



Research Article

Bone Marrow Examination: A Three Year Study At A Tertiary Care Centre

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ABSTRACT

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Background: Bone marrow study is a highly informative diagnostic test in evaluating hematological & non-hematological disorders. The final interpretation requires the integration of bone marrow aspiration and trephine biopsy findings, together with the results of supplementary tests such as immunophenotyping and molecular genetic studies in the context of clinical findings. **Aims & Objectives:** To study the spectrum of lesions diagnosed on bone marrow examination and to identify the frequency of various causes leading to bone marrow evaluation.

Methods: This was a retrospective study carried out at Terna Speciality Hospital and Research Centre over three years (January 2022 to December 2024). A total of 45 cases in which simultaneous bone marrow aspiration and bone marrow trephine biopsy were performed were included in the study

Result: Of the 45 cases were bone marrow examination was done, the mean age was 54.42 years with a male-to-female ratio of 1.25:1. The most common indication for a bone marrow examination was pancytopenia (28.9%), followed by evaluation for anemia (22%). In our study, the most common findings were hematological disorders (86.7 % cases), followed by non-hematological cases (2.22% cases), and a normal marrow was seen in 11.1% cases. Of the hematological disorders, non-malignant hematological causes (53.8%) were more common than malignant (46.2%).

Conclusion: Bone marrow examination plays a vital role in understanding and diagnosing a variety of marrow diseases as well as other diseases affecting the bone marrow.

Keywords: Bone marrow aspirate, Bone marrow biopsy, Hematological disorders, Flow cytometry, Karyotyping, Fluorescence in situ hybridization (FISH).

INTRODUCTION

Bone marrow is the major site of hematopoiesis, giving rise to the three cellular elements: red blood cells, white blood cells, and platelets. Various hematological disorders may arise from an abnormality in one of the three lineages & sometimes bone marrow examination becomes essential to determine the actual etiology. The history of in vivo bone marrow examination dates back to as early as 1876 when Mosler used a regular wood drill to aspirate bone marrow particles from a patient with leukaemia. ^[1]

The two most important techniques used for the diagnosis of these hematological diseases are bone marrow aspiration and trephine biopsy, along with review of peripheral blood smear. ^[2]

Bone marrow is involved in nutritional deficiency diseases, leukaemias, myeloproliferative neoplasm, hematomphoid neoplasm, and some of the non-hematological causes include parasitic infections, tuberculosis, and metastatic deposits. ^[3] Bone marrow examination includes the process of obtaining the cells from the bone marrow blood (aspirate) and a solid bony piece (biopsy) of bone marrow. Aspiration of the marrow is primarily utilized for cytological assessment for morphology and obtaining a differential cell count. Aspirate can also be useful for additional investigations like flow cytometry, immunophenotyping, cytogenetics, and molecular studies. Trephine biopsy provides more comprehensive information regarding the marrow cellularity, architectural patterns, and overall hematopoiesis. ^[4]

The final interpretation requires the integration of peripheral blood, bone marrow aspirate, and biopsy findings, together with other ancillary tests, and in the context of the clinical picture, which can lead to a definitive diagnosis. [4]

The main objectives of the present study were to identify the frequency of various causes leading to bone marrow examinations and to analyze the spectrum of lesions that are encountered in bone marrow examinations of patients attending a tertiary care hospital.

MATERIALS AND METHODS

This is a retrospective study that was performed in the Department of Pathology, TernaSpeciality Hospital & Research Centre, Navi Mumbai, over three years from January 2022 to December 2024. Institutional ethical clearance was obtained (TMCHRC/Surg/2025/IEC Protocol-03/34)

All the cases where simultaneous bone marrow aspiration (BMA) and bone marrow trephine biopsy (BMB) were performed were included in the study; a total of 45 cases, which fulfilled the above criteria, were included.

BMA&BMB were done from the posterior superior iliac spine, smears were prepared immediately following aspiration and stained with Leishman stain, while the biopsy bits were initially kept for decalcification for 24 hours, and then processed and stained with Haematoxylin and Eosin stain.

Records about the demographic details, clinical indication for the procedure, complete blood count, peripheral blood smear reports, and bone marrow examination findings were retrieved from the hospital information system. The collected data was entered into the Microsoft Office Excel software and tabulated in percentages and frequency.

Inclusion Criteria

- All the cases where simultaneous BMA and BMB were performed

Exclusion Criteria

- Cases where only BMA or only BMB was performed were not included.

RESULTS

A total of 49 cases underwent bone marrow examination during the study period of three years from January 2022 to December 2024. Of these 45 cases who had undergone both BMA and BMB simultaneously were studied. The age of the patients ranged from 16 to 85 years, with a mean age of 54.42 years. The most common age group of presentation in our study was 51 to 60 years, which accounted for 11% of the cases. Of the 45 cases, the majority of the patients were males [55% (n=25 cases)], with a male-to-female ratio (M: F) of 1.25:1.

The most common indication for a bone marrow examination was pancytopenia (n=13, 28.9%), followed by anemia (n=10, 22%). (**Table 1**)

Of the 45 cases where bone marrow evaluation was performed, the majority had hematological disorders (86.7 %; n=39), and a sole case of adenocarcinoma metastases to the marrow was identified (2.22%), while the remaining five patients did not have any significant abnormality or identifiable pathology. (**Figure 1**) Of the hematological disorders (n=39), the non-malignant hematological causes accounted for the majority of the cases (n= 21, 53.8%) while the rest were malignant (n=18, 46.2%). **Table 2** shows the results of the bone marrow evaluation.

Out of 45 cases studied, **hematologic malignancy** was found in 40% (n=18) cases. Acute Leukemias, Myelodysplastic Syndrome (MDS) & Plasma cell dyscrasias, were the commonest hematological neoplasms in the current study, comprising of 26.7% of overall cases (n=45) and 66.7% (n=12) of malignancies (n=18). The next common malignancies in this study were Myeloproliferative neoplasms (MPN) followed by Lymphoproliferative disorders (CLPD). **Table 3** shows the distribution of the hematological malignancies encountered in our study

Of the four cases of **acute leukemia**, three were diagnosed as Acute Myeloid Leukemia (AML) & one was Acute Lymphoblastic Leukemia (ALL). Further evaluation was done in only two cases. One was a 16-year-old male presenting with intracranial bleed, peripheral smear was showing 70% abnormal promyelocytes suggesting Acute Promyelocytic Leukemia (APML), flow cytometry performed showed positive moderate expression of the myeloid markers CD13, CD33, CD117, cMPO & CD64 while the FISH analysis revealed positive (variant) status for PML RARA: t(15;17) in 75% of interphase cells, further confirming the diagnosis of APML. (**Figure 2**)

The other was a 56-year-old female who came with pancytopenia; the peripheral smear did not show any abnormal cells. However, to our surprise, the bone marrow showed the presence of blast cells, Further immunohistochemistry (IHC) was done on BMB (IHC results- CD 20, CD 34, Tdt & CD10 were diffusely positive) and was labelled as CALLA positive B-Acute lymphoid leukemia. (**Figure 3**)

Four patients presenting with pancytopenia on blood findings turned out to be **Myelodysplastic Syndrome (MDS)**. Further testing was done in two cases. In one case karyotype analysis revealed a composite karyotype in 15 cells out of 20, The other 5 metaphase cells revealed apparently normal karyotype. tp53 was positive. In other case karyotyping revealed normal Karyotype & MDS Panel by FISH analysis revealed negative status for deletion.

Anemia was the common mode of presentation in all four cases of **plasma cell dyscrasias**, bone marrow was hypercellular in all the cases with an increase in the plasma cells noted. IHC performed on the biopsy had a strong and diffuse positivity for CD138 and MUM1 with either Kappa or Lambda restriction. Serum protein electrophoresis in three cases showed the presence of Monoclonal band suggestive of multiple myeloma. **(Figure 4)** One case was diagnosed as monoclonal gammopathy of undetermined significance (MGUS).

There were three cases of **Myeloproliferative neoplasms (MPN)**, further evaluation was done in all three. In one case Karyotyping by G-Banding revealed Philadelphia chromosome & FISH analysis also revealed BCR/ABL: Ph positivity (9q deletion variant) in 95% of interphase cells analysed. suggestive of Chronic Myeloid Leukemia (CML). **(Figure 5)**

In second case FISH analysis revealed **JAK-2 Mutation (V617F) Positive, {BCR/ABL:Ph, JAK-2 Exon 12, CALR and MPL mutation were negative}** & karyotype was normal, findings were suggestive of **JAK2 positive MPN**.

In third case Karyotyping revealed 47,XY,+8- Karyotype - showed Trisomy 8 & MPN Panel (BCR/ABL1, JAK-2, CALR & MPL Mutation) was negative suggestive of triple negative MPN (MPN unclassified).

Two elderly male patients came with leucocytosis, marrow was hypercellular in both showing excess of mature looking lymphoid cells. Flow cytometry performed showed CD20, CD5, CD23 and BCL2 positivity and negative for Cyclin D1 & BCL6 features suggesting of a B cell chronic lymphoproliferative disorder.

A 45 years female presented with massive splenomegaly and bicytopenia, bone marrow aspiration showed 35% atypical lymphoid cells. On IHC, CD 20, CD5 and BCL2 were Positive in neoplastic lymphocytes and CD 3, CD 10, CD23 and BCL6 were Negative. Ki67 showed Low index suggestive of bone marrow involvement by Low grade B cell Non Hodgkin Lymphoma. Further flow cytometry was done - CD5 & CD10 Negative Mature B - Cell Neoplasm. • In view of co-expression of CD19 with bright CD20 and Kappa restriction, a possibility of **Splenic Marginal Zone Lymphoma (SMZL)** was considered.

In the current study, **non-malignant hematological disorders** amounted to 21 cases. Among them, the commonest disorder was Immune Thrombocytopenic Purpura (ITP) (n=7) followed by erythroid hyperplasia (n=5), reactive plasmacytosis (n=5) & Aplastic anemia (n=4).

Seven cases in the study had severe thrombocytopenia (n=7, 15.6%) on presentation, all the secondary causes of thrombocytopenia were ruled out. Bone marrow examination revealed an increase in the megakaryocytes with immature forms seen. All the features pointing out to the diagnosis of **ITP**. Karyotyping was done in one case which was showing normal karyotype.

Of the five patients with anemia, bone marrow examination revealed erythroid hyperplasia which on further analysis were labelled as nutritional anemia in four cases (two each of megaloblastic anemia and iron deficiency anemia) and one patient had autoimmune hemolytic anemia (AIHA) which was direct Coombs test positive and had evidence of cold agglutinins in the serum.

Reactive plasmacytosis was seen in 5 (11.1%) cases, in all these cases plasma cells were in the range of 3-6% in the bone marrow without any light chain restriction or any other features otherwise suggesting of myeloma.

Aplastic anemia was seen in 4 (8.9%) cases, all these cases had pancytopenia, BMA and BMB were hypocellular with all the three cell lines suppressed. Karyotyping was done in two patients which showed normal karyotype. **(Figure 6)**

Non-hematological disorders were encountered in 2.22 % of cases (n=1). There was a single case of **metastasis** to the bone marrow, 25% atypical large cells were seen in marrow aspirate. Features were consistent with Metastatic Adenocarcinoma. Reflex IHC: Pan CK, CK 7, CEA were positive. CK 20, TTF 1, CDX 2, PAX 8 were negative, Findings were suggestive of primary in gastric or pancreaticobiliary tract.

FIGURE WITH LEGENDS

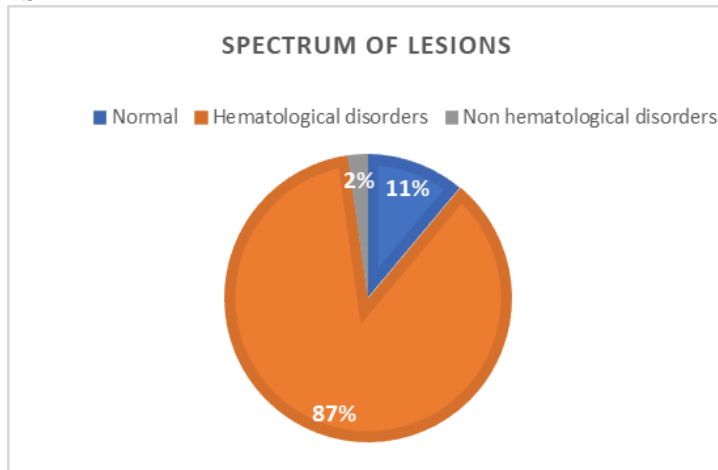


Figure 1

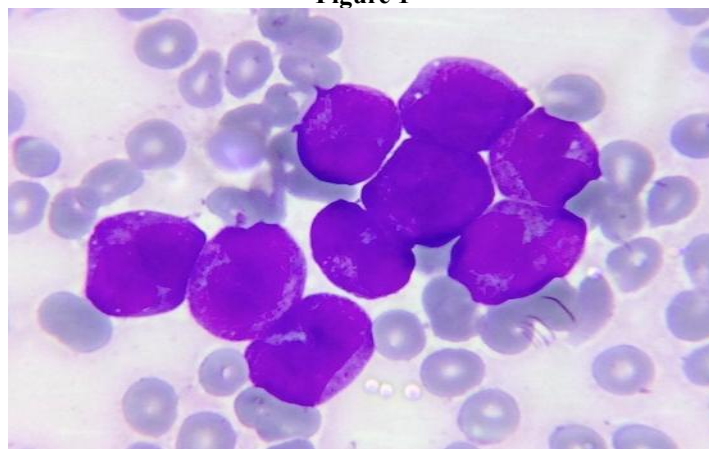


Figure 2

BMA Leishman Stain (Oil Immersion) -Acute promyelocytic leukemia

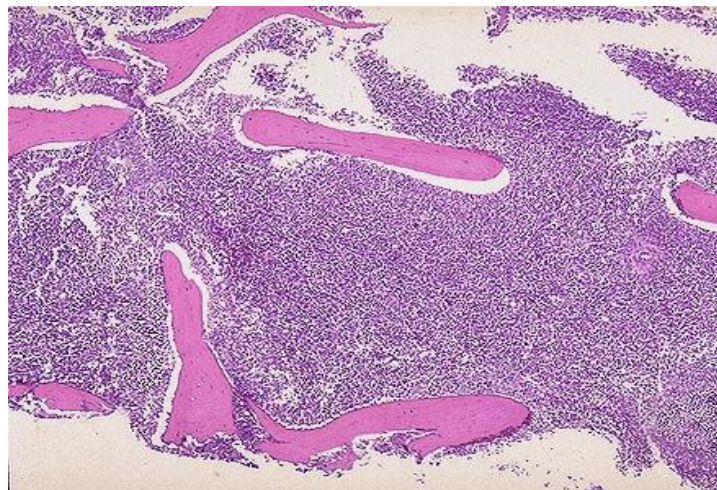


Figure 3

BMB Hematoxylin & Eosin Stain (40x) -Acute Lymphoblastic leukemia (ALL)

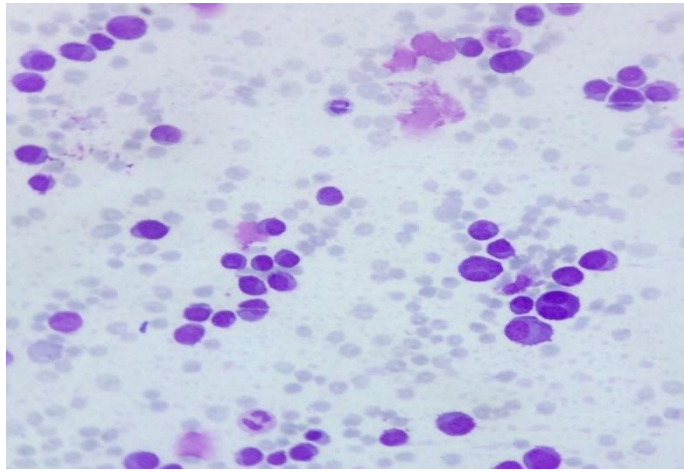


Figure 4

BMA Leishman Stain (400x)- Multiple Myeloma

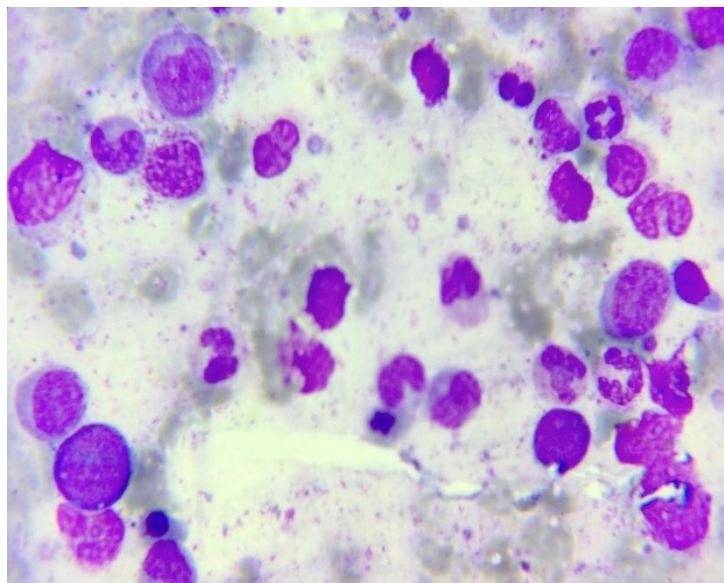


Figure 5

BMA Leishman Stain (Oil Immersion) - Chronic Myeloid Leukemia

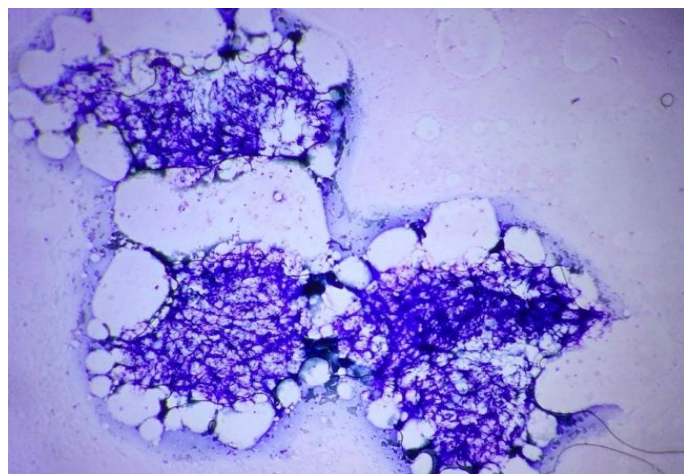


Figure 6

BMA Leishman Stain (100x) - Aplastic anemia

TABLES

Table 1: Indications for Bone marrow examination

Indications	n (%)
Anemia	10 (22%)
Thrombocytopenia	7 (15.6%)
Bicytopenia	8 (11.1%)
Pancytopenia	13 (28.9%)
Leucocytosis	9 (20%)
Organomegaly	2 (4.5%)

Table 2: Spectrum of diseases on bone marrow evaluation

Sr No	Spectrum of diseases	Total	n=45 (%)
1)	No Significant abnormality	5	5 (11.1%)
2)	Hematological disorders		
2A)	Malignant Hematological disorders		
	Acute Leukemia	4	4 (8.9%)
	Plasma cell dyscrasia	4	4 (8.9%)
	MDS	4	4 (8.9%)
	MPN	3	3 (6.7%)
	CLPD	2	2(4.5%)
	Lymphoma	1	1 (2.2%)
2B)	Non-Malignant Hematological disorders		
	ITP	7	7 (15.6%)
	Erythroid hyperplasia	5	5 (11.1%)
	Reactive plasmacytosis	5	5 (11.1%)
	Aplastic anemia	4	4(8.9%)
3)	Non Hematological disorders		
	Metastasis	1	1 (2.2%)

Table 3: Distribution of hematological malignancies

Sr No	Hematological Malignancies	Total	n=45 (%)
1	Acute Leukemia	4	4 (8.9%)
	AML	2	2 (4.5%)
	B-ALL	1	1 (2.2%)
	APML	1	1 (2.2%)
2	Plasma cell dyscrasia	4	4 (8.9%)
	Multiple Myeloma	3	3 (6.7%)
	MGUS	1	1 (2.2%)
3	Myelodysplastic syndrome (MDS)	4	4 (8.9%)
4	Myeloproliferative Neoplasm (MPN)	3	3 (6.7%)
	CML	1	1 (2.2%)
	MPN JAK2 positive	1	1 (2.2%)
	MPN Unclassified	1	1 (2.2%)
5	Chronic Lymphoproliferative disorder (CLPD)	2	2(4.5%)
6	Lymphoma	1	1 (2.2%)

DISCUSSION

Bone marrow examination is an essential and effective diagnostic technique for identifying a variety of hematological diseases. The diagnosis is established with the aid of clinical examination, peripheral smear findings and bone marrow investigations.^[5]

In our present study, in 45cases simultaneous BMA and BMB was done. In our study M: F was 1.25:1. Similar studies by Atchyuta et al, Vala et al showed M: F of 1.1:1 and 1.25:1 respectively. ^[6,1] In a study by Rathod et al (n= 572), 56.99% were males and 43 % were females with M:F=1.32:1 which was similar to our study. ^[3]

The **age** of the patients in our study **ranged** from 16 to 85 years, with a mean age of 54.42 years. In a similar study by Piplani G et al, the age ranged from 9 to 85 years, with a mean age of 50.22 years ^[7] A similar study conducted by Sanjana et al showed age range distribution from 4 years to 78 years. ^[5]

In our study the most common age group of presentation was 51 to 60 years, which accounted for 11% of the cases which was similar to study by Vala et al and Piplani G et al. ^[1,7]

The **most common indication** for a bone marrow examination was **pancytopenia** in our study (28.9%) which was similar to study done by Ojha et al & Chowdhury et al which also showed a similar finding where pancytopenia accounted for 27.8% & 33.67% of indications respectively. ^[8,9] Second most common indication in our study was anemia which accounted for 22 % cases. Our findings were concordant with Rathod et al, in which the most common indication was pancytopenia of 201 cases (35.13%) followed by anemia 167 cases (29.19%). ^[3]

In our study **hematological disorders** comprised 86.7 % cases, **non hematological** causes constituted 2.22% cases and the rest had no significant abnormality (11.1%). Similar findings were observed by Piplani G et al where hematological disorders were accounting for 92% followed by 6% of non hematological disorders and 2% were normal bone marrow. ^[7] Another study by Chowdhury et al (n=196 cases), 155 (79.08%) were hematological diseases and 10 (5.10%) were non-hematological diseases and normal marrows were 31 (15.82%). ^[9]

In our study among the hematological disorders (n=39, 86.7 %) the non-malignant hematological disorders [n=21 (46.7%)] were slightly more common than the malignant haematological conditions [n=18, (40 %)]. This was in contrast to the study by Chowdhury et al where malignant hematological conditions were more common [n=108 (55.10%)] and non malignant hematological conditions were [n=47 (23.98%)], however as per Atchyuta et al (n=375) cases, hematological diseases were 310 (82.66%), of which malignant cases were 100 (26.66%), non malignant were 210 cases (56.0%) and normal marrows were 39 (10.4%) findings which were similar to our study. ^[9,6]

Further literature search had studies by Anita T et al, Rajendra N et al; Fazlur R et al and Al –Ghazaly J et al in which the incidence of hematologic malignancies was to 18 %, 20.28%, 27.12% and 47.48% respectively. ^[6]

In our study **normal bone marrow** biopsy accounted for 11 % cases which was similar to studies by Atchyuta et al (10.4%), Vala et al (10.62%), Chowdhury et al (15.82%). ^[6,1,9]

In our study Acute leukemia accounted for 8.9% of overall cases and 22.2% of all hematological malignancies. It was similar to studies by Rathod et al, Dhanlaxmi et al where acute leukemia accounted for 8.77% and 9.6% of overall cases respectively. ^[3,5]

Out of four cases (8.9%) in our study of **acute leukemia**, three (6.7%) were AML and one (2.2%) case of ALL. In acute leukemias, AML was more common than ALL, similar findings were seen in study by Sanjana et al. ^[5]

In our study **MDS** accounted for 8.9% (n=4) of overall cases and 22.2% of all hematological malignancies. It was similar to study by Pudasani et al, Chowdhury et al and Ojha et al. where it was 3.5%, 6.45% and 7.1% respectively. ^[6,9,8]

In our study **plasma cell dyscrasias** accounted for 8.9% (n=4) of overall cases and 22.2% of all hematological malignancies. Similar studies by Anita et al, Vala et al, Kibria et al & Chowdhury et al showed similar values of 7%, 8.12%, 9.04% and 12.3% respectively. ^[6,1,9]

In our study **MPN** accounted for 6.7% of overall cases and 16.66 % of all hematological malignancies. Similar studies by Rathod et al, Vala et al, Rajendra N et al showed 4.13 %, 8.12 % and 8.57% respectively. ^[3,1,6]

In our study **CLPD** accounted for 4.5% of overall cases and 11.1% of all hematological malignancies, similar to study by Vala et al, Al-Ghazaly Jet al where it was 5.62% and 5.74% . ^[1,6]

There was a single case of lymphoma in our study accounting to 2.2% of overall cases and 5.55% of all hematological malignancies. Similar studies by Fazlur R et al, Rajendra N et al, Atchyuta et al where lymphoma cases were accounting to 1.17%, 1.42% and 1.8% of overall cases. ^[6]

In our study, **non-malignant hematological disorders** amounted to 21 cases (46.7%).

In our study **ITP** accounted for 15.6% of overall cases, these findings were similar to study by Chowdhury et al, Pudasaini et al, Piplani et al, Vala et al where it accounted for 9.68 %, 10.5 %, 10.86 % and 11.25 % respectively. ^[9,6,7,1]

In our study **Aplastic anemia** accounted to 8.9 % cases. In other studies by Atchyuta M et al, Pudasaini S et al, Gayathri BN et al, Khodke K & Ojha et al et accounted to 3.2 %, 5.3%, 19%, 14 % & 14 % of overall cases. ^[6,8]

In study by Chowdhury et al Aplasticanaemia (n=28, 14.29%) and immune thrombocytopenia (n=15, 7.65%) were the most common non-malignant hematological disorders which was similar to our study.^[9]

In **non-hematological disorders**, there was a single case of **metastasis** to the bone marrow accounting to 2.22 % of overall cases & its features were consistent with metastatic adenocarcinoma. In study by Piplani et al, Vala et al, Toi et al & Nandu et al the incidence was 2.18% , 2.5%, 2.6 and 2.8 % respectively.^[7, 1, 10]In study by Lalitha S M et al out of 1.7 % cases of metastatic tumors, one case was SCC & another was adenocarcinoma on biopsy.^[10]

CONCLUSION

Bone marrow aspiration and biopsy examination are complementary to each other and for diagnostic purpose both the procedures can be done simultaneous. In our study, pancytopenia was the most indication for bone marrow examination and the spectrum among these hematologic diseases showed that non malignantheamatological diseases were more common than hematologic malignancies. A complete clinical examination, patient history and investigations like peripheral blood examination along with bone marrow examination & ancillary test complete the diagnostic workup for many hematological conditions.

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