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Bone Marrow Fibrosis (BMF): A New Proposal for Grading System

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Semi-quantitative evaluation of Bone Marrow Fibrosis (BMF) in trephine biopsies is important in laboratory workup of many haematological lesions. Various grading systems for the study of patterns and extent of Fibrous Tissue Content (FTC) have been used previously. In this paper, a new grading system for FTC has been proposed and studied in trephine biopsies of 160 cases of various haematological lesions. BMF was observed in 59% of cases. The grading system applied in this study was found quite comprehensive, and it was easy to segregate BMF into various grades with the help of this system. Grade 1 fibrosis was the commonest (35.1%), followed by Grade 2 (28.7%), Grade 3 (24.5%) and Grade 4 (11.5%), respectively. Van Giesen's trichrome stain was found useful for demonstration of collagen in Grade 4 fibrosis.

Key words: Bone Marrow Fibrosis; Grading System; Fibrous Tissue Content.

Introduction

Bone Marrow Fibrosis (BMF), a common morphological finding in trephine biopsies, is observed with variable grades of severity in various lesions, including primary as well as secondary myelofibrosis.[1] The study of pattern and extent of bone marrow Fibrous Tissue Content (FTC) is important not only in diagnosis, but also in evaluation of prognosis.[2]

Many workers have contributed to the semi-quantitative and quantitative evaluation of reactive FTC in the bone marrow. Burston and Pinniger classified FTC of bone marrow into two patterns:[3]

1) That essentially found in normal marrow, and
2) Coarse network of argyrophil fibres which are thicker than those found in the normal reticulin network.

Kundel et al graded reticulin fibrosis in their 40 cases of ALL as follows:[4]

Trace (N): When only an occasional fibre was found.
+1 : Slight increase in reticulin.
+2 : Moderate increase in reticulin.
+3 : Marked increase in reticulin.
+4 : Same as +3, with presence of collagen.

Amaki et al classified argyrophil fibres in trephine biopsies of their 157 cases of haematological disorders into 4 types.[5]

Type 1: Represents normal bone marrow without any increase of argyrophil fibres.

Type 2: Slight increase in argyrophil fibres in the bone marrow around the trabeculae and sinuses.

Type 3: Moderate increase of argyrophil fibres in the marrow in the form of abundant fibre network.

Type 4: Marked increase of argyrophil fibres with bundles of thick fibres.

Bauermeister et al examined 121 bone marrow specimens for quantitation of marrow reticulin.[6] They adopted a grading system, modified from Kundel et al, as follows:

0: No reticulin fibres demonstrable.
N: Occasional fine individual fibres only.
+1: Occasional fine individual fibres plus foci of fine fibre work.
+2: Fine fibre work throughout most of the sections, no coarse fibres demonstrable.
+3: Diffuse fibre network with scattered thick

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coarse fibres but no true collagen.

+4: Diffuse often coarse fibre network with areas of collagenisation.

Manorahan et al[7] in their study on 44 patients of acute leukaemia, modified the grading system as follows:

N: Occasional fine and individual fibres, or occasional coarse individual fibres with foci of perivascular fibre network or reticulin associated with benign lymphoid follicles.

+1: Fine fibre network (with occasional coarse fibres) or a local increase in reticulin away from vessels and lymphoid follicles.

+2: Diffuse fibre network with an increase in scattered coarse fibres.

+3: Diffuse often coarse fibre network with no evidence of collagenisation.

+4: Diffuse coarse network with areas of collagenisatin; trichrome stain positive.

Clough et al,[8] graded histological changes in 44 cases of CGL as follows:

Grade 1: Highly cellular marrow. No reticulin increase.

Grade 2: Similar, but with a focal increase in reticulin.

Grade 3: Similar, but a diffuse increase in reticulin.

Grade 4: Cellularity diminished with persistent prominence of megakaryocytes. There was increase in reticulin and the collagen was demonstrable.

Grade 5a: Histologically not distinguishable from IMF. There was diffuse increase in collagen and distortion of architecture.

Grade 5b: Similar to 5a but new bone formation was also observed.

Lazzarino et al[9] studied BM trephine biopsies in 139 patients of CGL and graded the FTC as follows:

Grade 0: No reticulin increase.

Grade 1: Focal minimal fibrosis.

Grade 2: Multifocal or diffuse non-confluent fibrosis.

Grade 3: Marked, diffuse fibrosis with absence (3a) or presence (3b) or collagen.

Dekmezian et al[10] studied the relevance of reticulin fibrosis at diagnosis in 138 patients of chronic myeloid leukaemia and classified the results into four grades.

Grade 1: Either minimal reticulin or reticulin fibres occupying less than 25% of the areas showing no haemorrhage or artefactual depletion of cells.

Grade 2: Reticulin fibres seen in 25-50% of areas examined.

Grade 3: Reticulin fibres seen in 50-75% of areas examined.

Grade 4: Greater than 75% of marrow containing reticulin.

The aim of the present study was to study the pattern of bone marrow FTC in various haematological disorders, and to give recommendations for grading of BMF, after comparison with published literature.

Materials and Methods

All the cases of BMF numbering 160, studied at Armed Forces Institute of Pathology, Rawalpindi in a period extending over a year were included in this study. The patients were from all age groups and both sexes. They were referred from various hospitals in Rawalpindi & Islamabad.

All the patients were interviewed and information was recorded in a proforma. Age, sex, and the main clinical manifestations, such as the rise of temperature, general weakness, loss of weight and appetite, haemorrhagic manifestations and symptoms due to infections were particularly noted. A thorough systemic examination was carried out with particular attention to organomegaly and lymphadenopathy.

Complete blood picture, bone marrow aspiration and trephine biopsies were performed in all the cases. Three bone marrow smears were stained with Leishman's stain. Where required (acute and chronic leukaemias) special stains, i.e., Myeloperoxidase, Periodic Adid-Schiff, Acid Phosphatase, Sudan Black-B, Naphthol AS-D chloroacetate, Naphthol AS-D Acetate Esterase with and without NaF and Alkaline Phosphatase were also performed.

FTC of bone marrow was evaluated semiquantitatively from bone marrow trephine biopsies. These biopsies were subjected to H&E, Gomori's reticulin and Van Giesen's stains for routine, reticulin and collagen demonstration respectively.

In the present study, the following grading system was adopted. for semi-quantitative evaluation of FTC in myelofibrosis:

No Fibrosis (Normal)	Small amount of fine argyrophilic fibres seen around blood vessels and alongside the bony trabeculae. Rest of

the marrow tissue shows occasional fine argyrophilic fibres. No collagen demonstrated.

Grade 1 Focal network of fine argyrophilic fibres away from the normal sites. Total area involved is upto 25%. No collagen demonstrated.

Grade 2 Diffuse network of fine argyrophilic fibres involving more than 25% but upto 50% of marrow space. Occasional coarse fibres are also present. No collagen demonstrated.

Grade 3 Diffuse network of both fine and coarse argyrophilic fibres involving more than 50% but upto 75% of total marrow space. No collagen demonstrated.

Grade 4 Diffuse network of mostly thick argyrophilic fibres involving more than 75% of marrow space. Collagenisation is demonstrable.

Results

In a total of 160 cases studied, 94 (59%) showed fibrosis of varying grades, while 66 cases (41%)
showed no bone marrow fibrosis. The majority, i.e. 87 (92%) of the cases were of secondary myelofibrosis. Primary myelofibrosis was diagnosed only in 7 (7.5%) cases.

The patients showing BMF belonged to all age groups with a median age of 43.5 years and both sexes (77 males & 17 females), with a 4.5:1 male: female ratio.

Major clinical features recorded at the time of presentation have been shown in Table 1.

Table 1: Clinical Features at Presentation.

Clinical Features	No. of cases	Percentage
General weakness	33	35.12
Fever	24	25.53
Abdominal distension	19	20.21
Pain and aches	18	19.15
Loss of weight	10	10.64
Pain abdomen	09	09.57
Bleeding	07	07.45
Breathlessness	07	07.45
Pallor	68	7.2
Splenomegaly	59	63
Hepatomegaly	53	57
Lymphadenopathy	44	47

Table 2 gives a breakup of various disorders associated with BMF, alongwith the grades of fibrosis in each group. The FTC of the biopsied specimens was evaluated according to the proposed grading system (Figs. 1-4).

All grade 4 and a few grade 3 cases in these haematological disorders had a marked degree of fibroblastic proliferation. The quality of FTC also varied with increase in quantity and thicker fibres were seen with increase in the number of fibres. In some of the cases, quantity of reticulin fibres also certainly displayed a direct relationship with the cellularity of the areas under view, i.e. more cellular areas had more fibres.

Discussion

Marrow Fibrosis is a proliferation of fibroblastic cells and an increase in marrow fibrous tissue content. It accompanies many haematological and non-haematological disorders which may be both benign and malignant.[11]

Silver stains are important in establishing the pattern and amount of F.T.C in trephine biopsy.[12] All clonal haematological diseases may have increased marrow reticulin fibres, but only infrequently have collagen fibrosis.[13, 14]

In this study, FTC in the biopsied bone marrows was observed in 160 patients with various haematological disorders. Reticulin was the connective tissue fibre selected for semiquantitative evaluation, since it has been shown that the demonstration of an increase in the reticulin content of bone marrow is of value in a wide range of haematological disorders.[3] The quality and relationship of reticulin fibres with cellularity, fibrablastic and megakaryocytic proliferation was also studied in these biopsies.

It has been previously emphasized that silver

impregnation (which imparts a black colour to reticulin) and tirchrome stain (which gives a bluish green colour to collagen) are suitable for semi-quantitative evaluation of FTC.

Many workers have proposed grading systems for the semiquantitative evaluation of FTC. They used different stains for the demonstration of reticulin and collagen. Some of these workers have dealt only with a particular disorder e.g., chronic granulocytic leukaemia[8-10] and acute lymphoblastic leukaemia.[4] While others have studied acute leukaemias (both myeloid and lymphoblastic)[2] and haematological disorders in general.[3, 5, 6] The description of these grading systems is so heterogenous that the same disorder may be evaluated in a variety of ways. None of these systems takes into account simultaneously some important aspects like quality, quantity, distribution and the area involved by the fibrous tissue. Moreover the description in some grading systems is inadequate[3] or is lengthy

Table 2: Grading of Fibrous Tissue Content (FTC) and its correlation with associated disorders.

S #	Haematological Disorders	Cases under study	Fibrosis Grades					Cases showing BMF	% of BMF
			No Fibrosis (Normal)	Grade 1	Grade 2	Grade 3	Grade 4		
1.	Erythroid Hyperplasia	30	30					Nil	Nil
2.	Myeloid Hyperplasia	12	12					Nil	Nil
3.	Aplastic Anaemia	16	16					Nil	Nil
4.	Acute Lymphoblastic Leukaemia	18	3	5	3	6	1	15	83.33
5.	Acute Myeloid Leukaemia	14	4	3	5	2		10	71.42
6.	Chronic Lymphatic Leukaemia	7		3	3	1		7	100.00
7.	Chronic Granulocytic Leukaemia	13		2	4	6	1	13	100.00
8.	Hairy Cell Leukaemia	4			2	2		4	100.00
9.	Non Hodgkin Lymphomia	20		10	7	3		20	100.00
10.	Hodgkin's Disease	4		3			1	4	100.00
11.	Multiple Myeloma	4		3	1			4	100.00
12.	Myeloproliferative Disorders	4	1	1	1	1		3	75.00
13.	Polycythemia Vera	2		1		1		2	100.00
14.	Essential Thrombocythemia	1		1				1	100.00
15.	Myelodysplastic Synordome	3		1			2	3	100.00
16.	Pure Red Cell Aplasia	1			1			1	100.00
17.	Idiopathic Myelofibrosis	7				1	6	7	100.00

Total:	160	66	33	27	23	11	94

in others.[2, 8] This poses a descriptive problem in the correct evaluation and a uniform staging of FTC in heterogenous haematological disorders. Anyhow, demonstration of collagen with the help of trichrome stains in the extreme degrees of fibrosis is met across within all these grading systems.

In many previous studies, the results of grading of bone marrow fibrosis in case of erythroid hyperplasia, myeloid hyperplasia and aplastic anaemia and various morphologic types of leukaemias have been dissimilar. When looked into critically, this non-conformity was significantly contributed by dissimilar grading systems used in various studies. This discrepancy was especially more prominent when the fibrosis was mild to moderate.

The proposed system designed for the evaluation of FTC in these 160 cases is comprehensive and covers all such pitfalls. Thus the FTC in different haematological disorders can be evaluated in depth and more clearly. The first degree is of 'No fibrosis' (Normal) and it was adopted after a thorough study of 'Normal' described by many authors[3-6] and adopted by other.[2]

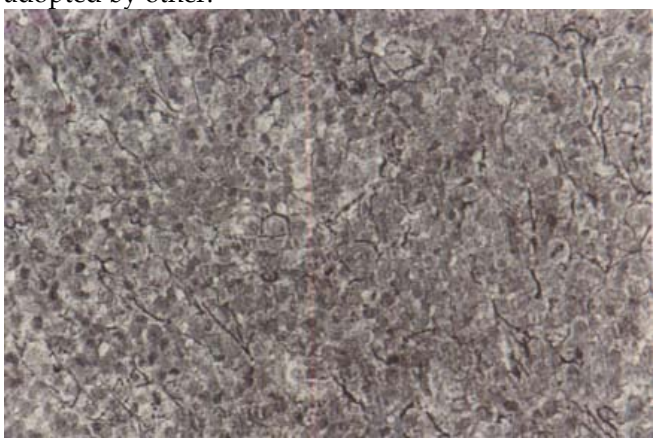

Fig. 1: Microscopic Appearance of Grade 1 Fibrosis

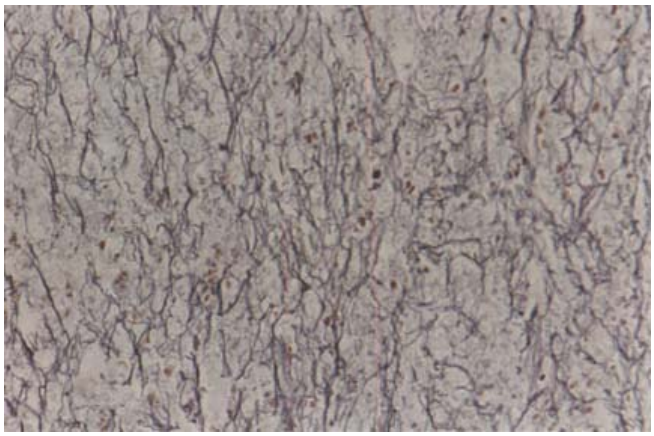

Fig. 3: Microscopic Appearance of Grade 3 Fibrosis

Fig. 2: Microscopic Appearance of Grade 2 Fibrosis

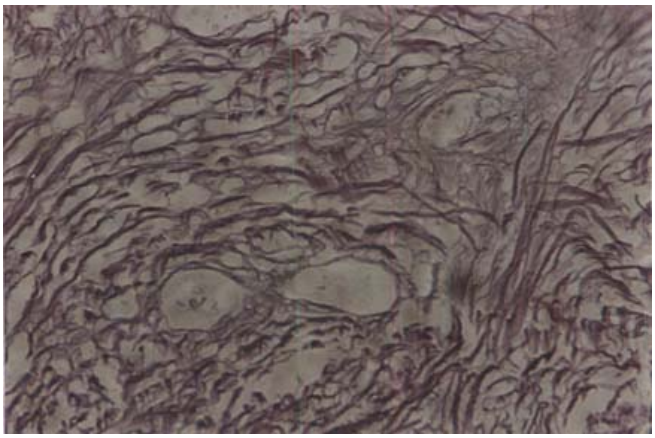

Fig. 4: Microscopic Appearance of

Grade 4 Fibrosis

The presence of reticulin fibres more than normal in quantity, thicker in quality and away from the normal sites was termed as 'Fibrosis'. The fibrosis was sub-divided into four grades on the basis of the same i.e. quantity, quality and site. Any demonstrable collagen was termed as grade 4 fibrosis.

It is presumed that since the proposed grading system takes into account both 'quality' as well as 'quantity' of FTC in a more logical and reproducible manner, therefore, if adopted widely, this system will bring a greater uniformity in assessment of FTC in trephine biopsies performed in all types of haematological and non-haematological lesions.

Conclusion

It was concluded that

1. Fifty nine percent of haematological disorders were accompanied by some degree of marrow fibrosis.
2. Secondary BM fibrosis was present in 87 (92%) of 94 cases.
3. Primary (idiopathic) myelofibrosis is relatively uncommon condition i.e. 7 in overall 160 cases (4.4%) and its incidence among the 94 cases showing fibrosis is 7.5%, i.e., 7 in 94 cases.
4. Grading system adopted for this study is quite comprehensive and it is easier to segregate BMF into various grades with the help of this system.
5. Grade-1 fibrosis is most common (35%) with a gradual decrease in percentage incidence with the increase in degree of fibrosis i.e. grade-2 (28.7%), grade 3 (24.5%) and grade 4 fibrosis (11.7%).
6. Grade-4 fibrosis was most common in idiopathic myelofibrosis.
7. Van Gieson's trichrome stain is useful for the demonstration of collagen in grade-4 fibrosis but cases with collagen fibrosis also give a reddish tinge even with Gomori's reticulin stain.

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