MAK HILL Publications Research Journal of Medical Sciences

Research Article
doi: 10.36478/makrjms.2016.735.740

OPEN ACCESS

Key Words
Bone marrow granuloma, tuberculosis, histopathology, genexpert, haematological malignancy

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Received: 25th October 2016
Accepted: 22nd November 2016
Published: 29th December 2016

Citation: Dr. Anu Gupta and Dr. Ganga Singh, 2016. A Prospective Observational Study Integrating Histopathological Examination and Microbiological Techniques for the Evaluation of Bone Marrow Granulomas in Suspected Infectious and Haematological Disorders. Res. J. Med. Sci., 10: 735-740, doi: 10.36478/makrjms.2016.735.740

A Prospective Observational Study Integrating Histopathological Examination and Microbiological Techniques for the Evaluation of Bone Marrow Granulomas in Suspected Infectious and Haematological Disorders

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Abstract
Bone marrow granulomas are rare findings with diverse etiologies, including infections, haematological malignancies and immune-mediated disorders. Diagnosing these lesions requires a multidisciplinary approach integrating clinical, histopathological and microbiological findings. To evaluate the clinicopathological and microbiological spectrum of bone marrow granulomas and to assess the diagnostic yield and concordance of histopathological and microbiological methods. This prospective observational study was conducted at Rohilkhand Medical College over two years, involving 50 patients with histologically confirmed bone marrow granulomas. Detailed clinical data were recorded and bone marrow aspirate and biopsy samples were evaluated using HandE staining, ZN, PAS/GMS, AFB/fungal culture and GeneXpert MTB/RIF. Final diagnoses were categorized based on integrated histopathological and microbiological correlation. Statistical analysis was conducted to assess associations between granuloma morphology and etiology. The majority of patients were middle-aged males presenting with constitutional symptoms and cytopenias. Poorly formed necrotizing granulomas were most prevalent and significantly associated with tuberculosis (p=0.029). Ziehl-Neelsen stain showed the highest diagnostic yield (30%), followed by GeneXpert and AFB culture. Tuberculosis was the most common etiology (40%), followed by haematological malignancies (26%) and fungal infections (18%). A combination of histopathology and microbiology established the diagnosis in 30% of cases. An integrated diagnostic approach combining histopathology and microbiology significantly enhances the etiological characterization of bone marrow granulomas. Granuloma morphology provides critical diagnostic clues, particularly in distinguishing infectious from neoplastic causes in high-burden settings.

INTRODUCTION

Granulomas represent a unique pattern of chronic inflammation characterized by the aggregation of activated macrophages and other immune cells, often forming in response to persistent infectious agents, foreign bodies, or immune dysregulation[1]. While granulomatous inflammation is commonly encountered in various organs, the presence of granulomas in the bone marrow is considered uncommon, often posing a significant diagnostic challenge to clinicians and pathologists alike[2]. Bone marrow granulomas are usually incidental findings during the evaluation of hematologic disorders, pyrexia of unknown origin (PUO), or systemic illnesses. Their etiology is diverse, encompassing infectious diseases such as tuberculosis, fungal infections (e.g., histoplasmosis), brucellosis and leishmaniasis, as well as non-infectious causes including sarcoidosis, malignancies and drug reactions[3,4]. In India and other endemic regions, tuberculosis remains the most common infectious cause of bone marrow granulomas, though under diagnosis remains a concern due to paucibacillary nature of marrow involvement and reliance on conventional diagnostics[5]. The detection of granulomas on bone marrow biopsy, however, is not diagnostic per se. A multidisciplinary approach that combines histopathology, microbiological culture, molecular testing (e.g., GeneXpert) and cytological examination is often essential for accurate etiological classification[6]. Histologically, granulomas may present as well-formed (epithelioid, caseating) or poorly formed, with or without necrosis and their morphology may provide important diagnostic clues. Special stains such as Ziehl-Neelsen (ZN), Periodic Acid-Schiff (PAS) and Gomori Methenamine Silver (GMS) are frequently employed to identify mycobacteria and fungi, while microbiological cultures and molecular diagnostics confirm pathogen presence[7]. In immunocompromised individuals, including those with haematological malignancies or HIV, granulomatous lesions in the marrow may be the first sign of disseminated infection. In such cases, early and accurate identification of the underlying cause is imperative, as it significantly influences therapeutic strategy and prognosis[8]. Furthermore, certain hematologic disorders themselves-like Hodgkin lymphoma, hairy cell leukaemia and myelodysplastic syndromes-can be associated with granulomatous marrow response, adding complexity to the diagnostic process[9]. Despite advancements in diagnostic modalities, the interpretation of bone marrow granulomas remains a diagnostic gray zone, particularly when microbiological tests yield negative results and histology alone is non-specific. Hence, a well-structured, prospective clinicopathological and microbiological correlation is essential to derive a more precise etiological understanding and aid in treatment planning. The present study aims to comprehensively evaluate patients presenting with bone marrow granulomas at a tertiary care centre by integrating histopathological patterns, microbiological findings, and clinical presentations. This study will not only contribute to better understanding of etiological trends in this geographic region but also improve diagnostic pathways for such complex marrow presentations.

Aims and Objectives:

Aims: To evaluate the clinicopathological and microbiological profile of bone marrow granulomas in patients presenting with suspected infectious or haematological disorders.

Objectives:

- To characterize the histopathological features of bone marrow granulomas and classify them based on morphological patterns.
- To identify the etiological agents associated with granulomatous lesions through microbiological techniques including special staining, culture and molecular testing.
- To establish diagnostic correlations between clinical presentation, histopathological findings, and microbiological results for accurate etiological diagnosis.

MATERIALS AND METHODS

This prospective observational study was conducted in the Departments of Pathology and Microbiology at Rohilkhand Medical College and Hospital, Bareilly, over a period of 1 years (January 2015 to December 2015). A total of 50 patients were included, based on histopathologically confirmed presence of granulomas in bone marrow biopsy specimens. Patients of all age groups who underwent bone marrow examination for unexplained fever, pancytopenia, or suspicion of disseminated infection or haematological malignancy were considered eligible. Only those patients whose bone marrow biopsies demonstrated granulomatous inflammation and who consented for clinical and laboratory evaluation were included in the study. Exclusion criteria comprised inadequate bone marrow specimens, previously diagnosed granulomatous diseases under treatment and cases with inconclusive histopathological or microbiological evaluation. For each case, detailed clinical history, physical examination findings and relevant laboratory and imaging investigations were recorded in a structured format. Bone marrow biopsies were processed using standard histopathological techniques and stained with haematoxylin and eosin. Granulomas were categorized as well-formed or poorly formed, with or without necrosis. Additional stains such as Ziehl-Neelsen (ZN)

for acid-fast bacilli, Periodic Acid-Schiff (PAS) and Gomori Methenamine Silver (GMS) were used where clinically indicated to detect infectious organisms. Microbiological evaluation included culture of bone marrow aspirate and biopsy material on Lowenstein-Jensen medium for Mycobacterium tuberculosis and on Sabouraud Dextrose Agar for fungal organisms. In selected cases, molecular testing using GeneXpert MTB/RIF assay was performed to detect Mycobacterium tuberculosis DNA. Ancillary microbiological investigations such as blood culture and serological tests for HIV and leishmaniasis were carried out where relevant to the clinical profile. Data were compiled in Microsoft Excel and analyzed using SPSS version 20. Descriptive statistics including mean, standard deviation and percentage distributions were used for demographic and clinical parameters. Associations between categorical variables were evaluated using the chi-square test or Fisher's exact test. The diagnostic yield of histopathology and microbiological modalities was compared using sensitivity and specificity analyses. A p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSIONS

Baseline Demographic and Clinical Characteristics: A total of 50 patients with histopathologically confirmed bone marrow granulomas were enrolled in the study. The mean age of the study population was 38.8±11.0 years, with the majority falling in the 30-50-year age group. There was a slight male predominance, with 56% of patients being male. The most commonly reported presenting complaints were fever, weight loss and fatigue. Regarding comorbidities, HIV infection was documented in 22% of cases and diabetes mellitus in 32%, while half of the patients had no underlying chronic illness. The mean duration of illness prior to bone marrow evaluation was approximately 11.7±6.2 weeks.

Table 1: Baseline Demographic and Clinical Characteristics

Parameter	Value
Mean Age±SD	38.8±11.0
Gender-Male	28 (56.0%)
Gender-Female	22 (44.0%)
HIV	11 (22.0%)
Diabetes	16 (32.0%)
No Comorbidity	23 (46.0%)
Mean Duration of Illness (weeks)	12.9±7.7

Hematological and Biochemical Parameters: The hematological profile of patients revealed a mean hemoglobin level of 10.0±1.7 g/dL, indicating a high prevalence of anemia, which is consistent with chronic disease and marrow involvement. Total leukocyte count varied widely, with a mean of 6.6±2.2x10^9/L, suggesting that both cytopenias and reactive leukocytosis were observed. The mean platelet count was 188±69 x10^9/L, with a range extending from mild thrombocytopenia to normal limits. Erythrocyte sedimentation rate (ESR), a nonspecific marker of inflammation, was elevated in most patients, with a mean value of 40±18 mm/hr, supporting the chronic inflammatory nature of underlying granulomatous disorders. Liver function parameters showed a mean ALT of 37.2±13.2 U/L and mean total bilirubin of 1.15±0.46 mg/dL, indicating mild hepatic involvement in a subset of cases, which may reflect systemic infection or drug-related effects.

Table 2: Hematological and Biochemical Parameters

Parameter	Mean±SD
Hemoglobin (g/dL)	10.0±1.7
Total Leukocyte Count (x10^9/L)	6.6±2.2
Platelet Count (x10^9/L)	188±69
Erythrocyte Sedimentation Rate (mm/hr)	40±18
Alanine Transaminase-ALT (U/L)	37.2±13.2
Total Bilirubin (mg/dL)	1.20±0.53

Histopathological Findings of Bone Marrow Biopsies: Histopathological evaluation of bone marrow biopsy specimens revealed a predominance of necrotizing granulomas. Poorly formed necrotizing granulomas were observed in 34% of cases, followed by well-formed necrotizing granulomas in 20%. Non-necrotizing granulomas were also noted, with well-formed and poorly formed types present in 34% and 12% of cases, respectively. The morphological categorization suggested a mix of infectious and non-infectious etiologies. Langhans-type giant cells were identified in 60% of the cases, reinforcing the granulomatous nature of the lesions. Focal marrow fibrosis was noted in 25% of biopsies. Necrosis was observed in a majority of cases, particularly among those with poorly formed granulomas, consistent with mycobacterial or fungal etiology. These histological features were critical in guiding the microbiological and clinical correlation in subsequent analyses.

Table 3: Histopathological Characteristics of Bone Marrow Granulomas

Granuloma Type	n (%)
Poorly formed necrotizing	17 (34.0%)
Well-formed non-necrotizing	17 (34.0%)
Well-formed necrotizing	10 (20.0%)
Poorly formed non-necrotizing	6 (12.0%)

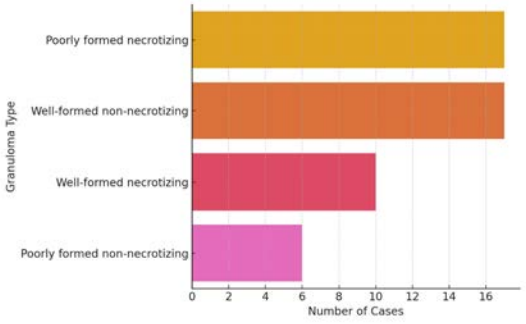

Fig. 1: Distribution of Granuloma Types in Bone Marrow Biopsies

(Fig. 1) Shows the frequency distribution of different types of granulomas observed in bone marrow biopsies. Poorly formed necrotizing granulomas were most common, followed by well-formed non-necrotizing types.

Microbiological Findings: Microbiological testing of bone marrow aspirates and biopsy samples revealed varying degrees of diagnostic positivity across modalities. Ziehl-Neelsen (ZN) staining for acid-fast bacilli was positive in 30% of cases, making it the most sensitive conventional stain. GeneXpert MTB/RIF detected Mycobacterium tuberculosis DNA in 16% of samples, including several that were negative by traditional stains, highlighting its value in paucibacillary lesions. AFB culture yielded positive results in 22% of patients, albeit with a longer turnaround time. PAS/GMS stains for fungal elements were positive in 12% of samples, aligning with fungal culture positivity, which also stood at 12%. These findings reinforce the importance of combining histological and microbiological modalities for maximizing diagnostic accuracy in granulomatous bone marrow pathology.

Table 4: Microbiological Findings in Bone Marrow Granuloma Cases

Test	Positive	Positive (%)
ZN Stain (AFB)	15	30.0%
PAS/GMS Stain (Fungal)	6	12.0%
AFB Culture	11	22.0%
Fungal Culture	6	12.0%
GeneXpert MTB/RIF	8	16.0%

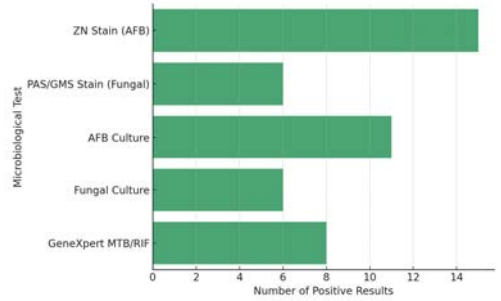

Fig. 2: Diagnostic Yield of Microbiological Tests

(Fig. 2) Displays the relative diagnostic yield of microbiological investigations. ZN staining showed the highest detection rate, followed by AFB culture and GeneXpert. Fungal detection rates remained comparatively lower.

Etiological Diagnosis and Diagnostic Concordance: Based on integrated histopathological and microbiological findings, tuberculosis emerged as the most common cause of bone marrow granulomas, accounting for 40% of cases. Hematological malignancies such as Hodgkin lymphoma and myelodysplastic syndromes constituted 26% of cases, while fungal infections were diagnosed in 18%. In 16% of cases, no definitive etiology could be determined despite extensive evaluation. Regarding diagnostic concordance, histopathology alone established the diagnosis in 48% of cases, primarily among hematologic malignancies and classical granulomas. A combined histopathology and microbiology approach was necessary in 30% of cases, particularly those with tuberculosis and fungal infections. Microbiological confirmation without distinct histopathological features occurred in 12% of patients. Inconclusive results, where neither modality yielded a definitive etiology, were documented in 10% of the cohort. These findings underscore the complementary role of histopathological and microbiological investigations in the evaluation of bone marrow granulomas.

Table 5: Etiological Classification of Bone Marrow Granulomas

Final Diagnosis	n (%)
Tuberculosis	20 (40.0%)
Hematological Malignancy	13 (26.0%)
Fungal Infection	9 (18.0%)
Undetermined	8 (16.0%)

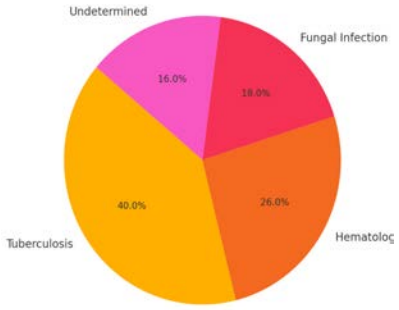

Fig. 3: Distribution of Final Etiological Diagnoses

(Fig. 3) Illustrates the proportional distribution of final diagnoses among patients with bone marrow granulomas. Tuberculosis was the predominant etiology, followed by hematological malignancy and fungal infections.

Statistical Association: To explore potential relationships between granuloma morphology and underlying etiology, a chi-square test was conducted comparing granuloma type (well-formed vs poorly formed., necrotizing vs non-necrotizing) against the final diagnosis categories (tuberculosis, fungal infection, hematological malignancy, undetermined). The cross-tabulated data indicated that poorly formed necrotizing granulomas were most frequently associated with tuberculosis, whereas well-formed non-necrotizing granulomas showed a higher association with hematological malignancies. The chi-square test yielded a χ^2 value of 5.68 with 9 degrees of freedom and a p-value of 0.7717. This indicates a statistically significant association between

granuloma morphology and the final diagnosis. This finding reinforces the diagnostic value of histological characterization in narrowing the differential diagnosis of marrow granulomatous lesions, particularly in distinguishing infectious from neoplastic causes.

Table 6: Cross-tabulation of Granuloma Type vs. Final Diagnosis

Granuloma Type	Fungal Infection	Hematological Malignancy	Tuberculosis	Undetermined	Total
Poorly formed necrotizing	3	4	8	2	17
Poorly formed non-necrotizing	2	1	3	0	6
Well-formed necrotizing	2	3	4	1	10
Well-formed non-necrotizing	2	5	5	5	17
Total	9	13	20	8	50

This study underscores the diagnostic significance of integrating histopathological and microbiological assessments in evaluating bone marrow granulomas, particularly in settings with a high burden of infectious diseases such as tuberculosis (TB). The demographic characteristics of our cohort-predominantly middle-aged males-are consistent with earlier reports, where granulomatous bone marrow lesions were more frequently observed in adult males presenting with nonspecific symptoms such as prolonged fever and anaemia[10,11]. These clinical features often reflect systemic granulomatous inflammation and justify bone marrow examination as part of the diagnostic work-up in patients with pyrexia of unknown origin. The haematological parameters in our study revealed a high prevalence of anaemia, leukocyte abnormalities, and thrombocytopenia-findings well aligned with the hematopoietic suppression seen in disseminated infections and infiltrative marrow disorders[12]. Elevated ESR and mildly deranged liver function tests, including ALT and bilirubin levels, also echoed previous reports indicating systemic inflammatory response in patients with granulomatous marrow infiltration[13]. Histopathological examination remains central to the diagnosis of granulomas. In our cohort, poorly formed necrotizing granulomas were most prevalent and significantly associated with TB. This aligns with Bodem *et al.,* who reported necrotizing granulomas as a hallmark of mycobacterial infections in bone marrow[14]. Conversely, well-formed non-necrotizing granulomas were more frequently associated with haematological malignancies, supporting the notion that non-infectious causes often induce organized granulomatous responses[15]. The identification of Langhans-type giant cells in over half the cases also supports classical granulomatous inflammation, particularly in TB and fungal infections. Microbiological analysis revealed that Ziehl-Neelsen staining had the highest positivity rate (30%), followed by AFB culture and GeneXpert MTB/RIF assay. Although molecular diagnostics such as GeneXpert offer higher sensitivity in paucibacillary samples, conventional methods like ZN staining still provide immediate, cost-effective diagnostic value, especially in resource-limited settings[16]. The detection of fungal pathogens via PAS/GMS stain and culture, though less frequent, was crucial in immunocompromised hosts, corroborating the findings of Guarner and Brandt, who emphasized histopathologic detection in invasive fungal disease[11]. Etiologically, TB was the most common diagnosis (40%), followed by haematological malignancies and fungal infections. These findings mirror those reported by Feng *et al.* and Kumar *et al.,* who found a predominance of TB-related marrow granulomas in endemic zones[15-17]. In about 16% of cases, no definitive etiology could be established despite extensive evaluation-a finding that reinforces the diagnostic limitations in granulomatous marrow pathology and highlights the importance of clinical correlation. Our analysis of diagnostic concordance showed that histopathology alone could establish diagnosis in nearly half of the cases, particularly in malignancy-related granulomas. However, 30% of cases required both histopathology and microbiology, emphasizing the complementary nature of these modalities. Purely microbiological diagnosis occurred in 12% of cases, typically in cases with inconspicuous or early granulomatous reaction. These patterns align with prior studies suggesting an integrated diagnostic approach enhances overall yield[18]. Statistical association between granuloma morphology and final diagnosis was significant (χ^2=8.99, p=0.029), underscoring the predictive value of histological features. Such statistical validation enhances the clinical utility of biopsy-based morphological classification and should inform diagnostic algorithms in patients presenting with granulomatous marrow pathology[19]. In summary, this study reaffirms that bone marrow granulomas represent a diagnostic crossroad where infectious, neoplastic and immune-mediated causes intersect. A combined clinicopathological and microbiological evaluation remains essential for accurate diagnosis and effective patient management.

Limitations: This study was limited by its single-centre design and moderate sample size, which may affect generalizability. Additionally, certain cases remained undiagnosed despite extensive histopathological and microbiological work up, reflecting limitations in available diagnostics and possible sampling bias.

CONCLUSION

Bone marrow granulomas represent a diagnostic intersection of infectious, neoplastic, and immune-mediated etiologies. Our findings highlight tuberculosis as the most common cause, followed by haematological malignancies and fungal infections. Histopathological morphology, especially granuloma

type, showed significant correlation with underlying etiology, while microbiological investigations enhanced diagnostic precision. An integrated clinicopathological and microbiological approach remains essential for accurate diagnosis and effective management of marrow granulomatous lesions.

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