present study was limited by its small sample size; however, the results of this study open up opportunities for exploring EndMT in bone marrow fibrosis using a wider panel of antibodies and possibly incorporating other ancillary techniques.

CONCLUSION(S)

BMF was a significant finding, even in the early stages of the majority of the lesions studied, and it was closely linked with angiogenesis. This study demonstrated that angiogenesis plays an important role in the pathogenesis of haematological neoplasms, with VEGF identified as a prominent stimulus in the majority of these disorders. Additionally, the results suggested that EndMT had a possible role in the pathogenesis of BMF. Further evaluation of the role of EndMT in BMF, using a wider panel of antibodies along with molecular studies, will help achieve a better understanding of the pathogenetic mechanisms associated with fibrosis in bone marrow disorders.

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PARTICULARS OF CONTRIBUTORS:

1. Resident, Department of Pathology, ICMR, National Institute of Pathology, Safdarjung Hospital Campus, New Delhi, India.
2. Scientist E, Department of Pathology, ICMR, National Institute of Pathology, Safdarjung Hospital Campus, New Delhi, India.
3. Assistant Professor, Department of Pathology, Hamdard Institute of Medical Sciences and Research, Jamia Hamdard University, New Delhi, India.
4. Professor, Department of Pathology, Hamdard Institute of Medical Sciences and Research, Jamia Hamdard University, New Delhi, India.
5. Scientist E, Department of Pathology, ICMR, National Institute of Pathology, Safdarjung Hospital Campus, New Delhi, India.
6. Professor, Department of Pathology, ICMR, National Institute of Pathology, Safdarjung Hospital Campus, New Delhi, India.
7. Scientist D, Department of Pathology, ICMR, National Institute of Pathology, Safdarjung Hospital Campus, New Delhi, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Zeeba S Jairajpuri,
Professor, Department of Pathology, Hamdard Institute of Medical Sciences and Research, Jamia Hamdard University, New Delhi-110062, India.
E-mail: jairajpurizs@gmail.com

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