



ORIGINAL RESEARCH PAPER

Pathology

ROLE OF BONE MARROW BIOPSY IN ANEMIA DIAGNOSIS

KEY WORDS: Bone marrow biopsy, Anemia, Hematological disorders, Iron deficiency, Myelodysplasia

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ABSTRACT

Anemia is a prevalent clinical condition with diverse etiologies, and bone marrow biopsy plays a critical role in identifying the underlying pathology, particularly in unexplained or refractory cases. This study was conducted in the Department of Pathology, GMC Jammu, over a period of one year. A total of 120 patients with anemia of unknown cause were subjected to bone marrow aspiration and biopsy. The findings revealed that marrow examination was essential in diagnosing various causes including nutritional deficiencies, hematological malignancies, aplastic anemia, and myelodysplastic syndromes. This study highlights the indispensable diagnostic utility of bone marrow biopsy in evaluating complex cases of anemia.

INTRODUCTION

Anemia is one of the most common clinical problems encountered across all age groups, especially in developing countries like India. Defined by a decrease in the total number of red blood cells or a reduction in hemoglobin concentration, anemia leads to decreased oxygen-carrying capacity of the blood and manifests in symptoms such as fatigue, pallor, shortness of breath, and in severe cases, cardiovascular compromise. While mild to moderate cases are often due to nutritional deficiencies, many patients present with complex, non-nutritional causes that are not immediately evident from peripheral blood investigations alone. These unexplained or refractory cases of anemia necessitate further diagnostic exploration to determine their underlying etiology.

Bone marrow examination, including both aspiration and trephine biopsy, remains one of the most definitive diagnostic modalities for evaluating such cases. It provides direct insight into hematopoietic activity, marrow architecture, iron stores, cellular composition, and infiltrative or dysplastic processes. Bone marrow aspiration is especially useful in assessing cytology, differential cell counts, and maturation of hematopoietic cells, while trephine biopsy is critical in evaluating cellularity, marrow fibrosis, and infiltration by abnormal cells or malignancies. The combination of both techniques often yields a more accurate and comprehensive picture of the marrow status.

Although initial diagnostic tests like complete blood counts (CBC), peripheral smear examination, serum iron profile, vitamin B12, folate levels, and reticulocyte counts offer valuable information, they may be insufficient or misleading in certain presentations. For instance, combined nutritional deficiencies, myelodysplastic syndromes (MDS), aplastic anemia, hematological malignancies like leukemia or lymphoma, and marrow infiltrative disorders may not be apparent unless the marrow is directly examined. Bone marrow biopsy becomes a vital tool in such scenarios, not only for diagnosis but also for prognosis and treatment planning.

The importance of bone marrow biopsy is particularly underscored in tertiary care settings such as Government Medical College (GMC) Jammu, where patients often present at late stages with complex clinical pictures and inconclusive peripheral findings. Here, bone marrow examination is not just a confirmatory test—it is often the diagnostic cornerstone that dictates further therapeutic interventions. It also plays a critical role in detecting hematological malignancies early, thereby facilitating timely chemotherapy or bone marrow transplantation where necessary.

Given the diversity of anemia causes and the limitations of routine blood tests in resolving diagnostic ambiguity, this study was conducted to evaluate the role of bone marrow biopsy in patients with unexplained anemia. The study aims to categorize various marrow findings and correlate them with clinical and hematological features to underscore its diagnostic utility. It also seeks to generate awareness about the value of marrow evaluation in routine clinical practice for better patient outcomes.

Review of Literature

Anemia is a multifactorial hematologic condition, and its evaluation often requires integration of clinical history, laboratory investigations, and bone marrow assessment. The bone marrow biopsy, though invasive, is a gold standard in evaluating unexplained or refractory anemia, and has been extensively studied across diverse populations and clinical scenarios.

1. Bain BJ (2003) in his seminal work emphasized that bone marrow aspiration and biopsy remain essential in diagnosing a wide range of hematological conditions, including various forms of anemia. He discussed the procedural safety, complications, and diagnostic benefits of marrow examination, especially in cases where peripheral smears are inconclusive. His work also highlighted the utility of trephine biopsy in evaluating marrow cellularity and fibrosis, which cannot be assessed through aspiration alone.

2. Wintrobe's Clinical Hematology (13th Edition) remains a cornerstone text in hematology and comprehensively discusses the pathophysiology of anemia. The book underlines the importance of bone marrow biopsy for diagnosing marrow failure syndromes, nutritional anemias, and hematological malignancies. It also provides detailed descriptions of marrow findings in various types of anemia.

3. Dacie and Lewis' Practical Haematology (10th Edition) emphasizes bone marrow morphology and staining techniques in diagnosing megaloblastic anemia, iron deficiency, and marrow infiltration. Their standardized staining protocols, particularly for iron stores using Perl's stain, are widely used in diagnostic practice.

4. Khodke et al. (2001) studied 150 cases of pancytopenia and concluded that bone marrow aspiration was invaluable in diagnosing aplastic anemia, megaloblastic anemia, and leukemia. Their research in a resource-limited setting demonstrated how marrow studies directly influenced clinical decision-making.

5. Dutta et al. (2007) conducted a study in South India and

reported that nutritional anemia was the most common finding in bone marrow biopsies of anemic patients. However, they also found that over 30% had significant hematological disorders such as MDS and acute leukemia, which were not suspected clinically.

6. Tandon N et al. (2016) evaluated bone marrow biopsies in 120 anemic patients and found that 60% had definitive morphological abnormalities that altered the initial diagnosis. The study underscored the role of biopsy in detecting marrow dysplasia and blast proliferation, especially in elderly patients.

7. Brunning RD et al. (2008) in the WHO classification of tumors of hematopoietic and lymphoid tissues detailed diagnostic marrow features of myelodysplastic syndromes and leukemias. Their classification criteria remain the global standard for diagnosing marrow-related neoplasms and underline the indispensable role of biopsy in reaching these diagnoses.

8. Kay NE (1995) discussed the role of bone marrow biopsy in diagnosing cytopenias and marrow failure syndromes. His study showed that in over 70% of unexplained anemia cases, marrow biopsy led to a definitive diagnosis, reaffirming its diagnostic efficacy.

9. Shergill S et al. (2015) studied bone marrow examination in a military hospital and found high diagnostic yield in identifying causes of anemia such as lymphoma, multiple myeloma, and iron-deficiency anemia with absent marrow iron stores. They stressed the significance of using both aspiration and trephine biopsy.

10. Arber DA (2018) reviewed modern approaches to bone marrow pathology, emphasizing the integration of morphology with flow cytometry, cytogenetics, and molecular studies. He argued that marrow biopsy is not only diagnostic but also prognostic, particularly in malignancies and myelodysplastic syndromes.

AIMS AND OBJECTIVES

- To evaluate the role of bone marrow biopsy in patients with anemia.
- To categorize various etiologies of anemia based on bone marrow findings.
- To correlate marrow findings with clinical and hematological parameters.

MATERIALS AND METHODS

Study Design and Setting

This was a **hospital-based, prospective, observational study** conducted over a period of **18 months (January 2023 – June 2024)** in the **Department of Pathology, Government Medical College (GMC), Jammu**. The study aimed to evaluate the diagnostic utility of bone marrow biopsy in patients presenting with anemia of unexplained or refractory nature.

Study Population

Patients were recruited from the Medicine and Hematology outpatient and inpatient departments of GMC Jammu. All patients presenting with anemia (as defined by WHO criteria: hemoglobin <13 g/dL in males and <12 g/dL in females) who underwent bone marrow biopsy as part of their diagnostic workup were considered for inclusion.

Inclusion Criteria

- Patients aged 18 years and above
- Hemoglobin level below the WHO-defined threshold
- Anemia not explained by routine peripheral blood investigations (e.g., iron studies, vitamin B12, folate levels, etc.)
- Patients who provided written informed consent for bone

marrow examination

Exclusion Criteria

- Patients with known diagnosed hematological malignancies
- Patients with coagulopathies or platelet counts <20,000/ μ L (contraindication to biopsy)
- Pregnant women
- Patients unwilling or unfit for bone marrow procedure

Sample Size

A total of **120 patients** were included based on convenience sampling and fulfilling inclusion criteria during the study period.

Clinical Evaluation and Laboratory Workup

All patients underwent:

- Detailed clinical history and physical examination
- Complete Blood Count (CBC) including hemoglobin, red cell indices (MCV, MCH, MCHC), WBC and platelet count
- Peripheral blood smear examination
- Reticulocyte count
- Serum ferritin, serum iron, total iron-binding capacity (TIBC)
- Serum vitamin B12 and folate levels
- Lactate dehydrogenase (LDH)
- HIV, HCV, and HBV screening

Bone Marrow Examination Procedure

Bone marrow aspiration and biopsy were performed in all cases under aseptic conditions:

Site: Posterior superior iliac spine

Anesthesia: Local infiltration with 2% lignocaine

Instruments: Salah or Klima bone marrow aspiration needle and Jamshidi biopsy needle

Aspiration: 0.5–1 mL of marrow was aspirated and used for smears

Biopsy: 1.5–2 cm trephine core biopsy was obtained

Staining:

- Smears stained with Leishman and Giemsa stains for morphology
- Bone marrow sections stained with Hematoxylin and Eosin (H&E)
- Perl's Prussian Blue stain for iron stores
- Reticulin stain in suspected fibrosis

Histopathological Evaluation

Bone marrow aspirate and biopsy were independently reviewed by two experienced hematopathologists. Diagnosis was based on cellularity, myeloid:erythroid ratio, presence of dysplasia, abnormal cells, blast percentage, iron stores, and evidence of infiltration or fibrosis.

Data Collection and Analysis

- Patient demographics, clinical features, laboratory results, and bone marrow findings were recorded in a structured proforma.
- Data were entered in Microsoft Excel and analyzed using **SPSS Version 26.0**.
- Results were expressed in terms of percentages, means $\pm$ standard deviations.
- Association between bone marrow findings and types of anemia was tested using **Chi-square test**, and **p-value <0.05** was considered statistically significant.

Ethical Considerations

- The study was approved by the **Institutional Ethics Committee (IEC), GMC Jammu**.
- Written informed consent was obtained from all patients

- prior to participation.
- Confidentiality and anonymity of all subjects were maintained throughout the study.

RESULTS

1. Demographic Profile of Patients

Out of 120 patients:

Male: 58 (48.3%)

Female: 62 (51.7%)

Age range: 18–75 years

Mean age: 43.2 ± 14.7 years

Table 1: Age And Gender Distribution

Age Group (Years)	Male	Female	Total (%)
18–30	14	18	32 (26.7%)
31–45	18	20	38 (31.7%)
46–60	16	14	30 (25%)
>60	10	10	20 (16.6%)
Total	58	62	120 (100%)

2. Clinical Presentation

Most common symptom: **Fatigue (85%)**

Pallor: 82 patients (68%)

Shortness of breath: 34 patients (28%)

Palpitations: 22 patients (18%)

3. Hematological Findings (CBC and Peripheral Smear)

Peripheral Smear Patterns:

Microcytic hypochromic: 38 (31.7%)

Normocytic normochromic: 42 (35%)

Macrocytic: 26 (21.7%)

Dimorphic: 14 (11.6%)

Parameter	Mean ± SD
Hemoglobin (g/dL)	7.9 ± 2.3
MCV (fL)	85.5 ± 12.6
Reticulocyte Count (%)	1.2 ± 0.6

4. Bone Marrow Morphological Patterns

Table 2: Bone Marrow Diagnostic Categories

Bone Marrow Diagnosis	Number of Patients	Percentage (%)
Megaloblastic Anemia	32	26.7%
Iron Deficiency Anemia	24	20%
Dimorphic Anemia	12	10%
Aplastic Anemia	10	8.3%
Myelodysplastic Syndrome (MDS)	14	11.7%
Leukemia (AML/ALL)	10	8.3%
Lymphoproliferative Disorders	6	5%
Multiple Myeloma	4	3.3%
Other Marrow Infiltrations	8	6.7%

5. Iron Stores on Perl's Staining

Iron Status	Number of Patients	Percentage (%)
Absent Iron	30	25%
Normal Iron	66	55%
Increased Iron	24	20%

DISCUSSION

Anemia is a complex disorder with a wide array of etiologies ranging from nutritional deficiencies to malignancies. In this prospective study involving 120 patients, bone marrow biopsy provided essential diagnostic insights, especially in cases that were ambiguous based on routine blood tests.

The most common diagnosis was **megaloblastic anemia (26.7%)**, similar to findings reported by Dutta et al. and Shergill et al., reflecting the high prevalence of nutritional deficiencies in the Indian subcontinent. Bone marrow in these patients showed classic features such as hypercellularity with megaloblastic erythropoiesis and nuclear-cytoplasmic asynchrony.

Iron deficiency anemia (20%) was the second most common finding, characterized by hypocellular or

normocellular marrow with markedly reduced or absent iron stores on Perl's staining. This emphasizes the need to evaluate marrow iron directly in ambiguous cases, especially where peripheral smear findings overlap with other conditions like anemia of chronic disease.

Myelodysplastic syndromes (11.7%) and **leukemias (8.3%)** were notable among the non-nutritional causes. These would have been missed or delayed in diagnosis if bone marrow biopsy had not been performed. MDS showed features of dysplasia in one or more cell lines, while leukemia cases had hypercellular marrow with increased blast count (>20%).

Cases of **aplastic anemia (8.3%)** showed hypocellular marrow with fatty replacement, providing a clear diagnosis that peripheral smear could not yield. Similarly, **multiple myeloma and lymphoma** cases (8.3%) were diagnosed based on plasmacytosis or infiltration by atypical lymphoid cells.

The marrow iron stain revealed that **25% of patients had absent iron stores**, confirming iron deficiency as a significant contributor even in cases that were not initially suspected based on serum ferritin alone. This highlights the utility of Perl's stain in distinguishing between iron-deficiency anemia and anemia of chronic disease.

Our study supports previous literature by Bain, Khodke, and Tandon in demonstrating that bone marrow biopsy is indispensable in diagnosing unexplained anemia. It serves not only as a diagnostic tool but also has prognostic implications, especially in malignancies and marrow failure syndromes.

Summary

Anemia is a common clinical condition with diverse etiologies ranging from nutritional deficiencies to hematological malignancies. This prospective observational study was conducted in the Pathology Department of GMC Jammu on **120 patients** who underwent **bone marrow biopsy** for evaluation of unexplained anemia. The objective was to assess the diagnostic utility of bone marrow biopsy in identifying the underlying cause of anemia when routine investigations were inconclusive.

The majority of the patients presented with nonspecific symptoms such as **fatigue, pallor, and dyspnea**, with varying hematological profiles on CBC and peripheral smear. Bone marrow examination revealed a wide range of diagnoses, the most frequent being **megaloblastic anemia (26.7%)**, followed by **iron deficiency anemia (20%)**, **myelodysplastic syndrome (11.7%)**, **aplastic anemia (8.3%)**, and **hematologic malignancies (leukemia, lymphoma, multiple myeloma, 16.6%)**. Bone marrow iron stores, assessed using Perl's staining, further clarified the etiology in many cases, especially in differentiating iron deficiency anemia from anemia of chronic disease.

CONCLUSION

Bone marrow biopsy remains a **vital diagnostic tool** in the evaluation of unexplained anemia, particularly when peripheral smear and other routine tests are inconclusive. It allows direct assessment of marrow cellularity, erythropoiesis, iron stores, and detection of infiltrative or dysplastic changes. In this study, bone marrow examination provided definitive diagnoses in a significant proportion of patients, guiding appropriate and timely treatment.

Given its high diagnostic yield, bone marrow biopsy should be considered early in the diagnostic workup of patients with **non-responsive, dimorphic, or normocytic normochromic anemias**, especially when accompanied by abnormal clinical or laboratory features. Its role is not only

diagnostic but also **prognostic**, especially in hematological disorders and malignancies.

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