# Differential diagnosis of bone marrow fibrosis: A study from a tertiary care hospital center

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## Abstract

**Background:** The term 'Myelofibrosis' is used to describe any increase in bone marrow stromal fibers. There are several conditions, both neoplastic and non-neoplastic. There are 2 types of fibers which are deposited in the marrow.

1) Reticulin fibers
2) Collagen fibers

Increased reticulin is associated with many benign and malignant conditions while increased collagen fibrosis is particularly prominent in late stages of myeloproliferative neoplasia or following tumor metastasis to the bone marrow.

**Objectives:** To look for the etiology of myelofibrosis, correlating the clinical features with morphology and to classify the grade of reticulin fibrosis (grade 2 or 3) and look for collagen fibrosis and look for bone changes of osteosclerosis.

**Materials and Methods study design:** Combined retrospective & prospective observational study.

**Period:** January 2018-June 2020 (over a 2½ year period).

**Study area:** Department of Pathology (hematology laboratory), NIMS, Hyderabad, India.

**Sample size:** The study included 300 cases of myelofibrosis of varied aetiologias. The cases were retrieved from the Hematopathology records at a tertiary care center. The

clinical details, including all investigations, were obtained from the patient's medical records.

**Inclusion criteria:** All cases where the bone marrow biopsies with diffuse, ≥ 2 Grade reticulin condensation, were included in the study.

**Exclusion criteria:** Cases where peripheral smear/marrow cytology/trephine biopsy not available for review. Cases with incomplete clinical details.

**Statistical analysis:** Statistical analysis was done using Fisher's exact test and p value ≤0.05 is considered statistically significant.

**Results:** Age range from19 to 90 years with mean of 45. Ther male preponderance ratio of 1.2:1.The cases were classified into broad categories as myeloproliferative neoplasms 90(30%)cases, acute Leukemia in 71(23%), reactive fibrosis in 60 cases (20%), lymph proliferative disorders in 9%) plasma cell neoplasm 7% cases, MDS in 14 (4.6%), metastases-9 cases (3%), granulomatous inflammation 5 cases (1.6%), auto-immune myelofibrosis (AIMF)in 3 cases (1%).

**Conclusion:** The study included 300 cases with grade 2 or 3 reticulin fibrosis within trephine sections Even though diffuse reticulin fibrosis can be seen in nonneoplastic conditions, it is more commonly associated with clonally or neoplastic pathologies. Acute leukemia, MDS, lymphoproliferative disorders and plasma cell neoplasms can also be associated with reticulin fibrosis. Recognition and diagnosis of AIMF is important as it is a rare condition.

**Keywords:** Myelofibrosis, bone marrow fibrosis, reticulin stain, grading, bony changes

## Introduction

The term 'Myelofibrosis' is used to describe any increase in bone marrow stromal fibers. There are a number of conditions, both neoplastic and non-neoplastic, which can cause myelofibrosis. Myelofibrosis can be classified as Primary Myelofibrosis (PMF) or secondary to a wide range of conditions. PMF is a clonal myeloproliferative neoplasm, characterized by proliferation of abnormal bone marrow megakaryocytes and granulocytes and is associated with reactive deposition of fibrous connective tissue in the marrow. There are 2 types of fibers which are deposited in the marrow.1) Reticulin fibers 2) Collagen fibers. Increased reticulin is associated with many benign and malignant conditions while increased collagen fibrosis is particularly prominent in late stages of myeloproliferative neoplasia or following tumor metastasis to the bone marrow.

Myelofibrosis can be diffuse or patchy/focal. Diffuse fibrosis can occur in a wide variety of conditions and patchy/focal fibrosis usually occurs following localized marrow insult. Increasing marrow fibrosis leads to several clinical signs and symptoms and a proper diagnosis is required for management. Increased reticulin formation and sometimes collagen deposition can revert to normal if the causative condition is treatable.

**Objectives**

1. To look for the etiology of myelofibrosis, correlating the clinical features with morphology, culture studies, serology, cytogenetics, and molecular studies (wherever available).
2. To classify the grade of reticulin fibrosis (grade 2 or 3) and look for collagen fibrosis.
3. To look for bone changes of osteosclerosis.

**Materials and Methods**

**Study design:** Combined retrospective & prospective observational study over a 2½ year period. The study included 300 cases of myelofibrosis of varied aetiologias. The cases were retrieved from the Hematopathology records at a tertiary care center. The clinical details, including all investigations, were obtained from the patient's medical records. Approval for the study was taken from the Institutional Ethics committee.

**Inclusion criteria**

1. All cases where the bone marrow biopsies with diffuse, ≥ 2 Grade reticulin condensation, were included in the study.

**Exclusion criteria**

1. Cases where peripheral smear/marrow cytology/ trephine biopsy not available for review.
2. Cases with incomplete clinical details.
3. Cases with only focal area of fibrosis.
4. Cases aged below 18 years were excluded.

**Statistical analysis**

Statistical analysis was done using Fisher's exact test and p value ≤0.05 is considered statistically significant.

**Results**

**Demographic details:** The patients' age ranged from 19 years to 90 years with mean of 45.5 years. There was male preponderance with 168 males and132 females; male to female ratio of 1.2:1.

Correlating the patients` clinical details with the peripheral blood and marrow morphology, immunophenotyping, cytogenetic and molecular data, the cases were classified into broad categories as follows-myeloproliferative neoplasms (MPN, Fig 1)-90 cases (30%), followed by acute Leukemias-71 cases (23.6%) (Table 1), reactive hyperplasia of the marrow-60 cases (20%), lymphoproliferative disorders-27 cases (9%)(Fig 3), plasma cellneoplasms-21 cases (7%), MDS-14 cases (4.6%), metastases-9 cases (3%), granulomatousinflammation-5 cases (1.6%), auto-immune myelofibrosis (AIMF)-3 cases (1%).

**Myeloproliferative neoplasms (n=90)**

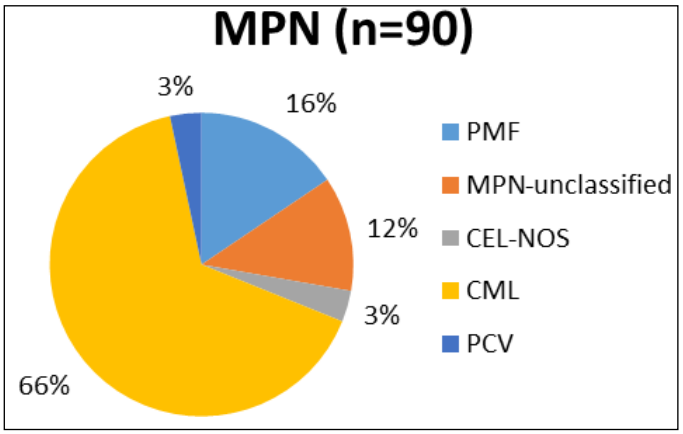


**Fig 1:** Pie chart showing the categories of myeloproliferative neoplasms (n=90)

**Primary Myelofibrosis(n=14)**

These patients presented with complaints of pain abdomen-8 cases (57%), fever-5 cases (35%), generalized weakness-3 (28%), skin rash-3 cases and decreased appetite-3 cases. On examination, splenomegaly was noted in 12 case (85%), pallor in 10 cases (71%), hepatomegaly in 3 cases (21%). Mutation studies were available in 5/14 cases (1 case was JAK2+, 2 cases were CALR+, other 2 were BCR-ABL and JAK 2 negative). LDH levels were elevated in 6 cases.

**Chronic Myeloid Leukemia (n=66)**

The major complaints were pain abdomen (42%), early satiety (25%), fever (27%).The major findings include splenomegaly (84%) and pallor (69%).Elevated LDH values were noted in 31 cases .BCR-ABL was positive in 53 cases, negative in 1 case.Cytogenetic report was not available in 5 cases.

**Other MPNs (Chronic Eosinophilic Leukemia, Polycythemia Vera, MPN-Unclassified)**

The major symptoms include abdominal pain (41%), weakness (23%), followed by fever and other constitutional symptoms. The major signs included splenomegaly (76%), pallor (35%) and hepatomegaly (29%). JAK 2 mutation detected had been detected earlier in 2 of the cases of PCV.

**Lymphoproliferative disorders(n=27)**

Fever and generalized weakness were the major symptoms seen in 9 cases (33%) each followed by bleeding manifestations, SOB, cough, pedal edema. The major signs noted

were pallor-20 cases (74%), splenomegaly-15 cases (55%), lymphadenopathy-8 cases (29%) and hepatomegaly 5-cases (18%). Immunophenotyping for lineage was performed by IHC on trephine sections in 15 cases, and by flow cytometry on aspirates in 4 cases. case distribution depicted in fig 2.

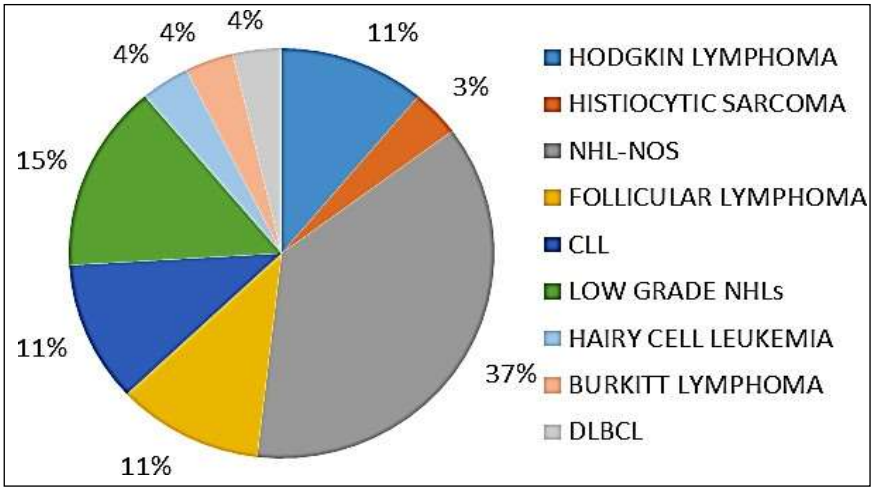


**Fig 2:** Depiction of distribution of Lymphoproliferative Disorders

**Acute Leukemia(n= 71)**

The major symptoms were fever- 50 cases (70%), followed by generalized weakness-14 cases (19%). The major signs were-pallor 55 cases (77%), followed by hepatomegaly-10 cases (14%), splenomegaly-14 cases (19%) and lymphadenopathy-7 cases (9%). Enzyme levels- ALP, LDH were elevated in 13 cases and 23 cases respectively. Immunophenotyping by flow cytometry was done in 14 cases and IHC on trephine sections were performed in 12 cases. The cases which could not afford further immunophenotyping were labelled as Acute Leukemia (unclassified).

**Myelodysplastic Syndromes (n=14)**

The major symptoms seen were fever in 7 cases (50%), bleeding manifestations-6 cases (42%) followed by cough, loss of appetite. Most prominent finding was pallor- 9 cases (64%).

**Plasma cell neoplasia(n=21)**

Predominant features include lytic lesions- 5 cases (23%), back pain-4 cases (19%). Examination of patient revealed pallor in 16 cases (66%), hepatomegaly in 2 cases, splenomegaly in 3 cases. M spike was present in 10 cases, ß 2 microglobulin levels were elevated in 5 cases; A/G ratio reversal was noted in 8 cases.

**Metastatic carcinoma(n=9)**

The major symptom was fever, weight loss, seen in 6 cases (66%); followed by back ache- 4 cases (44%).The major sign was pallor in 6 cases (66%). LFT was deranged in all the cases with elevation of ALP levels. LDH values were elevated in 4 cases.

**Granulomatous inflammation(n=5)**

The major symptoms were fever, cough, and loss of appetite followed by fatigue. The major signs included pallor-5 cases (100%), splenomegaly 4 cases (80%).Three cases had elevated ALP and LDH levels.

**Autoimmune Myelofibrosis(n=3)**

Two cases presented with bleeding manifestations and ITP was suspected. Pallor was noted in 2 cases, splenomegaly in 1.LDH was elevated in 2 cases. Collagen vascular profile-results were not available.

**Reactive Hyperplasia of marrow(n=60)**

There were 60 cases which showed no evidence of clonal disorders marrows showed > 2 reticulin condensation. These included18 cases with erythroid hyperplasia, 6 cases of ITP, 7 cases with bony changes suggestive of renal osteodystrophy and 7 cases of reactive hyperplasia with benign lymphoid aggregates and the remaining had no specific diagnosis, only a hypercellular marrow with increased reticulin.

The major symptoms were fever-24 cases (40%), bleeding manifestations-10 cases (16%), followed by fatigue, pain abdomen, renal failure. The major signs were pallor-50 cases (83%), splenomegaly-23 cases (38%), icterus-15 cases (25%), lymphadenopathy- 8 cases (13%), hepatomegaly -7 cases (11%).

The hematologic findings of the different conditions are summarized in Table 1 and Table 2. The bone marrow cytology and biopsy findings in the various neoplastic and non-neoplastic conditions have been summarized in Table 3 and Table 4, respectively.

**Table 1:** Summaries the hematologic findings in Acute leukemias, PCM, MDS, LPDs & metastasis

| | **Acute leukemias N=71** | | **MDS N=14** | | **Metastasis N=9** | | **LPD N=27** | | **PCM N=21** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Reticulin grade** | **2** | **3** | **2 N=12** | **3 N=2** | **2 N=4** | **3 N=5** | **2 N=17** | **3 N=10** | **2 N=18** | **3 N=3** |
| Hb <11gm/dl | 45 | 21 | 10 | 2 | 3 | 5 | 14 | 10 | 14 | 0 |
| 11-16gm/dl | 4 | 1 | 2 | 0 | 1 | 0 | 3 | 0 | 4 | 3 |
| >16gm/dl | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TC<4000cells/$mm^3$ | 20 | 9 | 9 | 0 | 0 | 0 | 3 | 2 | 3 | 0 |
| 4000-11000/$mm^3$ | 12 | 7 | 3 | 2 | 3 | 5 | 9 | 6 | 13 | 2 |
| >11000/$mm^3$ | 15 | 6 | 0 | 0 | 1 | 0 | 5 | 2 | 2 | 1 |
| Platelet<1.5L/$mm^3$ | 42 | 20 | 11 | 2 | 3 | 3 | 9 | 8 | 4 | 1 |
| 1.5-4.5l/$mm^3$ | 7 | 2 | 1 | 0 | 1 | 2 | 8 | 2 | 13 | 1 |
| >4.5l/$mm^3$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Nucleated RBC | 13 | 9 | 3 | 0 | 1 | 4 | 1 | 2 | 1 | 0 |
| Targets | 4 | 1 | 1 | 0 | 0 | 0 | 1 | 3 | 1 | 0 |
| Tear drops | 11 | 2 | 5 | 1 | 2 | 2 | 2 | 3 | 1 | 0 |
| Polychromatophils | 9 | 7 | 3 | 0 | 1 | 4 | 3 | 2 | 0 | 0 |
| Macrocytes | 9 | 3 | 9 | 1 | 0 | 4 | 3 | 2 | 0 | 0 |
| Microcytes | 6 | 3 | 1 | 0 | 1 | 1 | 1 | 3 | 1 | 0 |

**Table 2:** Summarizes hematological parameters of non-neoplastic conditions with fibrosis (n=8)

| | **Reactive hyperplasia N=60** | | **Granulomatous inf N=5** | | **AIMF N=3** | |
|---|---|---|---|---|---|---|
| **Reticulin grade** | **2 N=48** | **3 N=12** | **2 N=3** | **3 N=2** | **2 N=1** | **3 N=2** |
| Hb < 11gm/dl | 46 | 11 | 3 | 2 | 1 | 1 |
| 11-16gm/dl | 0 | 1 | 0 | 0 | 0 | 1 |
| >16gm/dl | 2 | 0 | 0 | 0 | 0 | 0 |
| TC <4000cells/$mm^3$ | 21 | 6 | 2 | 2 | 1 | 0 |
| 4000-11000/$mm^3$ | 25 | 6 | 1 | 0 | 0 | 2 |
| >11000/$mm^3$ | 2 | 0 | 0 | 0 | 0 | 0 |
| Platelet< 1.5L/$mm^3$ | 30 | 7 | 3 | 1 | 1 | 1 |
| 1.5-4.5L/$mm^3$ | 16 | 3 | 0 | 1 | 0 | 1 |
| >4.5l/$mm^3$ | 2 | 2 | 0 | 0 | 0 | 0 |
| N RBC | 1 | 5 | 0 | 0 | 0 | 0 |
| Targets | 5 | 0 | 0 | 1 | 0 | 0 |
| Tear drops | 16 | 10 | 1 | 0 | 0 | 0 |
| Polychromatophils | 13 | 6 | 0 | 0 | 1 | 1 |
| Macrocytes | 13 | 4 | 2 | 2 | 1 | 0 |
| Microcytes | 19 | 4 | 1 | 1 | 0 | 1 |

**Table 3:** Depicting bone marrow aspirate/imprint and biopsy findings in the neoplastic conditions (n=232)

| | | **PMFs N=14** | | **CMLs N=59** | | **Other MPNs N=17** | | **MDS N=14** | | **Acute leukemia N=71** | | **Metastases N=9** | | **CLPD N=27** | | **PCM N=21** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Grade of Fibrosis** | | **2 N=1** | **3 N=13** | **2 N=30** | **3 N=29** | **2 N=10** | **3 N=7** | **2 N=12** | **3 N=2** | **2 N=49** | **3 N=22** | **2 N=4** | **3 N=5** | **2 N=17** | **3 N=10** | **2 N=18** | **3 N=3** |
| Aspirate | Particulate | 0 | 0 | 9 | 5 | 6 | 2 | 12 | 1 | 27 | 2 | 2 | 0 | 11 | 5 | 16 | 1 |
| | Scant particulate | 0 | 0 | 8 | 3 | 2 | 1 | 0 | 0 | 6 | 6 | 1 | 0 | 3 | 2 | 2 | 1 |
| | A particulate | 1 | 13 | 13 | 21 | 2 | 4 | 0 | 1 | 15 | 14 | 1 | 5 | 3 | 3 | 0 | 1 |
| | Inc cellularity | - | - | 13 | 7 | 7 | 3 | 10 | 1 | 25 | 2 | 0 | 0 | 11 | 3 | 14 | 0 |
| Imprint cellularity | Increased | 1 | 0 | 25 | 18 | 10 | 3 | 6 | 0 | 29 | 8 | 1 | 0 | 7 | 4 | 12 | 0 |
| | Reduced | 0 | 9 | 2 | 3 | 0 | 1 | 1 | 0 | 7 | 7 | 1 | 2 | 0 | 3 | 2 | 2 |
| | Varying | 0 | 4 | 4 | 7 | 0 | 3 | 4 | 1 | 12 | 7 | 2 | 3 | 8 | 1 | 2 | 0 |
| | Hemorrhagic | 0 | 10 | 1 | 8 | 1 | 5 | 2 | 0 | 8 | 5 | 2 | 4 | 1 | 2 | 1 | 0 |
| Biopsy cellularity | <30% | 0 | 3 | 0 | 0 | - | - | - | - | 2 | 1 | - | - | 0 | 0 | 0 | 0 |
| | 30-90% | 0 | 9 | 6 | 7 | 3 | 2 | 7 | 2 | 16 | 9 | 4 | 5 | 15 | 5 | 14 | 3 |
| | >90% | 1 | 2 | 24 | 22 | 7 | 5 | 6 | - | 31 | 12 | - | - | 2 | 5 | 4 | 0 |
| Megakaryocyte number | ↓ | 0 | 1 | 3 | 3 | 1 | 2 | 2 | 1 | 39 | 15 | 4 | 4 | 11 | 6 | 12 | 0 |
| | N | 0 | 2 | 5 | 7 | 1 | 1 | 7 | 1 | 5 | 6 | 0 | 1 | 4 | 3 | 6 | 1 |
| | ↑ | 1 | 10 | 15 | 19 | 8 | 4 | 3 | 0 | 5 | 1 | 0 | 0 | 2 | 1 | 0 | 2 |
| Megakaryocytes | Bizarre | 1 | 12 | 0 | 3 | 2 | 0 | - | - | - | - | - | - | - | - | - | - |
| | Discrete nuclei | 0 | 0 | 0 | 1 | 3 | 1 | 2 | 0 | - | - | - | - | - | - | - | - |
| | Para trabecular | 0 | 2 | 0 | 1 | 2 | 1 | 6 | 1 | - | - | - | - | - | - | - | - |
| | Micromega | 0 | 1 | 25 | 20 | 0 | 0 | 4 | 1 | 0 | 1 | - | - | - | - | - | - |
| | Emperipolesis | 0 | 0 | | | 1 | 0 | 1 | - | - | - | - | - | - | - | - | - |
| | Clustering | 1 | 8 | 6 | 3 | 4 | 1 | | - | 0 | 1 | - | - | - | - | - | - |
| Bony changes | Thickened BT | 1 | 10 | 1 | 3 | 1 | 1 | | - | 0 | 1 | 1 | 3 | - | - | 0 | 1 |
| | Osteosclerosis | 0 | 8 | 1 | 4 | 1 | 1 | | - | - | - | - | - | - | - | - | - |
| | Osteoclast prominence | 1 | 1 | - | 1 | - | - | | - | - | - | - | - | - | - | 0 | 1 |
| | Osteoblastic rimming | 1 | 2 | - | 2 | - | - | | - | 0 | 1 | - | 1 | - | - | 0 | 1 |

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*Section A-Research paper*

Abbreviations in table-Inc=Increased, N-normal, BT-Bony trabeculae, ↓- Reduced, ↑-increased.

**Table 4:** Summarizes bone marrow findings in non-neoplastic conditions (n=68)

| | | **Reactive marrow(n=60)** | | **Granulomatous inflammation(n=5)** | | **AIMF (n=3)** | |
|---|---|---|---|---|---|---|---|
| **Grade of Fibrosis** | | **2(N=48)** | **3(N=12)** | **2** | **3** | **2** | **3** |
| Aspirate | Particulate | 42 | 3 | 3 | 1 | 1 | 0 |
| | Scant particulate | 4 | 2 | 0 | 0 | 0 | 0 |
| | A particulate | 2 | 7 | 0 | 0 | 0 | 2 |
| | Inc cellularity | 35 | 1 | 2 | 0 | 1 | 0 |
| Imprint cellularity | Increased | 32 | 3 | 0 | 0 | 1 | 0 |
| | Reduced | 4 | 8 | 1 | 1 | 0 | 0 |
| | Varying | 8 | 1 | 2 | 1 | 0 | 2 |
| | Hemorrhagic | 1 | 6 | 1 | 0 | 0 | 1 |
| Biopsy cellularity | < 30% | 1 | 4 | 0 | 0 | 0 | 0 |
| | 30-90% | 43 | 8 | 3 | 2 | 1 | 2 |
| | > 90% | 4 | 0 | 0 | 0 | 0 | 0 |
| Megakaryocyte Number | ↓ | 8 | 3 | 2 | 0 | 0 | 0 |
| | N | 18 | 6 | 0 | 0 | 1 | 2 |
| | ↑ | 17 | 3 | 1 | 2 | 0 | 0 |
| megakaryocytes | Bizarre | 0 | 2 | 0 | 1 | 0 | 0 |
| | Discrete nuclei | 0 | 0 | 0 | 0 | 0 | 0 |
| | Para trabecular | 1 | 1 | 0 | 0 | 0 | 0 |
| | Micro megakaryocytes | 5 | 2 | 0 | 0 | 0 | 0 |
| | Emperipolesis | 0 | 0 | 0 | 0 | 0 | 0 |
| | Clustering | 1 | 1 | 0 | 0 | 0 | 0 |
| Bony changes | Thickened BT | 5 | 4 | 0 | 0 | 0 | 0 |
| | Osteosclerosis | 2 | 2 | 0 | 0 | 0 | 0 |
| | Osteoclast prominence | 0 | 0 | 0 | 0 | 0 | 0 |
| | Osteoblastic rimming | 0 | 0 | 0 | 0 | 0 | 0 |

Abbreviations in table-Inc=Increased, N-normal, BT-Bony trabeculae, ↓- Reduced, ↑-increased.

**Statistical analysis**

All the cases with neoplastic and non-neoplastic etiologies were classified as Grade 2 and grade 3 reticulin and the following clinical features (hepatomegaly, splenomegaly) hematologic parameters (Hemoglobin, WBC and platelet), bone marrow cellularity, megakaryocyte numbers and bone changes) were compared using the Fisher's exact test. The parameters which had p value ≤ 0.05 are shown in Table 5.

**Table 5:** Parameters with significant p value (≤ 0.05)

| **Parameter** | **P value** |
|---|---|
| Anemia | 0.0068 |
| Leucocytosis | 0.056 |

| | |
|---|---|
| Aspirate-increased cellularity | 0.0237 |
| Imprint increased cellularity | 0.0228 |
| Biopsy cellularity (30-90%) | 0.0153 |

**Grading of myelofibrosis shown in fig 3**

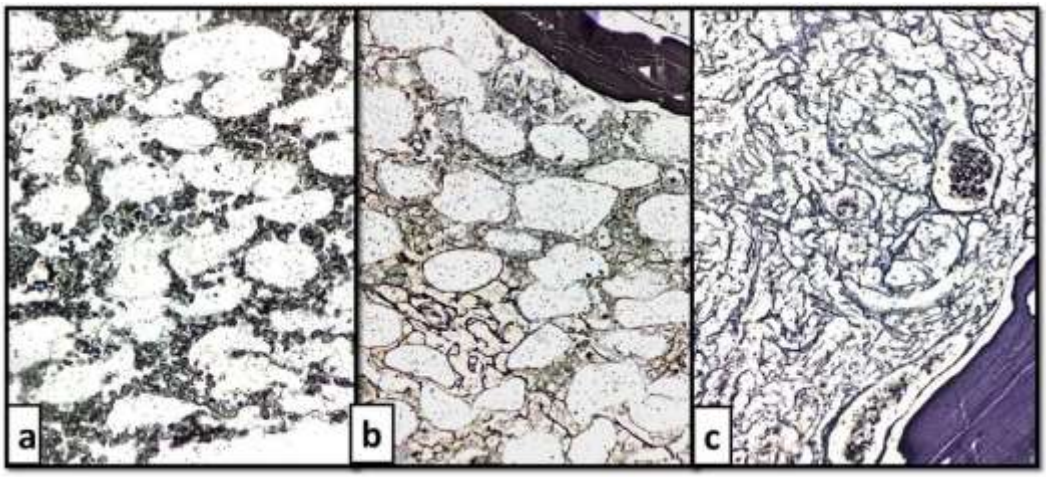



**Figure 3:** Reticulin staining grades:a.Grade 1. Few short, thin reticulin fibres but do not intersect to form a network; b. Grade 2. A focal network of thin reticulin fibres with fibre intersections; c. Grade 3: a dense network of thick reticulin fibres throughout the bone marrow. (Reticulin;400x)

**Peripheral smear and bonemarrow findings of different diseases with marrow fibrosis are shown in following figures from fig 4-fig13**

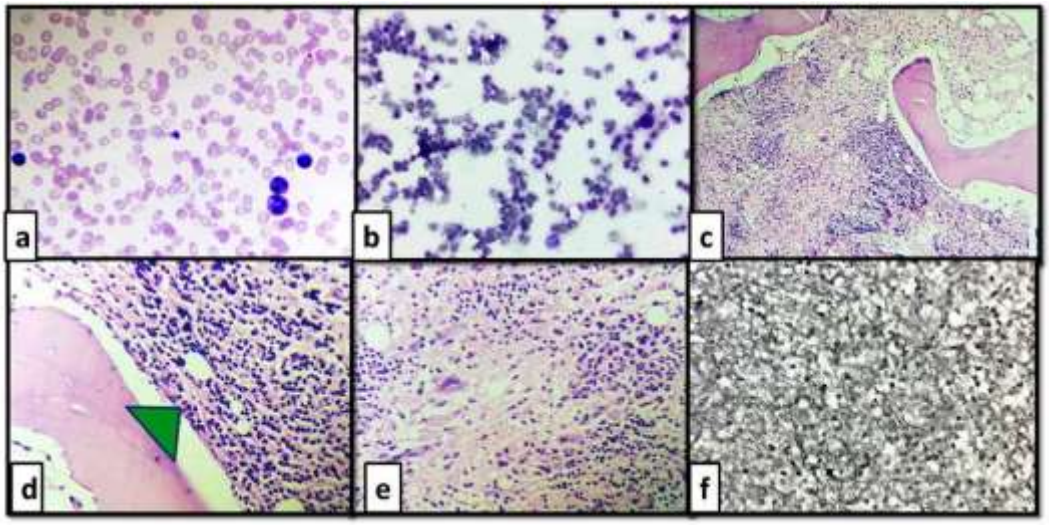



**Figure 4:** Autoimmune myelofibrosis: a. PS-Pancytopenia(Giemsa;400x);b.Hypocellular imprint smears with hemorrhagic background. c,d,e. Bx-hypercellular marrow with prominent megakaryocytes, lymphoid aggregates and mild fibrosis(H&E;100x,400x);f. Reticulin-Grade I condensation (Reticulin;400x)

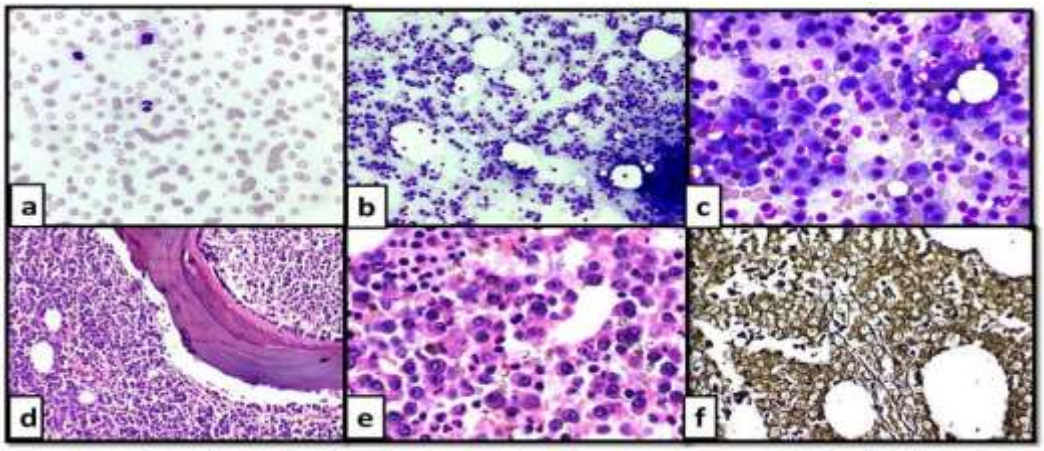



**Figure 5:** Plasma cell myeloma. a. PS- N/N RBC with rouleaux formation, WBC and platelets are normal (Giemsa;400x); b,c. BMA-hypercellularity with numerous plasmacells and few plasma blasts (Giemsa;100x, 400x); d,e. Bx-diffuse involvement of marrow by sheets of plasma cells (H&E;100x,400x);f. Reticulin-Grade I condensation.(Reticulin;400x)

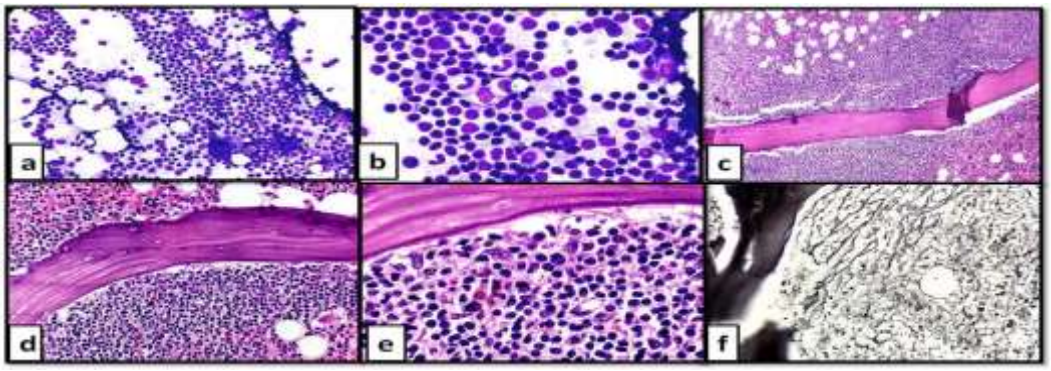



**Figure 6:** Non-Hodgkin Lymphoma. a,b. BMA-hypercellularity with numerous large lymphoid cells(Giemsa;100x, 400x); c,d,e. Bx-Para trabecular involvement of marrowreplaced by numerous monomorphic round cells (blasts) (H&E;40x,100x,400x);f. Reticulin-Grade II condensation. (Reticulin;400x)

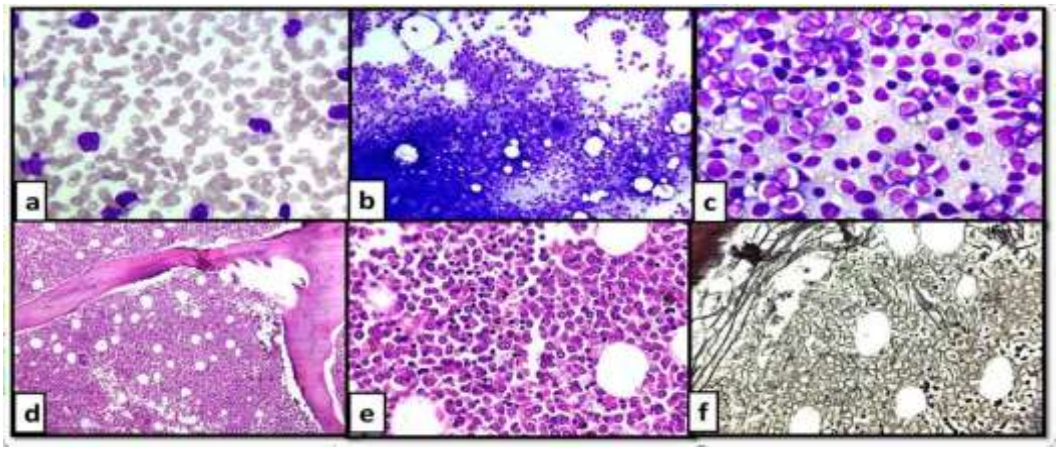



**Figure 7:** Acute leukemia. a. PS-N/N RBC, blasts with round to indented nucleus, increased N/C ratio, scant cytoplasm and platelets are reduced (Giemsa;400x); b,c. BMA-hyper cellularity with numerous blasts (Giemsa;100x, 400x); d,e. Bx-diffuse

involvement of marrow by numerous monomorphic round cells (blasts) (H&E;100x,400x);f. Reticulin-Grade II condensation. (Reticulin;400x)

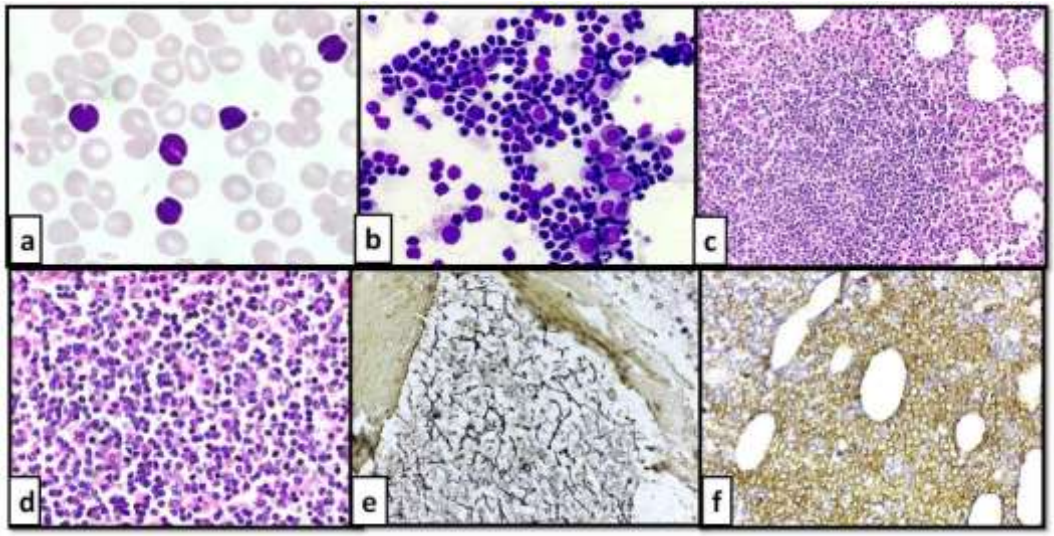



**Figure 8:** Follicular Lymphoma. a. PS-N/N RBC with few atypical lymphoid cells (buttock cells with nuclear clefts) and platelets are mildly reduced (Giemsa;400x); b. BMA-hypercellularity with numerous small lymphoid cells(Giemsa;400x); c,d.Bx-interstitial &Para trabecular involvement of marrow replaced by numerous monomorphic small round cells (H&E; 100x,400x);e. Reticulin-Grade II condensation. (Reticulin;400x);f. Diffuse Bcl2 positivity on immunohistochemistry (40x)

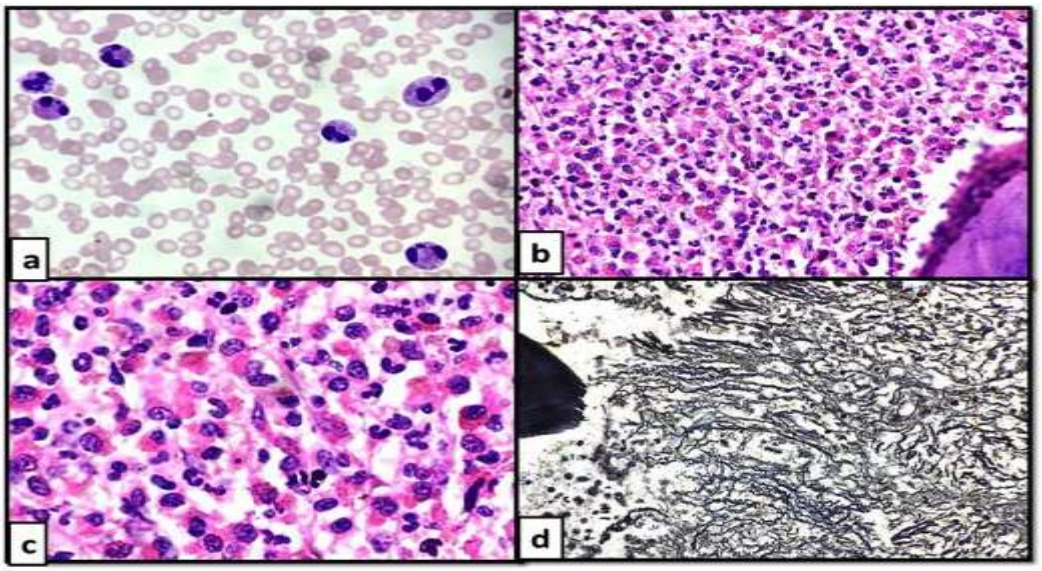



**Figure 9:** Chronic eosinophilic leukemia, NOS. a. PS-N/N RBC with eosinophilic leukocytosis and platelets are normal(Giemsa;400x); b,c.Bx-hypercellular marrow with marrow replaced by immature eosinophilic precursors (H&E;100x,400x);d. Reticulin-Grade III condensation. (Reticulin;400x)

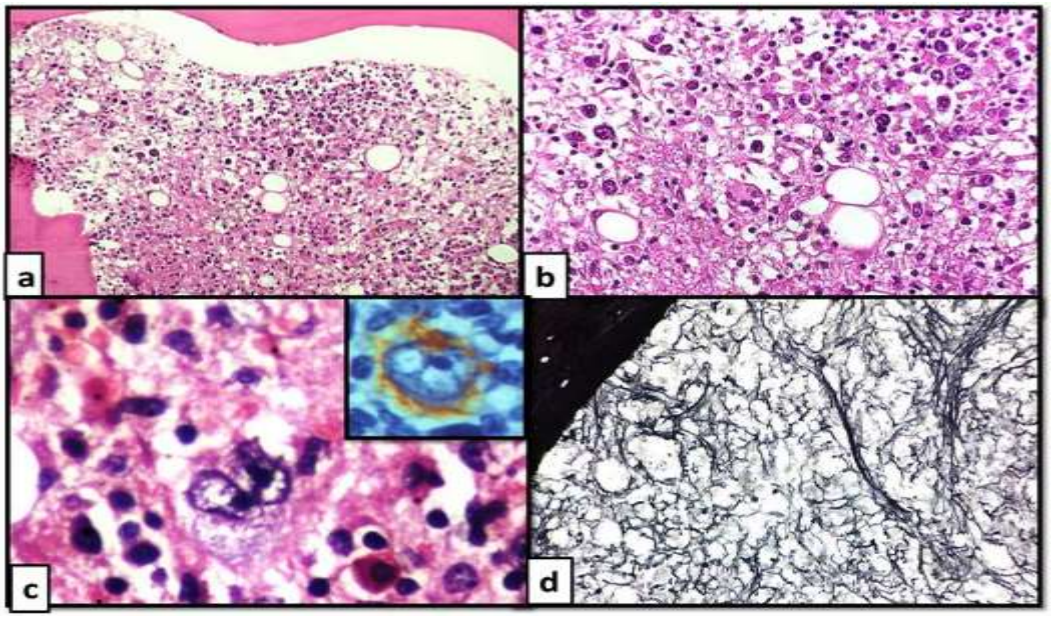



**Figure 10:** Hodgkin Lymphoma. a,b,c.Trephine biopsy-diffuse involvement of marrow with scattered binucleated and mononucleated RS cells against polymorphous background (H&E;40x,100x,400x) Inset shows CD30 positive RS cells (IHC 400x);d. Reticulin-Grade III condensation. (Reticulin;400x)

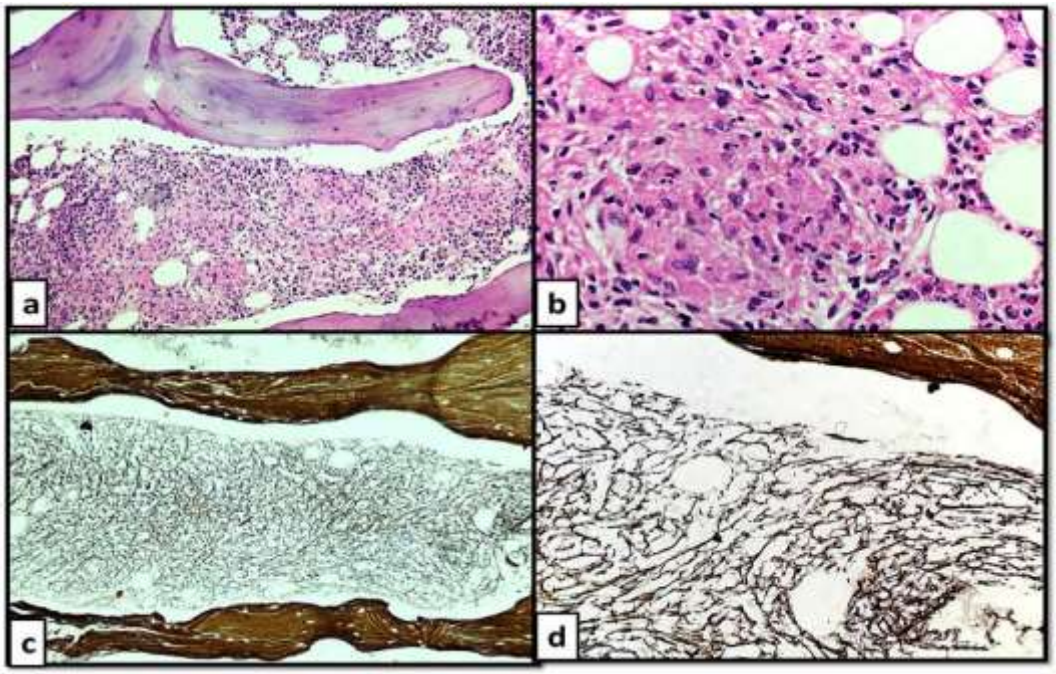
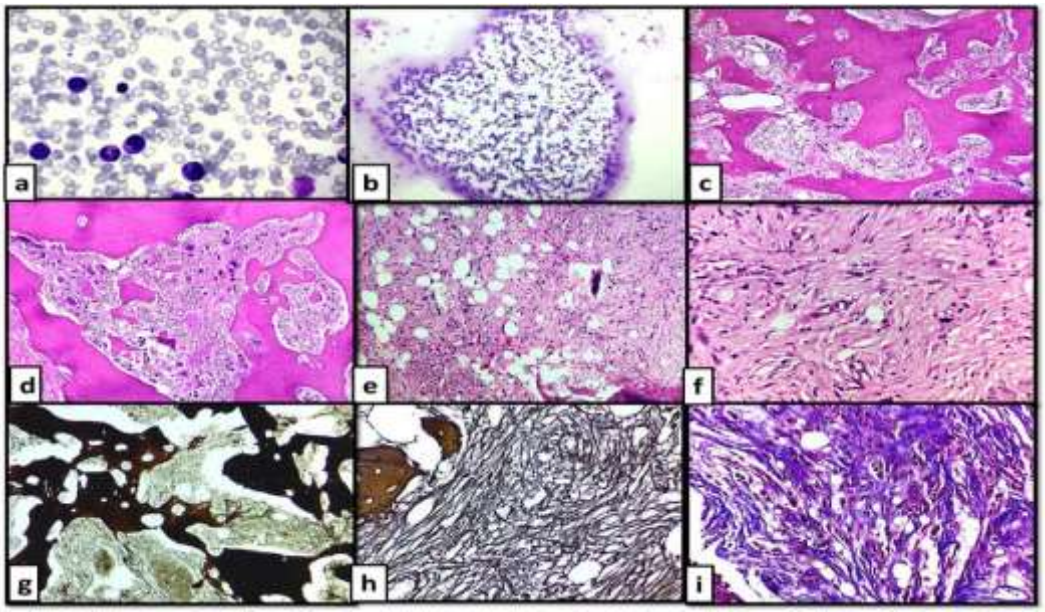


**Figure 11:** Granulomatous inflammation. a. Bx-mutiple confluent granulomas




(H&E;100x);b. Aggregates of epithelioid macrophages forming well defined granulomas (H&E;100x,400x);c,d. Reticulin-Grade III condensation. (Reticulin;100x,400x)

**Figure 12:** Primary myelofibrosis: a. PS- N/N RBC with teardrop cells, mild leukocytosis with few maturing myeloid precursors and platelets are reduced (Giemsa;400x);b. Hypocellular imprint smears with hemorrhagic background. c,d,e,f.

Bx-Osteosclerosis with irregularly thickened bony trabeculae, few pleomorphic megakaryocytes and diffuse marrow fibrosis (H&E;100x,400x);g,h. Reticulin-Grade III condensation with osteosclerosis. (Reticulin;100x, 400x); i.Massons trichrome stain-collagen fibrosis stained blue(400x)

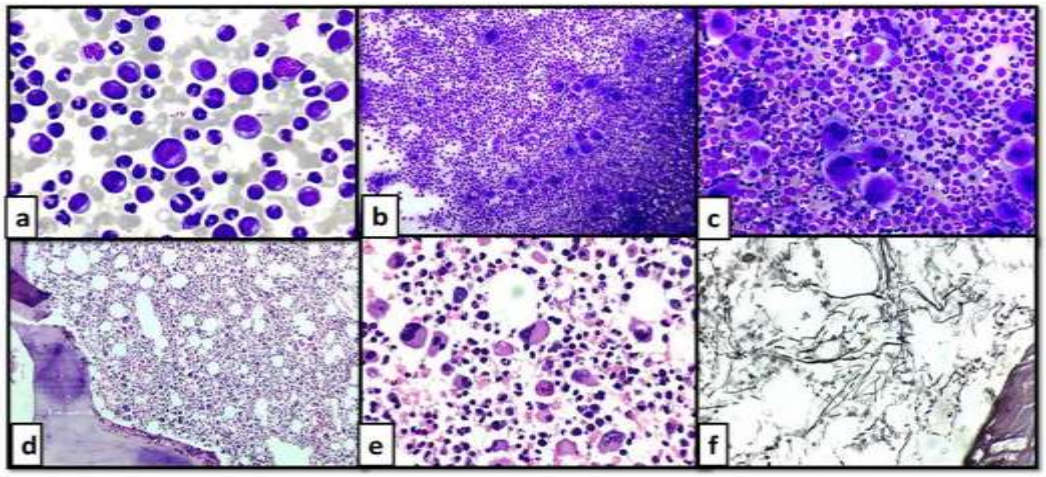



**Figure 13:** CML, chronic phase. a. PS-N/N RBC with overwhelming leukocytosis, maturing myeloid precursors with occasional blasts seen and platelets are normal (Giemsa;400x); b,c. BMA-hyper cellularity with myeloid prominence, occasional blasts, numerous micro megakaryocytes, and few show discrete nuclei (Giemsa;100x, 400x); d,e. Bx-hypercellular marrow with prominent micro

megakaryocytes (H&E;100x,400x);f. Reticulin-Grade II condensation. (Reticulin;400x)

**Discussion**

During the study period, 3460 bone marrow biopsies were done in the department, of which 300 cases (8.6 %) were found to have reticulin condensation ≥ grade 2. Of the 300, aspirates were particulate in 149 cases, scant particulate in 41 cases and a particulate in 108 cases. Reticulin condensation was grade 2 in 192 cases and grade 3 in 108 cases.

**Primary myelofibrosis (PMF)**

The present study included 14 cases of PMF (1 case with Grade 2 condensation and 13 cases with grade 3 condensation). There were 5 males and 9 females, with a median ageof 50 years. The clinical and hematologic parameters of these were compared with otherIndian case series and depicted in Table 6.[1,2,3].

**Table 6:** Comparison of PMF cases of present study with 3 other Indian studies

| | **Present study (2020)** | **Sazawal*et al.*[1](n=80) (2015)** | **Patil *et al.*[2](n=50)(2019)** | **Dixit *et al.*[3](n=28)(2019)** |
|---|---|---|---|---|
| Age (yrs)mean±SD | 50 ± 10.88 | 47. 5 ± 15.7 | 52 median | 53 ± 14.7 |
| Gender-M:F | 5/9 | N/A | 27/23 | 18/10 |
| Constitutional symptoms | 14 | 20/63(31.7%) | 30/50 | N/A |
| Splenomegaly (%) | 85 % | 63.7% | 68 % | 82 % |
| Hemoglobin/ gm dl Mean±SD | 9.6 ± 1.97 | 9.2 ± 3.5 | 10.2 | 8.7 ± 2.9 |
| Median TLC($\times 10^9$/l) | 12 (4.5-77) | 8.6 | 18.3 | 11.7 (1.1-64) |
| Median platelet count($\times 10^3$/µl) | 450(50-1800) | 196 | 313 | 150(10-1310) |
| Myelofibrosis Grade III (%) | 13/14(92%) | 15/80(18.75%) | N/A | 21/28(75%) |

The age range of the patients with PMF at first presentation was similar to that in the other studies. This study had more number of female patients as compared to the others. Mean haemoglobin values were similar in all the studies. In this study, majority of the cases 13/14 (92%) has shown grade 3 myelofibrosis, which is higher than other studies. This is possibly because only cases with grade 2 & 3 were included, without any cases of early PMF.Imprints show hemorrhagic background in 10 cases indicating that the biopsy was showing some stromal change (fibrosis).

In the study by Sazawal*et al.*[1], CALR positive patients are 9/80 and has shown significant association with younger age and larger spleen size with p=0.001.However, in this study, CALR positive cases were 2/14 cases. In contrast to the Sazawal study, these were seen in older age group (mean of 58 years and average spleen size of 7 cms (moderate splenomegaly). Twopatients were of young age, had severe anemia and were transfusion-dependent, similar to those in the study by Singh *et al.*[4]. Prominence of osteoblasts and osteoclasts were noted when there was associated osteosclerosis.

**CML with secondary fibrosis**

The present study included 59 cases of CML, which had developed myelofibrosis. Of these, 38 cases were newly diagnosed and 21 were known and treated cases of CML.

The distribution was as follows:-CML-CP-48 cases, 5 cases of CML-AP, 6 cases of CML-blast crisis. This indicates that there were large number cases in chronic phase, which had already developed myelofibrosis.

The mean age of the cases was 39 years with M: F of 1:1.95. CML seems to occur a decade earlier in India than the west, as is reported in studies done by Ambalathandi*et al*.[5]and Bhatti *et al*.[6].The data from UK and USA has shown peak incidence in older age group. Bhatti *et al*. attributed this fact to demographic differences between nations [6,7].

Saleem *et al*.[8]found anemia to be more common with advanced fibrosis, which is similar to the present study. WBC count and platelet count are similar to the study conducted by Gupta *et al*.[9].In the present study, leukocytosis was associated with increased fibrosis.

Nucleated RBC and tear drops were comparatively more in cases with grade 3 reticulin condensation. Grades of myelofibrosis at presentation had significant impact on outcomes in CML-CP. Hence, bone marrow examination for presence and grade of myelofibrosis, helps in prognosticating CML-CP.

Maximum cases of grade 3 fibrosis were a particulate as increased reticulin condensation yields less particles. Increased bone marrow cellularity, increased myelopoiesis, increased megakaryocyte number were noted and were in concordance with study conducted by Gupta *et al*., Khonglah*et al*.[10].

The cases in accelerated phase and blast crisis revealed grade 3 reticulin, as well as majority of the treated cases of CML. Cytogenetics report in available in 54 cases out of which 53 patients are BCR-ABL positive, one case was negative.

Other MPNs include 17 cases; grade 2 condensation was noted in 10 cases and grade 3 condensation in 7 cases.

**Acute leukemia with myelofibrosis**

Certain acute leukemias, especially AML M7 subtype have been identified as a cause of myelofibrosis [11]. Islam *et al*.[12]reported that some degree of bone marrow fibrosis is seen in few cases right from the disease onset. Similar results were also outlined by Kundel *et al*.[13] who reported myelofibrosis in 30% of cases with AML and ALL.

Uncommonly, myelofibrosis can be encountered in cases of APML and post chemotherapy for acute leukemia as seen in studies by Aventin *et al*., Batlle M *et al*. and Abou Dalle I *et al*.[14,15,16]. Mori *et al*. has reported that over expression of Transforming growth factor $\beta_1$ in the initial stages of APML resulted in myelofibrosis [17]. There were 7 APLs and 7 were treated AML cases. Of the 71 cases of acute leukemia, 49 marrows showed grade 2 reticulin condensation and 22 grade 3 condensation.

**MDS with Fibrosis**

In the present study, 12 of the 14 cases, had grade 2 reticulin, 2 had grade 3 and 5 were diagnosed as MDS-EB-F(Myelodysplastic syndrome with excess blasts with fibrosis. This was compared to study by Wang *et al*.[18].Wang*et al*. reported 24 (15.3%) cases of MDS-F as grade 1 and 10 (6.4%) patients as grade 2. In the current study, only cases with fibrosis of grade $\geq 2$ were included. In a study by Ramos *et al*., bone marrow fibrosis grade 2 or higher was observed in 17 (22.1%) patients [19]. MDS-F is associated with increased number of megakaryocytes with a high degree of dyspoiesis. This

feature was noted in 50% of our cases.

**Lymphoproliferative disorders with fibrosis**

The LPDs with fibrosis (27 cases) included NHLs-23 cases (85.1%), Hodgkin Lymphoma involving the marrow- 3 cases (11%), and 1 case of Histiocytic sarcoma. Of the NHLs, majority were of B-cell lineage. There was 1 case of hairy cell leukemia, which is known to have pericellular reticulin condensation.

**Plasma cell neoplasms with fibrosis**

There were 21 cases of plasma cell neoplasms with marrow fibrosis. Of these, majority (85%) showed grade 2 reticulin condensation.

**Metastatic carcinomas with fibrosis**

The study included 9 cases with metastatic carcinoma to the marrow with grade 2 condensation (4 cases) and grade 3 (5 cases).Majority showedthickened bony trabeculae, collagen fibrosis and metastatic deposits.

**ITP and myelofibrosis**

Myelofibrosis has been reported in cases of ITP being treated with thrombopoietin-receptor antagonists (TPO-RA) [20]. Studies found that TPO-RA induced myelofibrosis (grade2/3), occurred in one-fifth of patients of treated ITP, increasing with > 2 years of treatment. There are studies indicating that myelofibrosis is occasionally detected in cases of untreated ITP [21].

**Auto-immune myelofibrosis (AIMF)**

Autoimmune myelofibrosis (AIMF) is a fairly recently described entity and is an uncommon cause of bone marrow failure. The present study included study 3 cases of AIMF (M: F=1:2). There were more cases diagnosed as AIMF, but were excluded from the study, as they had only grade 1 reticulin condensation. The clinical and pathologic features of these 3 cases of AIMF were compared to 14 cases of PMF (present study) and a series of AIMF reported by Vergara *et al*.[22], depicted in Table 7.

**Table 7:** Comparisons between PMF, AIMF (present study) and AIMF (Vergara *et al*.) [22]

| | **Primary Myelofibrosis (n=14) Present study** | **AIMF(n=3) Present study** | **AIMF(n=34) Vergara *et al*.[22]2014** |
|---|---|---|---|
| Mean age±SD in years | 50 ± 10.88 | 26 ± 3.46 | Median age-43(22-78) |
| Male/female | 5/9 | ½ | 5/24 |
| Constitutional symptoms | 14/14 | 0/3 | 0/29 |
| Splenomegaly | 85% | 1/3 | Not included in study |
| Cytopenia | Seen | Seen | 27/29 |
| Nucleated RBC | 9/14 | 0/3 | 1/29 |
| Tear drops | 14/14 | 0/3 | 1/29 |
| Osteosclerosis | 8/14 | 0/3 | 0/29 |
| Megakaryocytic dysplasia | Seen in megakaryocytes | Not seen | 0/29 |
| Reticulin grade ≥2 | 14 | 3 | 4/29 |

In the present study, benign lymphoid aggregates were seen in on 2 cases and IHC revealed these as non-neoplastic aggregates.

Similar to the study conducted by Vergara *et al*.[22], our study shows similar features

like younger age of onset, female predominance, absence of constitutional symptoms, presence of cytopenia, minimal/absent RBC abnormalities, no megakaryocytic abnormalities and bony changes. In this study, only cases with reticulin grade ≥2 was included, whereas Verger *et al*. have included all cases from grade 1-3. In the study conducted by Vergara *et al*., on follow up, cytopenia of 29 patients responded to treatment with corticosteroids. For those patients who had no response or a minimal response, another immunosuppressive therapy was effective. In the present study, the 3 patients were put on steroids; however they were later lost to follow-up and response could not be evaluated.

**Limitations of the study**

Due to financial constraints faced by some of the patients, cytogenetics/molecular testing was not available in some of the cases of suspected myeloproliferative neoplasms. Many of the patients were lost to follow-up after the initial evaluation; hence details about treatment and outcome are not available.

**Conclusion**

The study included 300 cases with grade 2 or 3 reticulin fibrosis (232 neoplastic/clonal and68 of non-neoplastic etiologies). Though diffuse reticulin fibrosis can be seen in nonneoplastic conditions, but more commonly associated with clonal or neoplastic pathologies. Myeloproliferative neoplasms formed the largest group in this study. When bone marrow aspirate is scant or a particulate, biopsy imprint cytology plays an immense role in assessing cellularity, presence or absence of blasts or of atypical cells. The presence of a hemorrhagic background (either patchy or diffuse) indicates that some stromal change (e.g. fibrosis) has occurred and gives a clue about fibrosis, before the trephine biopsy is ready. Acute leukemias, MDS, lymphoproliferative disorders and plasma cell neoplasms can also be associated with reticulin fibrosis. Recognition and diagnosis of AIMF is important as it is a rare condition. The cytopenia encountered, are responsive to steroid therapy. All molecularand cytogenetic tests should be available to categorize the condition.

Ethics committee consideration: Institutional Ethics committee permission was taken prior to the commencement of the study.

Declared as no conflict of interest.

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