Original Article

Comparison of Hematoxylin and Eosin Stain with Reticulin and Van Gieson Stain in Confirming Presence, Amount and Type of Bone Marrow Fibrosis

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ABSTRACT

Objective: Comparison of Haematoxylin and Eosin stain, with Reticulin and Van Gieson stain in confirming presence, amount and type of bone marrow fibrosis.

Study Design: Descriptive / cross sectional study

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

Materials and Methods: Bone marrow trephine biopsies of eighty consecutive patients diagnosed with malignant disorders affecting bone marrow were taken, sections were made and stained with Haematoxylin and Eosin, Reticulin and Van Gieson trichrome stain. Identification and grading of bone marrow was done using European consensus 2005 (EC 2005). Types and grades of fibrosis were reported by using frequency and percentages. Data was entered and analyzed on SPSS 20.

Results: In a total of 80 patient studied, 47 (58.75%) patients showed bone marrow fibrosis on Haematoxylin and Eosin stain, while on Silver/Reticulin stain plus Van Gieson stain, 64(80%) of patients were positive for bone marrow fibrosis. If we grade bone marrow fibrosis on basis of Hematoxylin and Eosin stain ,33 (41.25%) patients had no fibrosis, 25 (31.25%) patients had mild fibrosis, 16(20%) patients had moderate fibrosis, and 6(7.5%) had severe fibrosis. While If we grade bone marrow fibrosis on basis of Silver/Reticulin plus 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.

Conclusion: Patients with various malignant disorders affecting bone marrow have various grades of bone marrow fibrosis, which can be identified more accurately by using special histochemical stains. Special histochemical stain like Reticulin stain plus Van Gieson stain identified 17 (21.25%) more patients having bone marrow fibrosis which were un-identified on Haematoxylin and Eosin stain.

Key Words: Hematoxylin and Eosin stain, Silver/Reticulin stain, Van Gieson Trichrome stain

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INTRODUCTION

Cancer is the third leading cause of death in developing economies, accounting for 9.5% of all deaths. It is also the second most common cause of death in the developed world next to cardiovascular disease with a mortality rate of 21%.[1] Haematological malignancies are one of the most frequent malignancies among males in Pakistan. Incidence of leukaemia is as high as 23.6 % followed by non-Hodgkin lymphoma (NHL) 15.1%[2].

Bone marrow fibrosis is a common morphological finding seen in patients with various haematological disorders especially haematological cancers.[3]

collagen may have differing relationships to disease has stimulated recent discussions on the importance of distinguishing between increases in these two types of fibres in bone marrow biopsies (Bain et al, 2001; Thiele et al, 2005). Stains that identify reticulin and collagen are routinely available and the use of both stains to evaluate a single biopsy specimen can provide a more complete picture of the amount and nature of bone marrow stromal fibres than performing either one of these stains alone Bone marrow fibrosis (BMF) is a step-wise evolution from physiologically normal state

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to focal deposition, leading to diffuse increase in reticulin, which finally progresses to collagen fibrosis with or without osteosclerosis[4]. Abnormal cytokines released from platelets and megakaryocytes seems to be essential but not sufficient for fibrosis to occur[5]

Increased in reticulin fibrosis is associated with many benign and malignant conditions while increased in collagen fibrosis is particularly prominent in metastatic cancers and in late stages of severe myeloproliferative disorders[5]

The clinical implications of increased collagen (collagen fibrosis) is quite different from increased in reticulin (reticulin fibrosis) [6]. Collagen fibrosis is not reversible while reticulin fibrosis is often reversible in conditions which are responsive to the treatment [7,8]

Accurate assessment of the bone marrow fiber content is clinically important for staging of MDS [9], MPN[10] and lymphoproliferative neoplasms[11].

Convincing evidence had been produced by several groups that linked degree of BM fibrosis to overall survival in patients with MPN[12] however, further studies are required to correlate changes in BM fibrosis with molecular markers of disease evolution[13].

Fibrous tissue of the bone marrow is not well appreciated on Hematoxylin and Eosin stain and require special stains. Van Gieson trichrome stain, Masson's trichrome stain or Mallory's trichrome stain is used to identify collagen[14], while reticulin can be stained by Gomori method or Gordon and Sweets method using silver impregnation technique[14,15].

Original staining method used by Gomori to stain reticulin, also enabled assessment of collagen fibers by staining them yellow, while automatic staining procedures with commercially available kits used now a days fails to stain collagen; [16] however, thick reticulin fibers usually represent bundles of collagen[17]. In practice, collagen is almost never revealed unless there is a marked increase in reticulin[18,19]. Therefore, to detect collagen fibers an additional histological stain such Mallory's trichrome, Van Gieson or Masson's trichrome stain has to be used[19].

MATERIALS AND METHODS

This cross sectional descriptive study was carried out in Histopathology and Haematology department of SZH, Lahore; during the period from January 2013 to December 2014.

First 80 patients irrespective of their sex and age 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. Patients with history of chemotherapy or radiotherapy and those on thrombopoietin analogues (TPO) were not included in the study

Three sections were made from each trephine block and stained with Haematoxylin and Eosin, Reticulin/Silver stain and Van Gieson stain.

Severity and percentage of bone marrow fibrosis was done on H and E stain Identification and grading of bone marrow fibrosis was also done on Reticulin/Silver stain and Van Gieson stain using European consensus 2005 (EC 2005).

All data will be entered and analyzed by using SPSS 20 (statistical package for social sciences).Types and grades of fibrosis will be reported by using frequency and percentages

Positivity on Van Gieson and Silver stain is compared with positivity on H and E stain and statistical significance will be calculated using Kappa statistics.

RESULTS

In a total of 80 patient studied, 47 (58.75%) patients showed bone marrow fibrosis when diagnosis was made on the basis of Hematoxylin and Eosin stain (table 1). On Hematoxylin and Eosin stain 100% of patients with Hodgkin lymphoma, Hairy cell leukaemia and fibrotic phase of MF show BMF. 83.3% of patients with AML and CML are positive for BMF. The percentage of positivity in patients with Multiple myeloma and NHL was 25% and 43.8% respectively, while 50% of patients with cellular phase of MF were positive for BMF. Results were positive in 66.7% and 60% of patients with MDS and CLL respectively, while no patient of ET was positive for bone marrow fibrosis on Hematoxylin and Eosin stain (table- 1, fig 1)

On Reticulin plus Van Gieson stain 64 (80%) of patients showed bone marrow fibrosis (table 2). Percentage of positivity in patients suffering from AML, MDS, Hairy cell leukaemia, Hodgkin lymphoma, and fibrotic phase of Primary Myelofibrosis was 100%. In CLL the percentage of bone marrow fibrosis was 80% while 83.3% of patients with CML showed bone marrow fibrosis on basis of this stain. In Multiple myeloma, bone marrow metastasis and NHL 75% patients showed bone marrow fibrosis. In ALL the percentage of fibrosis was 60%, while 50% of patients with cellular phase of MF are positive for BMF. Two patients with ET included in the study were negative for BMF on basis of Reticulin and Van Gieson stain (table 2, fig 2)

If we grade bone marrow fibrosis on basis of H and E stain, 33 (41.25%) patients had no fibrosis, 25 (31.25%) patients had mild fibrosis, 16(20%) patients had moderate fibrosis, and 6(7.5%) had severe fibrosis(table 1,fig 3)

While on Reticulin and Van-Gieson stain 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 2,fig3)

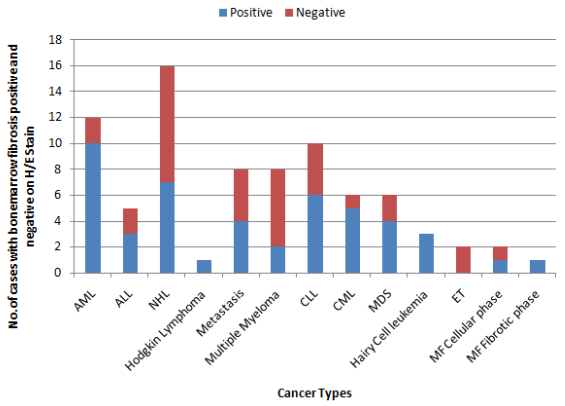

Figure No.1: Bone marrow fibrosis diagnosed on H and E stain in 80 cases of malignant disorders affecting bone marrow

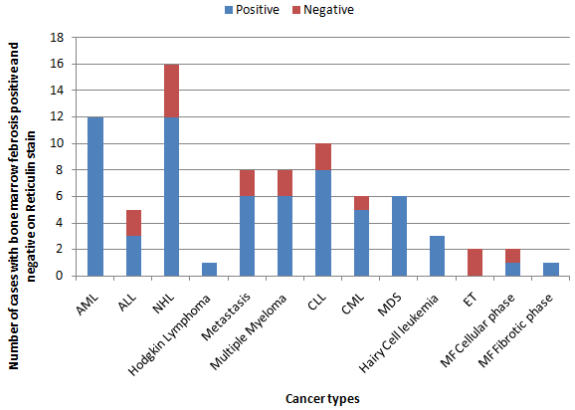

Figure No.2: Bone marrow fibrosis diagnosed on Reticulin +Van Gieson stain in 80 cases of malignant disorders affecting bone marrow

Table No.1: Severity and percentage of bone marrow fibrosis in 80 cases of malignant disorders affecting bone marrow on H and E stain

Malignant Disorders affecting B.M	No of cases	Cases of BMF	% of BMF	Severity of Bone Marrow Fibrosis			
				Absent	Mild	Moderate	Severe
AML	12	10	83.33	2	8	2	0
ALL	5	3	60.00	2	1	2	0
NHL	16	7	43.75	9	4	1	2
Hodgkin lymphoma	1	1	100.00	0	0	1	0
Metastasis	8	4	50.00	4	2	0	2
Multiple Myeloma	8	2	25.00	6	0	2	0
CLL	10	6	60.00	4	2	4	0
CML	6	5	83.33	1	2	3	0
MDS	6	4	66.60	2	3	1	0
Hairy cell leukemia	3	3	100.0	0	2	0	1
ET	2	0	0.00	2	0	0	0
MF : Cellular Phase Fibrotic Phase	2	1	50.00	1	1	0	0
	1	1	100.0	0	0	0	1
Total	80	47	58.75	33	25	16	6

Table No.2: Percentage and grades of bone marrow fibrosis according to European consensus on grading of bone marrow fibrosis using Reticulin and Van Gieson stain

Malignant Disorders affecting B.M	No of cases	Cases of BMF	% of BMF	Grades of Bone Marrow Fibrosis			
				MF-0	MF-1	MF-2	MF-3
AML	12	12	100.00	0	10	2	0
ALL	5	3	60.00	2	1	2	0
NHL	16	12	75,00	4	10	2	0
Hodgkin lymphoma	1	1	100.00	0	0	1	0
Metastasis	8	6	75.00	2	4	0	2
Multiple Myeloma	8	6	75.00	2	2	4	0
CLL	10	8	80.00	2	2	6	0
CML	6	5	83.33	1	2	3	0
MDS	6	6	100.00	0	6	0	0
Hairy cell leukemia	3	3	100.00	0	2	1	0
ET	2	0	0.00	2	0	0	0
MF : Cellular Phase Fibrotic Phase	2	1	50.00	1	1	0	0
	1	1	100.00	0	0	0	1
Total	80	64	80.00	16	40	21	3

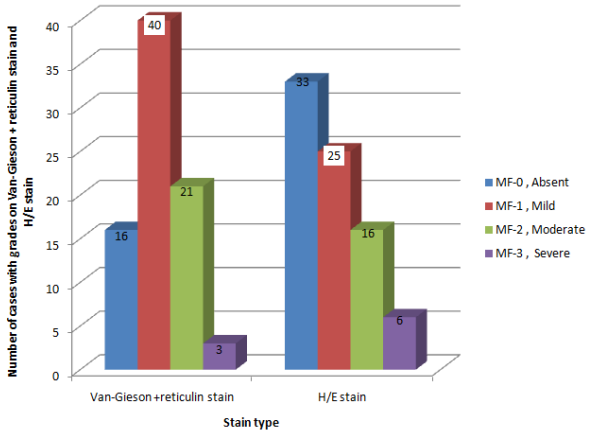

Figure No.3: Relation and comparison of two grading systems done on basis of both Reticulin+Van Gieson stain (EC-2005) and H/E stain alone in 80 cases of malignant disorders affecting bone marrow

Comparison of Hematoxylin and Eosin stain with Reticulin plus Van Gieson stain: Hematoxylin and Eosin identifies only 47 (58.75%) cases of BMF while Reticulin and Van Gieson stain identifies 64 (80.0%) cases that is 17 more positive cases of BMF (table 3). The 47 cases which were positive on Hematoxylin and Eosin stain were also positive on Reticulin+Van Gieson stain.16 cases were negative on and Reticulin+Van Gieson stain (table 3)

So the agreement between H and E and Reticulin + Van Gieson stains was 63/80 (78.8%) with Kappa statistic of 0.525 and a p-value <0.001 (table 3, table 4).

Table No..3: Comparison of results of H and E stain with Reticulin + Van Gieson stains together on trephine biopsies from 80 patients with malignant disorders affecting bone marrow

H/E stain	Reticulin and Van- Gieson stain together					
	Positive		Negative		Total	
	N	%	N	%	N	%
Positive	47	73.4	0	0.0	47	58.8
Negative	17	26.6	16	100.0	33	41.2
Total	64	100.0	16	100.0	80	100.0

Table No.4: Association between H and E stain and Reticulin+Van Gieson stains by using Kappa test in 80 cases of malignant disorders affecting bone marrow

Symmetric Measures					
		Value	Asymp. Std. Error[a]	Approx. T[b]	P-value
Measure of Agreement	Kappa	0.525	0.090	5.337	< 0.001
N of Valid Cases		80			
a. Not assuming the null hypothesis.					
b. Using the asymptotic standard error assuming the null hypothesis.					

DISCUSSION

For detection and grading of bone marrow fibrosis the use of Silver/Reticulin stain and Trichrome stain was much more effective when compared with H&E stain. We noticed that 41.25% of patients had no marrow fibrosis when their trephine biopsy sections were stained with H&E stain, while this percentage reduced to 20% when Reticulin and Van Gieson stain were used. Furthermore the H&E stain identified fibrosis without determining its exact degree, which is important for the definite diagnosis and evaluation of effectiveness of therapy and transformations in Myeloproliferative disorders (MPD) .

A study, very similar to our present study was conducted in Baghdad. Stored paraffin embedded trephine blocks from 132 patients with CMPD were taken Both males and females irrespective of age were included in the study. The grading system used, similar to our present study was European consensus on grading of bone marrow fibrosis. Trichrome stain and Reticulin stain was used for the demonstration of collagen and reticulin fibers respectively[20]. Although Masson's trichrome stain and Gomori reticulin stain was used for demonstration of collagen and reticulin respectively instead of Van Gieson trichrome stain and Gordon and Sweet reticulin stain used in our present study for demonstration of collagen and reticulin fibers respectively. The results revealed that when H and E stain was used 90 (68.18%) patients were negative for BMF but when Reticulin stain plus Van Gieson stain was applied on trephine biopsy sections, only 39 (29.5%) patients were negative for BMF. According to our own present study 33(41.25%) patients did not show BMF on H and E stain while only 16(20%) patients did not have BMF (MF-0) when Reticulin plus Van Gieson stain was used

Baghdad study showed that 15(11.36%) patients had mild fibrosis,15(11.36%) patients had moderate fibrosis and 12 (9%) patients diagnosed to have severe BMF on H and E stain, while according to our own present study 33 (41.25%) patients had no fibrosis, 25 (31.25%) patients had mild fibrosis, 16(20%) patients had moderate fibrosis, and 6(7.5%) had severe fibrosis

While on Reticulin plus Van-Gieson stain according to Baghdad study 46 (34.84%) patients had MF-1, 42(31.81%) patients had MF-2 and 5(3.78%) patient had MF-3. While according to our own study, 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 on Van Gieson plus Reticulin stain.

The difference in the results between these two studies is most likely due to the difference in study population which was CMPD patients in Baghdad study and patients with malignant disorders affecting bone marrow (which also includes CMPD patients) in our present study. Percentage of severe BMF is more on H&E stain than on Reticulin + Van Gieson stain in both these studies.

CONCLUSION

Patients with metastatic cancers and various haematological cancers have various grades of bone marrow fibrosis which may be negative on Haematoxylin and Eosin stain used during the initial diagnosis of the disease. The use of Silver/reticulin plus Van Gieson stain to identify reticulin and collagen respectively and use of proper grading system is essential to give more precise status of bone marrow fiber content. This is very important in distinguishing between various myeloproliferative neoplasms, the transition of one myeloproliferative condition to another, and in making correct therapeutic choices.

Author's Contribution:

Concept & Design of Study:	Maliha Asif
Drafting:	Sabeen Fatima, Naseem Akhtar
Data Analysis:	Ghazala Tabassum, Uzma Ishaq
Revisiting Critically:	Maliha Asif, Sabeen Fatima
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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