



Diagnostic Utility Of Bone Marrow Examination In Patients With Cytopenia In A Tertiary Care Hospital

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Abstract

Introduction: Bone marrow examination remains a cornerstone in diagnosing the underlying causes of cytopenia. The yield of bone marrow aspirates provides a valuable insight into the bone marrow cellularity, morphology and architecture which aid in identifying various haematological and non- haematological disorders. (1) Analysis of bone marrow biopsy is essential for correlation in cases especially where aspirate is haemodiluted or in cases of hypocellular marrows, fibrosis, lymphomas and granulomas amongst others. (2)

Aims and Objectives: This study aims to analyse the diagnostic utility of bone marrow examination in patients of cytopenia in a tertiary care hospital.

Materials and Methods: This retrospective study was carried out in the department of pathology, Lilavati hospital and Research Centre, Mumbai from January 2023 to March 2025. A total of 300 bone marrow aspirate samples of consecutive patients were morphologically analysed and assessed with bone marrow biopsy interpretation, immunophenotyping and molecular studies where applicable.

Results: Out of 300 cases, acute leukaemia (22%), reactive marrow (21%), myelodysplastic syndrome (15%), and plasma cell dyscrasia (11%) were the predominant diagnoses. Flow cytometry (n=83) most commonly identified acute leukaemia, mainly AML and ALL, while cytogenetic and FISH analyses revealed monosomy/deletion (5q, 7q, 20q) and translocations t(15;17) and t(8;21) as key abnormalities.

Conclusion: Bone marrow examination remains a vital tool for identifying the underlying causes of cytopenia, and guiding further diagnostic and therapeutic decisions. In our study, the most frequent marrow findings included acute leukaemia, myelodysplastic syndrome, plasma cell dyscrasia, megaloblastic anaemia, lymphomas, hypoplastic marrow, metastatic infiltration, and infections. Adjunct techniques such as immunophenotyping and cytogenetic analysis, significantly enhanced diagnostic precision, classification, prognostication, and specific management. Although advanced molecular assays and imaging modalities have reduced the routine need for marrow evaluation in conditions such as Hodgkin lymphoma or DLBCL, bone marrow examination continues to hold essential, focused value when non-invasive approaches are inconclusive or insufficient.

Keywords: Bone marrow, Aspiration, Biopsy, Flow cytometry, Leukaemia, Myeloma, Lymphoma, Cytopenia

Introduction

Bone marrow examination is a pivotal diagnostic procedure in the evaluation of both haematological and non-haematological disorders. Given the vast and diverse spectrum of haematological conditions, examination of the marrow serves as an indispensable tool in establishing a definitive diagnosis.

Bone marrow aspiration and bone marrow biopsy serve as complementary modalities in the evaluation of marrow pathology. Aspirate smears allow assessment of cellular morphology and differential cell counts, while simultaneously also enabling advanced ancillary investigations, including flow cytometry, cytogenetics, and molecular analysis. Bone marrow biopsy assumes particular diagnostic importance in instances where the aspirate results in a dry or haemodiluted tap, as it yields essential information regarding marrow architecture, overall cellularity, degree of fibrosis, and the pattern of distribution of abnormal infiltrates. [3]

Cytopenia may arise through various pathological mechanisms. It may result from ineffective haematopoiesis leading to premature intramedullary cell death, or from the production of morphologically or functionally defective cells. In certain instances, immune-mediated sequestration or destruction of hematopoietic elements contributes to cytopenia, while in others, otherwise normal cells become entrapped within a hypertrophied and hyperactive reticuloendothelial system. [4]

The etiological spectrum of cytopenia extends from reversible and treatable conditions, such as megaloblastic anaemia, infections to more complex and progressive disorders like myelodysplastic/myeloproliferative syndromes and lymphoproliferative disorders, which carry an increased risk of transformation to haematological malignancies. At the severe end of the spectrum lie overt malignant diseases, including leukaemia, lymphoma, multiple myeloma or invasive metastasis requiring prompt clinical intervention.

Aims And Objectives

This study aims to analyse the diagnostic utility of bone marrow examination in patients of cytopenia in a tertiary care hospital.

Primary Objective:

To evaluate the diagnostic result of bone marrow aspirate, flow cytometry and biopsy in patients presenting with cytopenia in a tertiary care hospital.

Secondary Objectives:

1. To classify the underlying disease causing cytopenia (e.g., aplastic anaemia, leukaemia, myelodysplastic syndromes, infections, etc.) identified through bone marrow examination.
2. To assess the role of ancillary tests such as bone marrow cytogenetics karyotyping and molecular studies on the bone marrow aspirate in the patients wherever applicable.

Materials And Methods

This study was conducted as a retrospective observational analysis over a period of two years in the Department of Laboratory Medicine and Histopathology at Lilavati Hospital and Research Centre from January 2023 to March 2025. The study population comprised both inpatient and outpatient cases referred for haematological evaluation in whom bone marrow aspiration was indicated. A total of 300 consecutive patients above the age of 18 years were included in this study.

Inclusion Criteria:

Patients with cytopenia (i.e. haemoglobin less than 11 g/dL in females and less than 12 g/dL in males, total leucocyte count less than 4000 and platelet count less than 1,50,000) undergoing bone marrow examination procedure. (2) If patient underwent bone marrow examination twice, data of only the first bone marrow (confirmation of diagnosis) was considered for the study.

Exclusion criteria: Patients with severe bleeding diatheses such as severe haemophilia or severe disseminated intravascular coagulopathy, local skin infections and in patients with severe osteoporosis. [5]

Procedure

Bone marrow aspiration procedure was carried out after obtaining written consent from the patients. Posterior superior iliac spine was the preferred site for aspiration under local anesthesia with supervision of

senior hematologist and all aseptic precautions were followed.

Clinical details including age, sex and clinical symptoms were noted from the patients requisition form. Patients with cytopenia were stratified based on haemoglobin, total leucocyte count and platelet count undergoing bone marrow examination procedure. Aspiration and imprint smears were air-dried and stained with Leishman-Giemsa stain and Perl's Prussian blue was used for assessing iron stores in cases of anaemia. The bone marrow trephine biopsy specimens were fixed in Acetic Zinc formalin (AZF) solution comprising acetic acid, zinc chloride and formalin for two to four hours and subjected to decalcification. They were then left overnight in Osteosoft solution which is mild decalcifying agent. Sections from the biopsy embedded in paraffin were cut and stained with haematoxylin and eosin. Immunohistochemistry (IHC) was also conducted in required cases for further categorization. Flow cytometry analysis: Immunophenotyping was performed on an eight-colour flow cytometer BD FACS Canto II using a monoclonal antibody panel for acute leukaemia, Chronic Lymphoproliferative Disorders (CLPD), Multiple myeloma and Paroxysmal Nocturnal Haemoglobinuria on bone marrow samples. Bone marrow aspirate was evaluated for cellularity, morphology, differential count, flow cytometry, FISH, Cytogenetics karyotyping where applicable and bone marrow biopsy interpretation. The 5th edition of WHO Classification of Haematolymphoid Tumours was used as a reference for diagnostic criteria and classifying disease entity. Interpretation of bone marrow aspiration, iron staining and flow cytometry were performed by two consultant hematopathologists who were unchanged throughout the process and the histopathology reporting was done by the same two consultant histopathologists throughout the assessment. Data was analysed using Microsoft Excel.

Results

In the present study, a total of 300 cases subjected to bone marrow examination over a period of two years were analysed retrospectively. The study population included patients above the age of 18 years, with a mean age of 54.5 years. The largest proportion of patients belonged to the 61 to 75 years age group, comprising 125 cases (41.67%), followed by the 31-

45 years age group with 61 cases (20.33 %). Among the 300 individuals evaluated, 138 (46%) were males and 162(54%) were females, yielding a male-to-female ratio of 0.85: 1, thereby reflecting a slight female predominance.

Among the 300 patients analysed, single-lineage cytopenia was the most frequent presentation, observed in 139 cases (46.3%), followed by bicytopenia in 98 cases (32.7%) and pancytopenia in 63 cases (21%).

Among the 139 cases assessed for single-lineage cytopenia, anaemia emerged as the most prevalent form of cytopenia, observed in 84 cases (60.4%). Bone marrow examination in cases presenting with anaemia predominantly revealed myelodysplastic syndrome, plasma cell dyscrasia, reactive marrow changes, megaloblastic anaemia, chronic myeloproliferative neoplasm, acute myeloid leukaemia, monoclonal lymphocytosis, and diluted marrow aspirates.

Thrombocytopenia was observed in 41 cases (29.5%). Bone marrow examination in these cases contributed to establish the diagnosis of plasma cell dyscrasia, hemophagocytic lymphohistiocytosis, immune thrombocytopenic purpura, acute myeloid leukaemia, reactive marrow changes, metastatic involvement, and diluted marrow aspirates. In our study, 14 cases (10.1%) were evaluated for leucopenia which resulted in diagnosis of acute lymphoblastic leukaemia, myelodysplastic syndromes, acute myeloid leukaemia, and plasma cell dyscrasia.

Analysis of clinical manifestations revealed that fever, often accompanied by generalized weakness and malaise, was the most frequently observed presenting symptom. Gastrointestinal complaints, including anorexia, nausea, and abdominal distension, were also commonly reported. Bleeding tendencies constituted another prominent feature with presentations such as epistaxis, gum bleeding, melena, hematemesis, haemoptysis, menorrhagia, and ecchymotic patches.

Examination findings revealed that pallor was the most frequently observed clinical sign among the study population, followed by hepatosplenomegaly. Icterus and oedema were also noted in a smaller proportion of patients.

On bone marrow examination, out of the 300 cases, 66 cases (22%) patients were diagnosed with acute leukemia, 63 (21%) were reactive marrow, 45 cases

(15%) were showing myelodysplastic changes and 33 cases (11 %) were diagnosed as plasma cell dyscrasia. In 3% cases the aspirate was either a dry tap or hemodiluted marrow samples and thus inconclusive for opinion.

Bone marrow biopsy interpretation showed that 63 cases (21%) were suggestive of acute leukemic infiltration, 54 (18%) were reactive marrow, 39 cases (13%) showed features of myelodysplastic syndrome and 36 cases (12 %) were diagnosed as plasma cell dyscrasia.

The six cases reported to have a metastatic involvement of bone marrow included breast carcinoma in three patients, carcinoma of the ovary, pulmonary adenocarcinoma and a poorly differentiated adenocarcinoma.

In cases where pyrexia of unknown origin constituted the primary indication for bone marrow examination, the underlying etiology identified included *Plasmodium falciparum* malaria, bone marrow involvement by Diffuse large B-cell lymphoma, tuberculosis, and parvovirus B19 infection.

These findings indicate strong concordance in the carefully selected cohort of patients undergoing bone marrow examination, reflected by a high diagnostic yield that facilitated accurate classification of hematologic disorders enabling timely and appropriate clinical management. This also highlights the value of judicious patient selection for bone marrow evaluation.

Table 1: Diagnostic findings of bone marrow aspirate and biopsy

S. No.	Diagnosis	Bone marrow aspirate findings (%)	Bone marrow biopsy findings (%)
1	Megaloblastic anemia	10	12
2	Acute leukemia	22	21
3	Myeloproliferative Neoplasm	6	4
4	Chronic Lymphoproliferative disorder	2	4
5	Hypoplastic marrow	4	5
6	Myelodysplastic syndrome	15	13
7	Plasma cell dyscrasia	11	12
8	Metastasis	1	2
9	Lymphoma infiltration	4	6
10	Parasitic Infection	1	1
11	Reactive Marrow	21	18
12	Others (Diluted marrow/ inadequate sample)	3	2

Table 2: Diagnostic findings on immunophenotyping of bone marrow aspirate samples

S. No.	Flow cytometry diagnosis	No. of cases (83)
1	Acute Myeloid Leukemia	23
2	Acute Lymphoid Leukemia	11
3	Plasma Cell Dyscrasia	22
4	Chronic Lymphocytic Leukemia	8
5	Others	19

Out of 300 cases, 83 cases were further analysed by flow cytometry, Acute Leukaemia (34%) was the most common finding on flow cytometry — primarily AML and ALL, showing characteristic antigenic profiles (e.g., CD34, CD117, HLA-DR, aberrant lymphoid markers). Plasma Cell Dyscrasias (22%) showed CD38+, CD138+, and kappa or lambda restricted light-chain patterns. CLPD (8%) was the next most frequent, commonly B-cell phenotypes typical of CLL/SLL.

Among the patients of acute leukaemia, AML (23%) was the predominant type, with 4 cases showing monocytic differentiation and one case was diagnosed as Mixed Phenotype Acute Leukaemia. Two cases of Acute Promyelocytic Leukaemia were identified by classic HLA-DR– and CD34– pattern and confirmed with morphology and translocation t(15;17)(q24;q21) (PML-RARA).

Analysis of Acute Lymphoblastic Leukaemia revealed B-ALL (8 cases) was the major lymphoid type, followed by T-ALL (3 cases).

Among the 124 cases analysed for bone marrow cytogenetics karyotyping, normal karyotype was the most frequent finding (74 cases), suggesting that most patients did not show major cytogenetic abnormalities detectable by routine karyotyping. Abnormal karyotypes were identified in a subset, predominantly showing monosomy or deletion abnormalities, specific translocations, trisomy, and complex karyotypes. These abnormalities were most frequently

associated with acute leukaemia and myelodysplastic syndromes, reflecting underlying clonal genetic instability. Monosomy 5/7 and complex karyotypes indicated poor prognosis, whereas translocations such as t(8;21) and t(15;17) correlated with favourable outcomes in specific AML subtypes. Trisomy, particularly trisomy 8 and trisomy 21, were observed in both AML and MDS, representing intermediate-risk changes. Overall, cytogenetic findings provided crucial diagnostic and prognostic insights complementing morphological evaluation especially when aspirate was inconclusive. A few cases had complex karyotypes or were inconclusive due to culture failure or poor metaphase yield.

FISH analysis of 112 cases out of 300 revealed the highest frequency of abnormalities in Myelodysplastic Syndrome (MDS), primarily involving deletions of 5q, Monosomy or deletion of 7q (–7 / del(7q), and 20q seen in 39 cases, making it the predominant abnormality. These changes are cytogenetic hallmarks of MDS, reflecting clonal hematopoietic stem cell disorders and often associated with ineffective haematopoiesis and cytopenia. Plasma Cell Dyscrasia (24.7%) was the next most frequent, with 13q, IGH, and TP53 abnormalities. Myeloproliferative Neoplasms (3.1%) showed BCR-ABL1 positivity. Acute leukaemia cases exhibited characteristic translocations such as t(15;17) and t(8;21). Others (8.2%) included scattered or unclassified abnormalities.

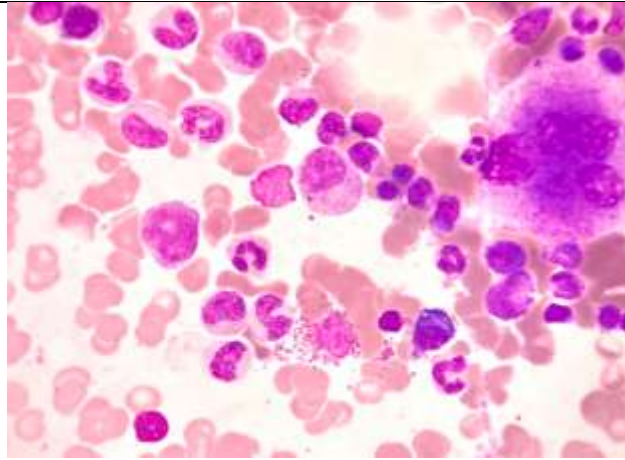


Image 1: Normal Bone Marrow Aspirate (100×): Normocellular marrow smear showing orderly trilineage haematopoiesis with normal maturation of erythroid, myeloid, and megakaryocytic precursors.

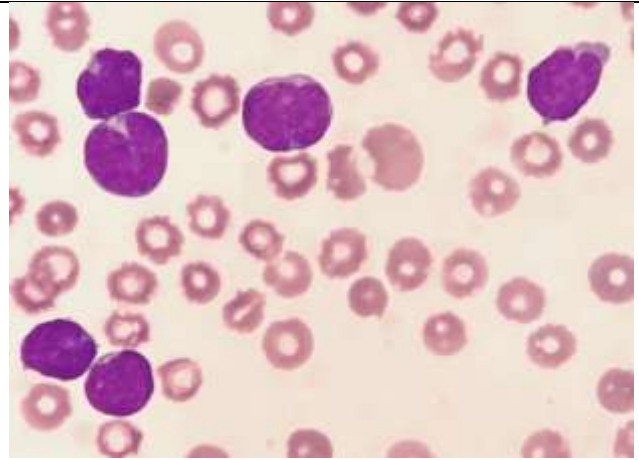


Image 2: Adult T-cell Acute Lymphoblastic Leukemia (100×): Hypercellular marrow with diffuse infiltration by lymphoblasts exhibiting high nuclear-to-cytoplasmic ratio, fine chromatin, and scant cytoplasm.

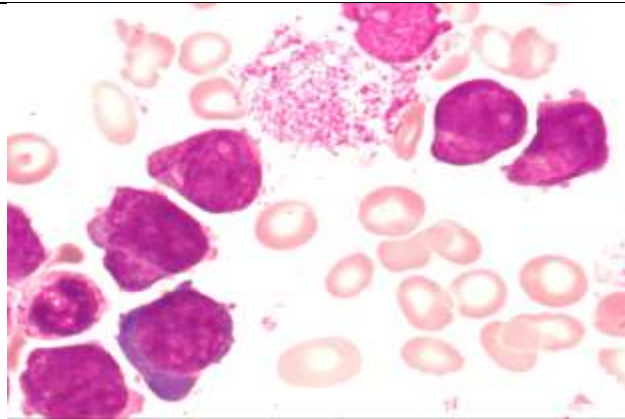


Image 3: Faggot Cell in Acute Promyelocytic Leukemia (100×): Abnormal promyelocyte containing multiple bundles of Auer rods (“faggot cell”), characteristic of acute promyelocytic leukemia.

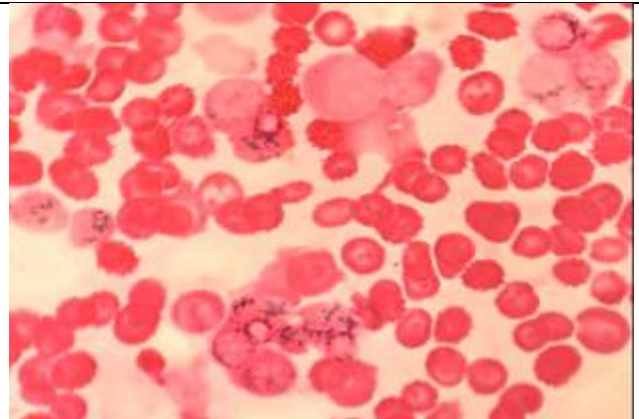


Image 4: Ring Sideroblasts in Myelodysplastic Syndrome (100×): Erythroid precursors demonstrating perinuclear iron-laden mitochondria arranged in a ring configuration (Prussian blue stain).

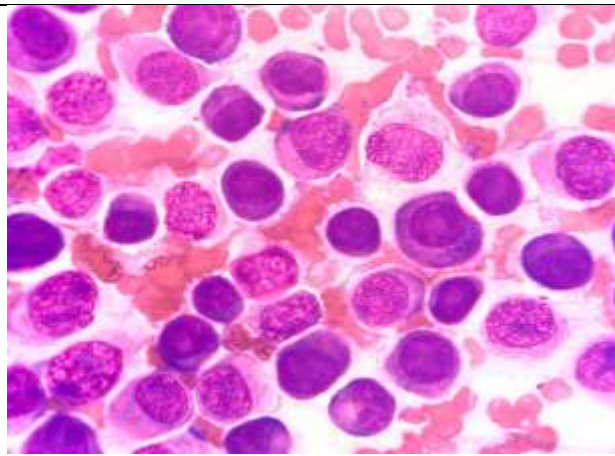


Image 5: Bone Marrow Metastasis – Carcinoma Breast (100×): Marrow infiltrated by cohesive clusters of malignant epithelial cells consistent with metastatic carcinoma.

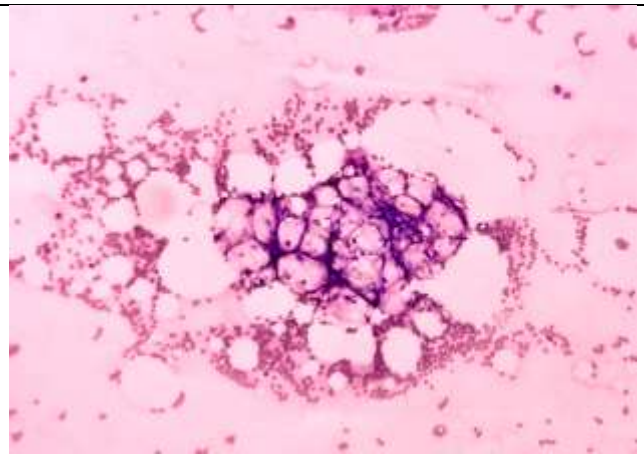


Image 6: Aplastic Anemia (10×): Hypocellular marrow with marked reduction of hematopoietic elements and replacement by adipose tissue.

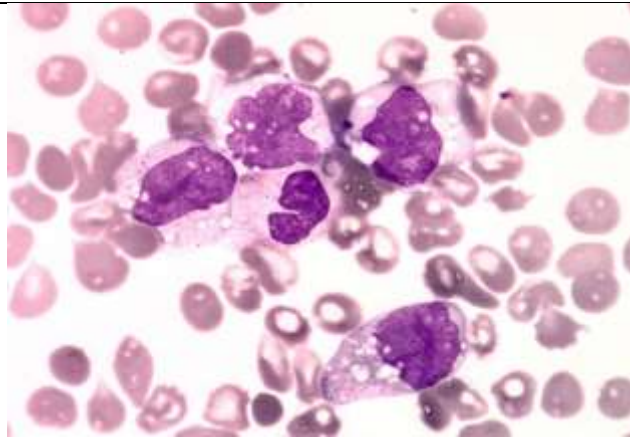


Image 7: Acute Myelomonocytic Leukemia (100×): Increased monocytic precursors with myeloid precursors, blasts and promonocytes, consistent with AMML.

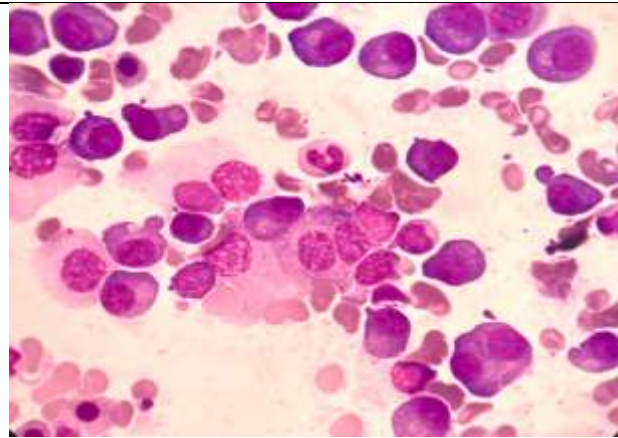


Image 8: Plasma Cell Dyscrasia (100×): Increased plasma cells with eccentric nuclei, coarse “clock-face” chromatin, and perinuclear hof, suggestive of plasma cell neoplasm along with binucleate forms.

Discussion

The bone marrow is involved in a multitude of haematological and non-haematological disorders. Bone marrow examination is a bedside; non-operative procedure performed on OPD basis or in the wards and intensive care unit under supervision using local anaesthesia. Among the haematological spectrum are conditions such as chronic anaemia, pancytopenia, aplastic anaemia, thrombocytopenic purpura, hypersplenism, acute leukaemia, myeloproliferative

neoplasms, and various haematolymphoid malignancies. Beyond these, the marrow may also bear the imprint of systemic, non-haematological diseases, including infections like tuberculosis, parasitic infestations, and secondary deposits from metastatic malignancies. Bone marrow examination is an important tool that aids in the diagnosis and directing the course of effective clinical management. [6]

Flow cytometry has emerged as a crucial tool in haematology owing to its rapid turnaround time and sensitivity in detecting aberrant cell populations. Immunophenotyping of bone marrow samples provides both quantitative and qualitative insights into hematopoietic cells, enabling precise characterization of their lineage and maturation patterns. Its principal applications in malignant haematology include the diagnosis, classification, and therapeutic monitoring of leukaemia, lymphomas, and plasma cell dyscrasias. Beyond these, it has also proven valuable in identifying disease-specific cell populations in paroxysmal nocturnal haemoglobinuria (PNH) expanding its role in diagnostic hematopathology. [7] Flow cytometry on peripheral blood samples can be diagnostic especially in elderly population and patients with severe thrombocytopenia, where marrow aspiration is avoided and treatment can begin sooner. In many cases in our study, flow cytometry for leukaemia was not done as no treatment was sought for various extraneous reasons.

Our current study included 300 consecutive patients above 18 years of age with male: female ratio of 0.85: 1 closely parallel those of Khunger et al. who conducted a study on 200 cases, reporting an age range of 2 to 70 years and a comparable female predominance with male-to-female ratio of 1.2:1.5 [8]. However, a study by Thiagarajan et al. [9] observed a similar age distribution pattern, with ages ranging from 8 to 90 years but a male predominance on comparison.

In the present study, acute leukaemia was the commonest haematological malignancy causing cytopenia (22%) [Table 1] which is in accordance with study of Jha et al in which it constituted 19.59% of total cases of pancytopenia. [10] In the study conducted by Mir et al acute leukaemia was the second most common cause present in 6.78% of total cases.[11] These results in the present study likely reflect that our institution functions as a tertiary referral centre for patients with haematological diseases. The concentration of these cases contributes to the increased diagnostic yield from bone marrow examinations observed in our study at our hospital in managing varied challenging hematological disorders.

In the study by Ranabhat S et al., 18.9% cases were diagnosed as haematological malignancy, 1.9% cases showed infection (1.9%), and 2.5% cases were other

miscellaneous diseases. [12] Dagdia et al. and Javalgi et al. in their studies found megaloblastic anaemia as the most common cause for pancytopenia seen in 29.3% and 72.6% cases, respectively [13,14]

In haematology, bone marrow examination remains an essential procedure for the differential diagnosis of various myeloproliferative and lymphoproliferative disorders, their prognostic classification, and the evaluation of disease status during and after therapy. It also plays a critical role in the grading of lymphomas and in identifying marrow infiltration by non-hematopoietic cells. In recent years, the scope of bone marrow investigation has expanded considerably, finding application not only in haematology but also in internal medicine, oncology, and osteology, where it serves as a valuable investigative tool in a wide range of systemic disorders. It plays a key role in diagnosis of malaria, parvovirus, CMV, LD bodies, Hemophagocytic Lymphohistiocytosis as well as in evaluating bone marrow in cases of Pyrexia of Unknown Origin.

However, the advent of FDG- positron emission tomography/computed tomography-staged treatment has largely rendered bone marrow aspiration and biopsy unnecessary for routine staging of *classical Hodgkin lymphoma*. Their role is now limited to exceptional or inconclusive cases, or where PET CT is not feasible. In classical Hodgkin lymphoma, the malignant Reed–Sternberg cells and their microenvironment are highly FDG-avid which means PET CT can reliably identify even small foci of marrow involvement. When PET CT shows bone marrow uptake, it almost always corresponds to true marrow disease confirmed histologically. In nearly all cases with marrow disease, PET CT already reveals advanced-stage disease elsewhere, so biopsy doesn't change staging or treatment. [15]

In many subtypes—especially *diffuse large B-cell lymphoma (DLBCL)*—PET CT now effectively identifies marrow involvement. Routine biopsy is no longer required if PET CT findings are conclusive. However, it may still be indicated in indolent lymphomas (e.g., follicular or marginal zone) where PET may underestimate low-volume marrow disease. [16]

In multiple myeloma, the need for repeat bone marrow biopsies has decreased with the introduction of serum free light chain assays, multiparametric flow

cytometry, and whole-body MRI or PET-CT including cases where biopsies aided by MRI or PET-CT have resulted in early diagnosis. Initial marrow evaluation remains essential for diagnosis, but serial monitoring is now often done via peripheral blood biomarkers and imaging rather than repeated invasive sampling. [17]

In chronic myeloid leukaemia (CML), bone marrow examination is no longer routinely performed other than at the time of initial diagnosis for assessing blast count, fibrosis and any additional cytogenetic abnormalities. In cases that come for follow up, quantitative PCR for BCR-ABL1 in peripheral blood provides accurate disease monitoring. [18]

Conclusion

Bone marrow aspiration is helpful for understanding the disease process and enabling the identification of the underlying etiology and in planning further investigations and management of cytopenia patients. In our study, bone marrow aspirate and biopsy results were used together with modern diagnostic technology to achieve a final diagnosis. The predominant findings observed included acute leukaemia, myelodysplastic syndrome, plasma cell dyscrasia, megaloblastic anaemia, lymphomas, hypoplastic marrow, marrow infiltration by metastatic disease and infection.

Moreover, flow cytometric immunophenotyping (FCM) serves as a crucial adjunct, providing precise characterization and classification of acute leukaemia, plasma cell dyscrasias and chronic lymphoproliferative disorders (CLPDs), thereby enhancing diagnostic accuracy and facilitating tailored management. Furthermore, cytogenetic evaluation provides definitive evidence of genetic alterations in various haematological malignancies, which help in understanding the prognostic index, patient's survival, and targeted therapy.

With the advent of advanced molecular and imaging modalities, the role of bone marrow examination has become more selective. In Hodgkin lymphoma and aggressive forms of Non-Hodgkin lymphoma such as DLBCL, PET/CT has largely replaced routine marrow biopsy for detecting marrow involvement. In chronic myeloid leukaemia (CML), quantitative PCR for BCR-ABL1 in peripheral blood now serves as the principal tool for disease monitoring, reducing the need for repeat marrow studies. Similarly, in multiple myeloma, modern assays such as serum free light

chain estimation, flow cytometry, and whole-body MRI/PET-CT have minimized the necessity for serial bone marrow evaluations. Overall, bone marrow examination remains an essential but increasingly targeted diagnostic procedure, reserved for cases where non-invasive modalities are inconclusive or unavailable.

1. Bone marrow examination has major utility in diagnosing a haematological disorder, malignant or otherwise.
2. It is a simple bed side or OPD procedure mostly performed on posterior superior iliac spine under local anaesthesia.
3. It enables diagnostic accuracy for timely and appropriate management of various haematological disorders.
4. It includes aspiration for morphological interpretation of cells, bone marrow biopsy for additional details to add to or corroborate aspiration findings and study of cytogenetics, immunophenotyping of malignant cells and other genetic studies along with iron staining for an accurate diagnosis so as to maximise an invasive procedure.
5. Bone marrow examination can be avoided if peripheral blood can provide an accurate diagnosis which allows appropriate management.
6. Hodgkins's lymphoma undergoing PET CT scan need not have routine bone marrow examination done, but it is still done for NHL and CML cases for additional information.
7. It has a high diagnostic utility in Hairy cell Leukaemia, low grade lymphomas and in pyrexia of unknown origin for better diagnosis.
8. With advent of better non – invasive diagnostics such as NGS, RTPCR, flow cytometry and PET CT scan, repeated marrow examination for disease monitoring may no longer be necessary.
9. Judicious selection of patients for bone marrow examination is vital.

Ethical Approval: Approval from the Institutional Ethics Committee for research was taken before the conduct of the study [Approval No. EC LHRC-252/ECBHR- 14/062023- 3 (a)(1)]

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