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"Reticulin Pattern in Adult Bone Marrow in Haematological Malignancies"- A Hospital Based Study"

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Abstract
Background:*: Bone marrow examination is necessary for diagnosis and management of many haematological malignancies. For bone marrow examination two types of samples, bone marrow aspiration and bone marrow biopsy can be obtained in the laboratory. The amount of bone marrow reticulin staining Often exhibits correlation to disease prognosis,while the presence of type1 collagen,as detected by trichome staining ,is often associated with more severe disease and a poorer prognosis . The use of reticulin and trichome stain was effective when compared with the use of H&E stain alone as a laboratory diagnostic method for detection and grading of marrow fibrosis.*
Aims and objectives*: 1. To study cytomorphology in adult bone marrow haematological malignancies.2. To study reticulin pattern in adult bone marrow haematological malignancies.*
Methods*: A total of 31 patients who were clinically suspected as having different haematological malignancies underwent bone marrow aspiration & trephine biopsy study in the department of pathology in a tertiary care hospital . Using Romanowsky stain the cellular morphology of the haematopoetic cells was studied in details. Cell size,characteristics of cytoplasm and nuclear characteristics were studied in details in bone marrow smear and PBS.*
Results: *Out of these 31 cases of haematological malignancies cases, 11 cases of CM , 9 cases of AML ,7 cases of multiple myeloma, 2 cases of ALL, 1 case of CLL and 1 case of JACK-2 negative PMF were diagnosed. Out of the 31 patients, 10 cases(32.2%) shows normal or physiological BMF and remaining 21 cases(67.7%) shows presence of pathological BMF of different grades after examination of trephine biopsy slides, stained with H&E stain and also special stain (Gomorri's Silver impregnation stain & Masson's trichome stain) for detection of BMF and based on modified Bauermeister BMF grading system.*
Conclusion: *: Bone marrow fibrosis may be a hidden condition in different haematological malignancies. This may not be detected by use of routine H&E stain alone on initial diagnosis. The use of reticulin stain and trichome stain is essential for detection of bone marrow fibrosis along with routine H&E stain. The present study emphasis the role of bone marrow biopsy and reticulin/trichome staining in haematological malignancies.*
Keywords: *Bone marrow aspiration, bone marrow biopsy, bone marrow fibrosis H&E Stain, Reticulin stain, Masson trichome stain.*

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I. Introduction

Bone marrow examination is necessary for diagnosis and management of many haematological malignancies. For bone marrow examination two types of samples, bone marrow aspiration and bone marrow biopsy can be obtained in the laboratory. Structural fibrils constitute a physiological component of the bone marrow stromal micro environment and contributes to providing a connective tissue structure and support for haematopoetic progenitor cells. The most common fibres in bone marrow are reticulin and collagen type1. Reticulin is a normal component of the bone marrow stromatolites and can be detected in 73% to 81% of healthy subjects.

In the bone marrow biopsies, stromal structural fibres are detected by reticulin and trichome stains, along with routine H&E stains performed on bone marrow biopsy specimens in diagnostic laboratories.

Fibrosis is usually of reticulin type, as detected by reticulin stain, in the Earl stages but it may progress to collagen fibrosis that is detected by trichome stain usually in the late stage[1.]. Increased reticulin staining is associated with many benign and malignant conditions while increased trichome staining is particularly prominent in late stages of severe MPD or following Timor metastasis to the bone marrow. Among haematological malignancies ,several myeloid neoplasm, as defined by WHO classification are associated with an increase in bone marrow fibrosis,including MPD (primary myelofibrosis , melofibrosis secondary to

essential thrombocythemia, or polycythemia Vera, CML),MDS, refractory anaemia with ringsideroblast and acute leukaemia.

Recent evidences have shown that the amount of bone marrow reticulin staining Often exhibits correlation to disease prognosis,while the presence of type1 collagen,as detected by trichomestaining,is often associated with more severe disease and a poorer prognosis .

The amount of bone marrow reticulin shows little correlation with blood counts or severity of the underlying disease [2,3,4] while the presence and amount of collagen fibres show a stronger correlation with abnormal blood counts and severity of underlying disease[1, 7]. Moreover,in conditions that are responsive to treatment, reticulin fibrosis is often reversible, while collagen fibrosis is less likely to be so. The use of reticulin and trichome stain was effective when compared with the use of H&E stain alone as a laboratory diagnostic method for detection and grading of marrow fibrosis. 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 [1,5,6].

AIMS AND OBJECTIVES:

1. To study cytomorphology in adult bone marrow haematological malignancies.
2. To study reticulin pattern in adult bone marrow haematological malignancies.

II. Method And Materials

The study was conducted for a period of one year from October 2016 to September 2017 in a tertiary care hospital of Assam. The laboratory work was done in the department of Pathology . Fresh peripheral blood and bone marrow samples were collected from the patients in the period of study. Using Romanowsky stain the cellular morphology of the haematopoetic cells was studied in details. Cell size,characteristics of cytoplasm and nuclear characteristics were studied in details in bone marrow smear and PBS. Diagnosis of the type of haematological malignancy and pattern of reticulin fibrosis in the bone marrow was based on morphology including cytochemistry and special cytochemical stain for reticulin and collagen fibres and whenever possible other investigations were done. Informed consent was obtained from patient party for participation in the current evaluation after having been advised about the purpose and investigational nature of the study.

The study design has been approved by the Research Ethics committee of the Institution prior to its initiation.

The results were calculated in Microsoft office excel 2007.

INCLUSION CRITERIA :

1. All adult patients presenting with clinical features of haematological malignancies referred for bone marrow study.

EXCLUSION CRITERIA:

1. Patients presenting with haematological malignancies in paediatric age group.
2. Patients presenting with conditions other than haematological malignancies referred for bone marrow study.
3. Unwilling patients.

III. Result

The present study was conducted from October 2016 to September 2017. Fresh peripheral blood sample and bone marrow sample both aspiration and biopsies were used in all of the cases. A total of 31 patients who were clinically suspected as having different haematological malignancies underwent bone marrow aspiration & trephine biopsy study in the department of pathology in a tertiary care hospital . Using Romanowsky stain the cellular morphology of the haematopoetic cells was studied in details. Cell size,characteristics of cytoplasm and nuclear characteristics were studied in details in bone marrow smear and PBS.

Table1: Types of stain

No	Stain	Purpose
1	Haematoxylin& eosin(H&E)	For demonstration of cytological details marrow pattern, pathological fibrosis
2	Gomorri's stain(reticulin stain)	For demonstration of reticulinfibres,collagen type 3; black colour
3	Masson's trichome stain (Bouin stain)	For demonstration of collagen type 1 fibres; blue colour

Out of these 31 cases of haematological malignancies cases, 11 cases of CM , 9 cases of AML ,7 cases of multiple myeloma, 2 cases of ALL, 1 case of CLL and 1 case of JACK-2 negative PMF were diagnosed.(Table 2) ;(Fig1)

Table 2: Comparative features of BMF in different haematological malignancies

Sl no	Diagnosis	Total no of cases	Normal fibrosis		Pathological fibrosis	
		Number	No	percentage	No	Percentage
1	Chronic myeloid leukaemia	11	03	27.3%	08	72.7%
2	Acute myeloid leukaemia	09	04	44.5%	05	55.5%
3	Multiple myeloma	07	02	28.6%	05	71.4%
4	Acute lymphoblastic leukaemia	02	00	0	02	100%
5	Chronic lymphocytic leukaemia	01	01	100%	00	0
6	JAK-2 negative Primary myelofibrosis	01	00	0	01	100%

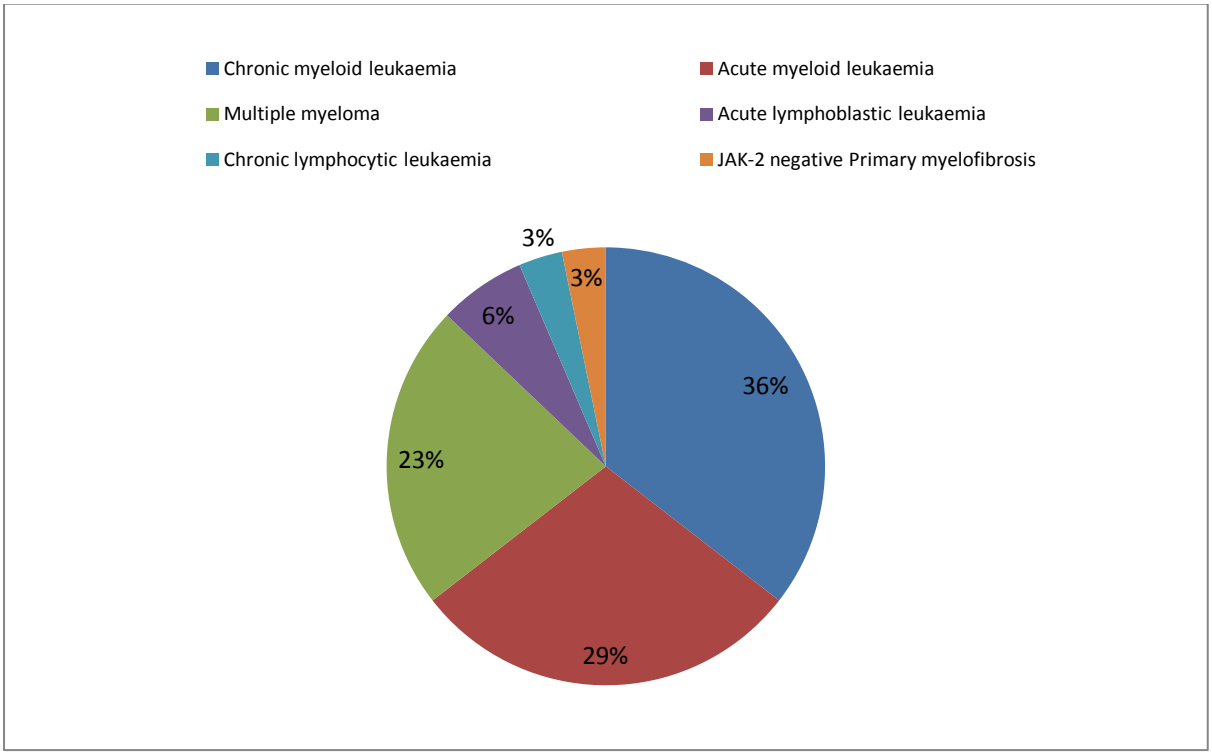

Fig1: Pie diagram depicting the no of haematological malignancies

Out of the 31 patients, 10 cases(32.2%) shows normal or physiological BMF and remaining 21 cases(67.7%) shows presence of pathological BMF of different grades after examination of trephine biopsy slides, stained with H&E stain and also special stain (Gomorri's Silver impregnation stain & Masson's trichome stain) for detection of BMF and based on modified Bauermeister BMF grading system.

Out of these 31 cases, 10 cases shows normal BMF, showing 4 cases of Grade 0 and 6 cases of grade1 BMF. Remaining 21 cases shows presence of Pathological BMF, showing 14 cases of grade 2 fibrosis, 5 cases of grade 3 fibrosis and 2 cases of grade 4 fibrosis.

Out of total 31 cases of haematological malignancies, 25 cases shows absence of BMF on trephine biopsy when only H&E stained slides were examined for the BMF (Fig2). But when along with H&E stained slides the Gomorri stain and Masson trichome stain slides were examined for the presence of BMF only 8 cases shows absence of BMF (Fig 3) with significant p value <0.05.(table 3)

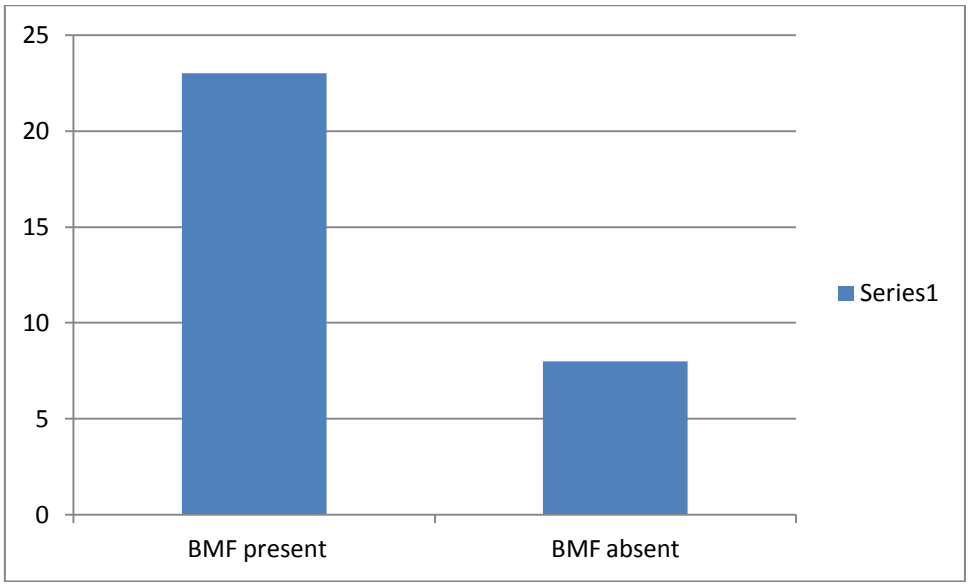

Fig2: Bar diagram showing bone marrow fibrosis on H&E stained slides

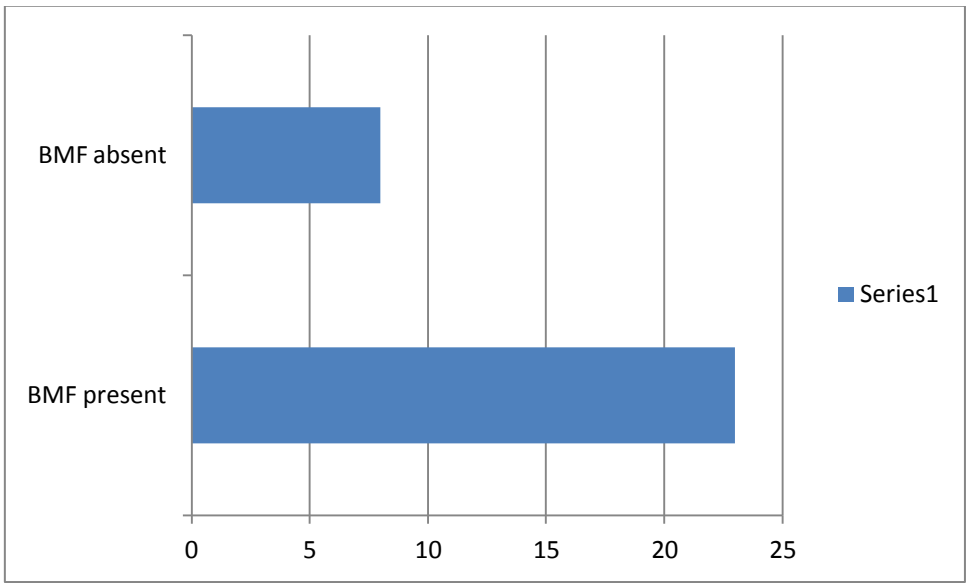

Fig3: Bar diagram showing BMF on special stained slides

Table 3: Comparison of BMF by H&E stain and special stain:

Sl no	Stain	Total no of cases	Fibrosis absent	
			Number	Percentage
1	H&E stain	31	25	80.6%
2	Special stain	31	08	25.0%

In CML 08 (72.7%) out of 11 cases shows presence of pathological BMF. Patients with CML in blast crisis shows pathological BMF of grade 4, while other patients shows BMF of grade 2 and grade 3.(table4)
In MM 5 (71.7%) out of 7 cases shows presence of pathological BMF . Out of these 5 patients , 3 cases show >50% plasma cells in the bone marrow aspirate and these 3 patients shows higher grade of BMF (grade 3), while other cases shows lower grade of BMF(grade 2).[table4]
In case of AML, 5 (55.5%) out of 9 cases shows presence of pathological BMF, all the 5 cases shows Grade 2 BMF. (table 4)
2 cases of ALL were diagnosed and both the cases shows presence of Pathological BMF, only one case of CLL was diagnosed and shows normal/ physiological BMF.
1 case of JACK 2 negative primary myelofibrosis was diagnosed and shows presence of pathological BMF (grade 4) [table4]. The patient required repeated blood transfusions and was having huge splenomegaly.

Table4: Different grades of BMF in different haematological malignancies based on Modified Bauermeister grading system:

Sl no	Diagnosis	Total no of cases (31)	Grade of bone marrow fibrosis				
			Normal		Pathological		
			0	I	II	III	IV
1	CML	11	1	2	5	2	1
2	AML	9	2	2	5	—	–
3	MM	7	1	1	2	3	—
4	ALL	2	–	—	2	—	—
5	CLL	1	—	1	—	—	—
6	JAK-2 negative primary myelofibrosis	1	—	—	—	—	1
Total			4	6	14	5	2

Fig4:Grade II BMF(Gomorri's stain).

Fig5: Grade III BMF (Gomorri stain)

Fig6:Grade IV BMF (Gomorri stain)

Fig7:Grade IV BMF (Masson trichome stain)

IV. Discussion:

Assessment of bone marrow fibrosis has been shown to have a clinical and prognostic implication in different haematological malignancies. Quantification of characteristic bone marrow biopsy features of fibre content is important to access the dynamics of disease processes with significant impact on risk stratification,survival patterns and specially therapy related changes.

The cellular morphology of the haematopoetic cells were studied in details using Romanowsky dyes. Cell size, characteristics of cytoplasm and nuclear characteristics were studied in details in bone marrow smear and PBS.

In bone marrow biopsies, stromal structural fibres are detected by reticulin stain, trichome stain in addition to routine H&E stain. Increased reticulin stain (reticulin fibrosis) is associated with many benign and malignant conditions while increased trichome staining (collagen fibrosis) is particularly prominent in late stages of severe myeloproliferative diseases or following tumour metastasis to bone marrow. Recent evidences have shown that the amount of bone marrow reticulin staining often exhibit correlation to disease severity, while presence of type 1 collagen as detected by trichome staining is often associated with more severe disease and poor prognosis [1,7,8].

Evidences from different studies suggest that the transforming growth factor B, factors elaborated by megakaryocytes, cytokines and growth factors released from other cells is also responsible for increased deposition of bone marrow stromal fibres. This suggests new avenues for investigation into pathogenesis of various disorders associated with increased bone marrow stromal fibres[8,9,10]

Ideally,bothtrichome stain and reticulin stain should be performed and both the type and amount of fibrosis should be described clearly using a defined grading scale. The original Bauermeister scheme-Bauermeister,(1971) used six different grades but has subsequently been simplified into a five grade system. The more recent Thiele (2005) scale includes only four categories [11]. The use of Gomorri stain and trichome stain was effective when compared with the use of H&E stain alone as a laboratory diagnostic method for detection and grading of marrow fibrosis in haematological malignancies.

Table5: Comparison of BMF with H&E stain and special stain.

SL no	Stain	Ali Khalil Al Khafaji et al 2011		Present study	
		Pathological fibrosis absent		Pathological fibrosis absent	
		Total no cases	Percentage	Total no cases	percentage
01	H&E	132	68.8%	31	80.6%
02	Special stain	132	31.2%	31	25.0%

BMF IN CML: In the present study, BMF was diagnosed in 72.7% of the cases which is in concordance with studies done by BirgeesMazharKazi et al; Ali Khalil I Ali et al.

Table6: BMF in CML in different studies

Study	no of cases	no of cases with pathological fibrosis	percentage
BirgeesMazaharKazi et al	13	13	100%
Ali Khalil I Ali et al	84	58	69%
Present study	11	8	72.1%

BMF in MM: In this study, it was found that there was a presence of increased bone marrow fibrosis in cases of MM and it was found that in cases of MM with increased percentage of plasma cells(>50%) having abnormal morphology and increased number of mitotic figures shows higher grade of bone marrow fibrosis on trephine biopsy. This is in concordance with other studies done by Richard Y Safari et al [12] and Bjorkholm Eva Rossman et al[13.]

The present study emphasise the role of bone marrow biopsy not only in establishing a diagnosis of multiple myeloma but also in providing prognostic information.

BMF IN AML: In this study, out of 31cases of haematological malignancies, 9 cases were diagnosed as AML and out of these 9 cases , 5 cases (55.5%) shows pathological fibrosis which is in concordance with studies done by Donald W Kunder et al[14]Anwarul Islam et al[15]

BMF IN ALL: In our study, two cases of ALL was diagnosed and both the cases showed pathological bone marrow fibrosis at diagnosis which is in concordance with studies done by BirgeesMazarKazi et al. In a study done by Donald W Kunder et al[14] out of 40 patients of ALL, 21 shows increased reticulin fibrosis in bone marrow. Remaining patients who did not have increased bone marrow fibrosis on admission later developed significant reticulinfibrosis,which was usually reversible after short duration of treatment.

BMF IN CLL: In this study, one case of CLL was diagnosed and the patient showed normal on bone marrow biopsy. In our study,as the number case is very less,so no definite conclusion could be made. Further studies with more number of cases to be made.

BMF IN PMF: In our study one case of JAK-2 negative Primary Myelofibrosis was diagnosed and trephine biopsy showed grade 4 fibrosis; which is in concordance with studies done by BirgeesMazarKazi et al, Paola Guglielmelli et al. Bone marrow fibrosis was also accompanied by increased number of megakaryocytes,supporting the theory that megakaryocytic proliferation and the interaction between megakaryocytes and bone marrow stroma have a role in bone marrow fibrosis. A higher grade of bone marrow reticulin content was also found to be an important prognostic parameter in a study of 50 PMF patients [16.]

LIMITATIONS:

1. The study is only one year and the number of cases are very less as compared to other studies.
2. Moreover post diagnosis follow up information of these patients are very limited.

V. Conclusion:

Bone marrow fibrosis may be a hidden condition in different haematological malignancies. This may not be detected by use of routine H&E stain alone on initial diagnosis of the haematological malignancy by examining bone marrow biopsy and aspiration sample.

The use of reticulin stain and trichome stain is essential for detection of bone marrow fibrosis along with routine H&E stain. The present study emphasis the role of bone marrow biopsy and reticulin/trichome staining in haematological malignancies. This is particularly essential in all cases of multiple myeloma with increased bone marrow fibrosis. The present study also emphasis that myelofibrosis is an integral part of CML. It is recommended not to forget MF when considering suboptimal response to treatment and BM biopsy is to be done at a regular interval in all cases MDS to check marrow fibrosis. The present study also found that there may be presence of increased BMF in other haematological malignancies.

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DECLARATIONS

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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