Original Article

Evaluation Typing and Grading of Bone Marrow Fibrosis in Malignant Disorders Affecting Bone Marrow

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ABSTRACT

Objective: To evaluate, type and grade bone marrow fibrosis in malignant disorders affecting bone marrow by using Reticulin and Van Gieson stain.

Study Design: Descriptive / cross- sectional study.

Place and Duration of Study: This study was conducted at the Haematology and Histopathology Department of Sheikh Zayed Hospital, Lahore from January 2013 to December 2014.

Materials and Methods: Paraffin embedded trephine blocks of 80 consecutive patients diagnosed with malignant disorders affecting bone marrow were taken, sections were made and stained with Reticulin and Van Gieson trichrome stain. Grading of bone marrow fibrosis was done using European consensus 2005 (EC 2005) on bone marrow fibrosis. All data was entered and analyzed by using SPSS 20 .Types and grades of fibrosis were reported by using frequency and percentages.

Results: In a total of 80 patient studied, 64 (80%) patients showed bone marrow fibrosis. Grade-1 fibrosis (MF-1) was seen in 50% grade-2 (MF- 2) was seen in 26.25% and grade-3 (MF- 3)was seen in 3.75 % of patients. Secondary bone marrow fibrosis was present in 62 (97%) of 64 cases and primary bone marrow fibrosis was seen in 2 (3%) of 64 cases.

Conclusion: Eighty percent of patients with various malignant disorders affecting bone marrow had some degree of bone marrow fibrosis. Grade-1 fibrosis (MF-1) was the most common, seen in 50% followed by grade-2 (MF- 2) seen in 26.25% and grade-3 (MF- 3) seen only in 3.75 % of patients..

Key Words: Bone marrow fibrosis, Reticulin stain, Van Gieson stain, thrombopoietin analogues

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INTRODUCTION

In recent years cancer has emerged as a serious health threat in many Asian countries resulting in tremendous loss of life in the region.[1,2] In year 2000 over 2 million people died of cancer in Asia and over 3 million new cancer cases were diagnosed[1]. Haematological malignancies are one of the five most frequent malignancies among males in Pakistan[3].

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Organophosphates (pesticides) has been linked to higher probability of childhood leukemia[4] and Non Hodgkin Lymphoma [5]

Fibrosis occurs in majority of patients with haematological malignancies [6]. When excessive it suppresses haematopoiesis and hence affects normal function of the bone marrow[7]

In principle bone marrow fibrosis may be either primary or secondary. Primary bone marrow fibrosis occurs on its own and is seen in primary myelofibrosis (PMF). Secondary bone marrow fibrosis develops during the course of other diseases like essential thrombocythaemia (ET), polycythaemia vera (PV), chronic myeloid leukaemia (CML) etc [8].

Abnormal cytokines released from platelets and megakaryocytes seems to be essential but not sufficient for fibrosis to occur. Platelets derived growth factor (PDGF), transforming growth factor-beta (TGF-beta), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (b-FGF), matrix tissue inhibitors of metalloproteinase plays a part in development of fibrosis[6]

It was initially thought that increase in bone marrow stromal fibers are responsible for the haematopoietic abnormalities seen in certain diseases but on the contrary recent studies have shown that haematopoietic

abnormalities themselves are the cause of bone marrow fibrosis rather than their outcome[9]

Evidence has shown that there is significant correlation between poor survival and grade of reticulin fibrosis [10]. Collagen fibrosis is strongly correlated with abnormal blood counts and poorer prognosis[11]

Fibrosis is a complication of the bone marrow neoplasm that not only affects the quality of life of the patient but also shortens his/her survival time[12].

Fibrous tissue of the bone marrow is not well appreciated on H and E stain and require special stains. Masson's trichrome stain, Mallory's trichrome stain or Van Gieson trichrome stain, are used to identify collagen[13], while reticulin can be stained by Gordon and Sweets method or Gomori method using silver impregnation technique[13,14]

In the era of targeted therapies like JAK2 inhibitors and realizing the role of bone marrow fibrosis in predicting disease outcome in various haematological malignancies the present study of evaluation, typing and grading of bone marrow fibrosis was done.

MATERIALS AND METHODS

This was a descriptive cross sectional study, which was carried out in Haematology and Histopathology department of SZH, Lahore.

First 80 patients of both gender irrespective of age and sex presenting in the indoor and outdoor department of Shaikh Zayed Hospital who were diagnosed with malignant disorders affecting bone marrow were included in this study. It includes 50 males and 30 females.

Patients with history of chemotherapy and radiotherapy or those on thrombopoietin (TPO) analogues were not taken

Sections were made from bone marrow trephine blocks and stained with Reticulin/Silver stain and Van Gieson stain.

Grading of bone marrow fibrosis was done using European consensus 2005 (EC 2005) on bone marrow fibrosis. All data was entered and analyzed by using SPSS 20 (statistical package for social sciences).Types and grades of fibrosis were reported by using frequency and percentages

RESULTS

When trephine biopsies from these eighty patients were stained with, Reticulin and Van Gieson stain the bone marrow fibrosis was found positive in 37(74.0%) of males and 27(90.0%) of females (table-1).

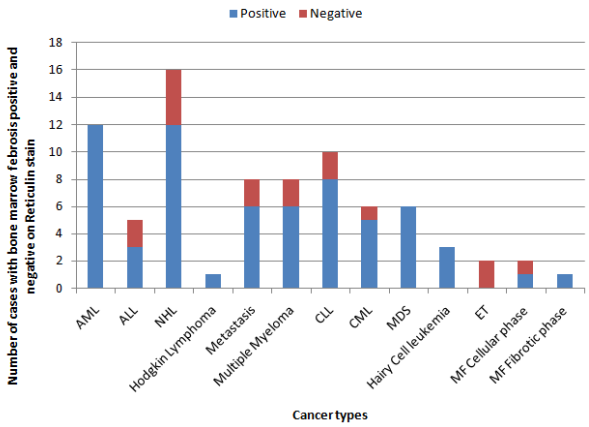

Figure No.1: Bone marrow fibrosis diagnosed on Reticulin +Van Gieson stain in 80 cases of malignant disorders affecting bone marrow

Table No.1: The distribution of cases with bone marrow fibrosis by gender in various malignant disorders affecting bone marrow

	Male				Female				Total			
	With BMF		Without BMF		With BMF		Without BMF		With BMF		Without BMF	
	N	%	n	%	N	%	n	%	n	%	n	%
AML	6	100.0	0	0.0	6	100.0	0	0.0	12	100.0	0	0.0
ALL	3	60.0	2	40.0	0	0.0	0	0.0	3	60.0	2	40.0
NHL	6	60.0	4	40.0	6	100.0	0	0.0	12	75.0	4	25.0
Hodgkin Lymphoma	1	100.0	0	0.0	0	0.0	0	0.0	1	100.0	0	0.0
Metastasis	1	33.3	2	66.7	5	100.0	0	0.0	6	75.0	2	25.0
Multiple Myeloma	4	80.0	1	20.0	2	66.7	1	33.3	6	75.0	2	25.0
CLL	4	100.0	0	0.0	4	66.7	2	33.3	8	80.0	2	20.0
CML	3	75.0	1	25.0	2	100.0	0	0.0	5	83.3	1	16.7
MDS	6	100.0	0	0.0	0	0.0	0	0.0	6	100.0	0	0.0
Hairy Cell leukemia	3	100.0	0	0.0	0	0.0	0	0.0	3	100.0	0	0.0
ET	0	0.0	2	100.0	0	0.0	0	0.0	0	0.0	2	100.0
MF Cellular phase	0	0.0	1	100.0	1	100.0	0	0.0	1	50.0	1	50.0
MF Fibrotic phase	0	0.0	0	0.0	1	100.0	0	0.0	1	100.0	0	0.0
Total	37	74.0	13	26.0	27	90.0	3	10.0	64	80.0	16	20.0

Table No.2: Distribution of bone marrow fibrosis by age in various malignant disorders affecting bone marrow

	≤30 yrs				31 – 45 yrs				46 – 60 yrs				> 60 yrs				Total			
	With BMF		Without BMF		With BMF		Without BMF		With BMF		Without BMF		With BMF		Without BMF		With BMF		Without BMF	
	N	%	n	%	n	%	N	%	N	%	n	%	n	%	n	%	n	%	N	%
AML	4	100.0	0	0.0	4	100.0	0	0.0	4	100.0	0	0.0	0	0.0	0	0.0	12	100.0	0	0.0
ALL	3	60.0	2	40.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	60.0	2	40.0
NHL	0	0.0	0	0.0	5	100.0	0	0.0	5	55.6	4	44.4	2	100.0	0	0.0	12	75.0	4	25.0
Hodgkin Lymphoma	0	0.0	0	0.0	0	0.0	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	1	100.0	0	0.0
Metastasis	0	0.0	1	100.0	0	0.0	1	100.0	4	100.0	0	0.0	2	100.0	0	0.0	6	75.0	2	25.0
Multiple Myeloma	0	0.0	0	0.0	4	100.0	0	0.0	2	100.0	0	0.0	0	0.0	2	100.0	6	75.0	2	25.0
CLL	0	0.0	0	0.0	2	100.0	0	0.0	4	100.0	0	0.0	2	50.0	2	50.0	8	80.0	2	20.0
CML	1	50.0	1	50.0	2	100.0	0	0.0	2	100.0	0	0.0	0	0.0	0	0.0	5	83.3	1	16.7
MDS	0	0.0	0	0.0	1	100.0	0	0.0	5	100.0	0	0.0	0	0.0	0	0.0	6	100.0	0	0.0
Hairy Cell leukemia	0	0.0	0	0.0	0	0.0	0	0.0	3	100.0	0	0.0	0	0.0	0	0.0	3	100.0	0	0.0
ET	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	2	100.0
MF Cellular phase	0	0.0	0	0.0	0	0.0	0	0.0	1	50.0	1	50.0	0	0.0	0	0.0	1	50.0	1	50.0
MF Fibrotic phase	1	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	100.0	0	0.0
Total	9	64.3	5	35.7	18	94.7	1	5.3	31	83.8	6	16.2	6	60.0	4	40.0	64	80.0	16	20.0

The mean age for BMF positive cases was 47.3 years (SD ±14.3) with a median age 50 (38.5 – 55.5) and for cases without BMF mean age was 46.1 years (SD ±17.9) and median age of 55 (29.0 – 59.5). (table: 2)

The bone marrow fibrosis was present in 9(64.3%) of cases with age below 30 years, 18(94.7%) in age group 31 – 45, 31(83.8%) in age group 46 – 60 years and 6(60.0%) in age group above 60 years. (table.2)

When Reticulin and Van Gieson stain was applied on trephine biopsies sample, 64 (80%) cases showed bone marrow fibrosis. (table 3), (fig 1).

Percentage of positivity in patients suffering from AML, Hodgkin lymphoma, MDS, hairy cell leukaemia and fibrotic phase of primary myelofibrosis was 100%.In CML the percentage of bone marrow fibrosis was 83.3% while 80% of patients with CLL showed bone marrow fibrosis on basis of these stains. In multiple myeloma, NHL and bone marrow metastasis 75% patients showed bone marrow fibrosis. In ALL the percentage of fibrosis was 60%, 50% of patients with cellular phase of MF are positive, while only two patients with ET included in the study were negative for BMF on basis of this stain . (table :3),(fig 1)

If we grade BMF on Reticulin and Van-Gieson stain using European consensus 2005, 16 (20%) patients had MF-0 (no fibrosis), 40(50%) patients had grade1 fibrosis, 21(26.25%) patients had grade 2 fibrosis, while grade 3 fibrosis was only seen in 3 (3.75%) patients (Table 4). Two patients having grade 3 fibrosis are of metastatic cancer while one patient belongs to fibrotic phase of PMF.

Table No.3: Results of Reticulin+Van Gieson stain in 80 cases of malignant disorders affecting bone marrow

Malignant disorders affecting bone marrow	Reticulin+Van Gieson stain					
	Positive		Negative		Total	
	N	%	N	%	N	%
AML	12	100.0	0	0.0	12	100.0
ALL	3	60.0	2	40.0	5	100.0
NHL	12	75.0	4	25.0	16	100.0
Hodgkin Lymphoma	1	100.0	0	0.0	1	100.0
Metastasis	6	75.0	2	25.0	8	100.0
Multiple Myeloma	6	75.0	2	25.0	8	100.0
CLL	8	80.0	2	20.0	10	100.0
CML	5	83.3	1	16.7	6	100.0
MDS	6	100.0	0	0.0	6	100.0
Hairy Cell leukemia	3	100.0	0	0.0	3	100.0
ET	0	0.0	2	100.0	2	100.0
PMF Cellular phase	1	50.0	1	50.0	2	100.0
PMF Fibrotic phase	1	100.0	0	0.0	1	100.0
Total	64	80.0	16	20.0	80	100.0

Table No.4: Grading of bone marrow fibrosis on Van Gieson and Reticulin stain using European Consensus-2005 grading system in 80 cases of malignant disorders affecting bone marrow

Malignant disorders affecting bone marrow	Reticulin stain and Van Gieson stain				Total
	MF-0	MF-1	MF-2	MF-3	
AML	0	10	2	0	12
ALL	2	1	2	0	5
NHL	4	10	2	0	16
Hodgkin Lymphoma	0	0	1	0	1
Metastasis	2	4	0	2	8
Multiple Myeloma	2	2	4	0	8
CLL	2	2	6	0	10
CML	1	2	3	0	6
MDS	0	6	0	0	6
Hairy Cell leukemia	0	2	1	0	3
ET	2	0	0	0	2
PMF Cellular phase	1	1	0	0	2
PMF Fibrotic phase	0	0	0	1	1
Total	16	40	21	3	80

DISCUSSION

Number of studies had been carried out to see the presence and prognostic implications of bone marrow fibrosis in various haematological disorders. Some studies were done
on haematological disorders in general [15], while others were done on some particular disorder e.g.CMPD[16], PMF[17],MDS[18],CML,CLL and Multiple Myeloma[19]etc.

First detailed study on fibrous tissue content of the bone marrow in patients with various haematological disorders was carried out decades ago. A total of 247 samples from 157 patients with various haematological disorders were studied. These also included 140 samples from patients with various haematological malignancies and metastatic cancers. Out of these 140 samples, 121(86%) biopsy specimen showed bone marrow fibrosis[15].Four different patterns of argyrophilic fiber were identified. Type 1, normal was seen in 19 (13.57%) biopsies; Type 2, slightly increase in fine fibers around the trabeculae and sinuses was seen in 28 (20%) biopsies; Type 3, moderate increase with abundant fiber network was identified in 51 (36.4%) biopsies; and Type 4, markedly increased argyrophilic fibers with bundles of thick fibers was seen in 42 (30%) biopsies. Our present study on trephine biopsies from 80 patients with malignant disorders affecting bone marrow 64(80%) biopsies showed bone marrow fibrosis of variable grades. MF-0, normal, was present in 16 (20%) biopsies; MF1, in 40 (50%) biopsies; MF-2, in 21 (26.25%) biopsies and; MF-3, was identified in 3 (3.75%) biopsies. Silver impregnation technique was used for demonstration of reticulin in both studies while Masson trichrome stain and Van Gieson trichrome stain was used for demonstration of collagen in the previous and our present study respectively. More than one biopsy sample was taken from every patient during the course of the disease in the previous study while our present study was performed on single biopsy specimen from each patient taken at the time of diagnosis. The grading system used in the two studies was also different. Difference in the study design and grading system used were the main factor behind the difference in the results observed in these two studies.

In a study conducted at Armed Forces Institute of Pathology (AFIP) on trephine biopsies from 160 patients with various haematological disorders concluded that 94 (59%) patients had some degree of bone marrow fibrosis. If we calculate the percentage of bone marrow fibrosis in haematological malignancies from this study we will find out that out of 101 patients with haematological malignancies included in this study 93 (92%) patient had bone marrow fibrosis of various grade.35.48% have grade 1 fibrosis,27.95% have grade 2 ,24.73% grade 3 and 11.82% have grade 4 fibrosis The percentage of secondary fibrosis was 92.47% and primary fibrosis was 7.53%[20]. According to our present study on 80 patients with malignant disorders affecting bone marrow 64 (80%) patients had bone marrow fibrosis of varying grades.20% of patients had MF-0 (normal), 50% patients had MF-1, 26.25% percent of patients had MF-2, while 3.75% of patients had MF-3,while the percentage of secondary fibrosis was 97% and of primary fibrosis 3% according to our present study. If we compare these two studies we will find out that both studies were done on consecutive samples, taken from both males and females irrespective of age. Van Gieson stain was used for demonstration of collagen in both studies. Both studies used silver impregnation technique for demonstration of reticulin although Gomori reticulin stain was used for demonstration of reticulin in the previous study while Gordon and Sweet method was used for the demonstration of reticulin in our present study. The difference in the percentage of fibrosis that is 92% in the previous study and 80% in our present study is due to the difference in the grading system used in these two studies. In the previous study grading of reticulin was done on 0-4 scale according to the new proposed grading system, while European consensus on grading of bone marrow fibrosis was used in our present study, and reticulin content of bone marrow was graded on 0-3 scale. The slight high percentage of primary fibrosis 7.5% in a study done in AFIP as compared to 3% in our

own present study among the patients of malignant disorders affecting bone marrow was due the reason that patients from all over Pakistan, with various haematological disorders are referred to AFIP for treatment, so the number of PMF patients referred to this centre and included in the study were also high 7 as compared to 3 included in our present study.

CONCLUSION

1. 64(80%) of 80 patients with various malignant disorders affecting bone marrow had some degree of bone marrow fibrosis.
2. Bone marrow fibrosis was seen in seventy four percent of males and ninety percent of females and its maximum percentage (94.7%) was seen in patients between the age of 31-45 years.
3. Grade-1 fibrosis (MF-1) was the most common, seen in 50% of patients followed by grade-2 (MF-2) seen in 26.25% and grade-3 (MF- 3) was seen only in 3.75 % of patients.
4. Secondary bone marrow fibrosis was present in 62 (97%) of 64 cases whereas primary bone marrow fibrosis was seen in 2 (3%) of 64 cases.

Author's Contribution:

Concept & Design of Study:	Maliha Asif
Drafting:	Sadia Taj, Sabeen Fatima
Data Analysis:	Naseem Akhtar, Yasmeen Batool
Revisiting Critically:	Maliha Asif, Sadia Taj
Final Approval of version:	Maliha Asif

Conflict of Interest: The study has no conflict of interest to declare by any author.

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