





Original Article

From nodal to marrow: The diagnostic trail of lymphoma infiltration

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ABSTRACT

Objectives: The objective of the study is to study the patterns and degree of BMI in various subtypes of lymphoma diagnosed at a tertiary care center in Northern India.

Materials and Methods: This was a cross-sectional, observational study of 96 patients diagnosed with lymphoma over a period of 5 years (3 years retrospectively, 2 years prospectively) at AIIMS, Rishikesh. Bone marrow aspirate, trephine biopsy, and immunohistochemistry (IHC) were evaluated to assess marrow involvement. Cases were categorized by lymphoma subtype, pattern of infiltration, and extent of involvement.

Results: Of 96 cases, 49 (51%) showed BMI. BMI was more frequent in non-Hodgkin lymphomas (NHL) (41/76, 54%) than Hodgkin lymphomas (8/20, 40%). Among NHLs, small cell type NHL (e.g., chronic lymphocytic leukemia/small lymphocytic lymphoma) demonstrated a higher frequency and diffuse pattern of marrow involvement. In contrast, large B-cell lymphomas and T-cell lymphomas showed predominantly focal or interstitial infiltration. IHC was essential in subtyping and identifying marrow involvement in challenging cases.

Statistical analysis: Data were entered in Microsoft Excel 2019 and analyzed using descriptive statistics. Results were presented as tables and figures. Statistical analysis was performed using R software, employing Fisher's exact test and the chi-square test.

Conclusions: BMI is frequent in NHL, especially small cell subtypes. The pattern of marrow involvement correlates with the lymphoma subtype and may guide diagnostic and therapeutic decisions. Early and accurate evaluation of bone marrow and biopsy is critical in lymphoma management.

Keywords: Bone marrow infiltration, Fibrosis, Granuloma, Lymphoma, Necrosis

INTRODUCTION

Lymphomas represent a heterogeneous group of hematological malignancies characterized by the potential to disseminate throughout various organs, including the spleen, liver, lungs, and bone marrow. They account for approximately 3% of all malignancies and are broadly categorized into two types: Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). Of these, NHL constitutes the majority, accounting for 90% of cases globally, while HL comprises the remaining 10%. In India, the incidence of NHL is approximately 2.2/100,000 individuals, resulting in over 23,800 new cases annually.^[1-3]

Bone marrow biopsy (BMB) remains a cornerstone in the diagnosis, staging, and treatment planning of lymphoma. This is primarily due to the hematogenous spread of lymphoma cells

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to the bone marrow, making it a frequent site of secondary involvement.^[4,5] BMB enables direct assessment of marrow architecture and infiltration patterns, offering vital insights into disease extent and aggressiveness.^[6] Although imaging modalities such as computed tomography (CT), magnetic resonance imaging, and positron emission tomography-CT contribute to lymphoma evaluation, they are not definitive in assessing bone marrow involvement (BMI). Only histopathological examination can confirm microscopic infiltration, positioning bone marrow trephine biopsy as the gold standard.^[7,8]

In addition to its role in staging, BMB is particularly valuable when lymph node involvement is absent or when lymph node biopsy fails to provide adequate material for classification.^[9] In cases of unexplained cytopenias, the BMB may even serve as the first site of lymphoma diagnosis.^[10] The Ann Arbor staging system, with Cotswold's modifications, remains the standard for lymphoma staging, categorizing disease from stage I to IV. Involvement of extranodal sites such as the liver, lungs, or bone marrow automatically classifies the disease as stage IV, indicating advanced systemic dissemination.^[4,11,12]

BMI is observed in ~40% of all lymphoma cases.^[13] Specifically, studies have reported that 16-40% of NHL cases exhibit bone marrow infiltration.^[3,5,9] NHL remains a global health concern, with 544,000 new cases and 260,000 deaths reported in 2020.^[14] Among NHL subtypes, T-cell lymphomas tend to have a higher frequency of marrow involvement compared to diffuse large B-cell lymphoma (DLBCL).^[15] BMI in NHL is generally considered a marker of poor prognosis, indicating widespread disease. Furthermore, the histological pattern of marrow infiltration, such as diffuse, interstitial, or paratrabeular, has been associated with varying prognostic implications.^[16]

The posterior superior iliac spine is the preferred site for bone marrow sampling due to ease of access and adequacy of yield. Optimal marrow evaluation includes a combination of aspirate, trephine biopsy with touch imprints, and peripheral blood smears, each providing complementary diagnostic information.^[4] The diagnostic yield of BMB is also influenced by biopsy length, with specimens between 17 and 20 mm shown to be most effective in detecting infiltration.^[18] Therefore, BMB remains indispensable in the comprehensive evaluation of lymphoma, enabling the detection of occult marrow disease that may not be visualized through imaging alone.

Although BMB is regarded as the gold standard for detecting marrow involvement in lymphoma, available literature largely reports overall incidence or selected subtypes, with limited correlation of infiltration patterns, extent of involvement, and diagnostic yield of aspiration versus biopsy, and sparse data from tertiary care centers in the Indian subcontinent. In the era of advanced imaging, the relevance of BMB, particularly in HL, remains debated. In this context, this study presents the experience of a tertiary care institute

in North India (AIIMS, Rishikesh) and evaluates the frequency and prevalence of BMI across lymphoma subtypes, analysing patterns and degree of infiltration, concordance between aspiration and biopsy, and associated secondary marrow changes, thereby providing region-specific evidence supporting the continued diagnostic value of BMB.

MATERIALS AND METHODS

This observational, cross-sectional, exploratory study was conducted in the Department of Pathology and Laboratory Medicine at AIIMS, Rishikesh, over 5 years, comprising three retrospective and two prospective years. A total of 96 diagnosed cases of lymphoma undergoing bone marrow examination as part of staging work-up were included. The sample size was calculated assuming a prevalence of 0.5, with a 95% confidence interval and 10% absolute precision. Inclusion criteria were a confirmed diagnosis of lymphoma on lymph node biopsy with immunohistochemistry (IHC), availability of relevant clinical details, and an adequate BMB. Cases lacking IHC confirmation or insufficient trephine tissue were excluded. Bone marrow biopsies were obtained from the posterior superior iliac spine using a standard Jamshidi needle (11G or 13G) following local anesthesia. The biopsy cores were fixed in Bouin's solution, decalcified in ethylenediaminetetraacetic acid (EDTA), routinely processed, embedded in paraffin, and sectioned at 2-4 μ m thickness. Sections were stained with hematoxylin and eosin and examined independently by two pathologists. Marrow was evaluated for the presence, pattern, and extent of lymphoma infiltration, along with background changes such as granulomas, necrosis, and stromal fibrosis. Reticulin grading was done (Grade 0-3). BMI was assessed semi-quantitatively based on the proportion of lymphoma cells relative to total marrow elements and categorized as <10%, 10-50%, or >50%. Infiltration patterns were classified as diffuse, nodular, focal, paratrabeular, interstitial, or mixed, based on morphological features. IHC was performed when required, using formalin-fixed paraffin-embedded tissue sections. Following deparaffinization and Tris-EDTA-based antigen retrieval (pH 9.0), staining was done using a polymer-based horseradish peroxidase (HRP) system with 3, 3'-diaminobenzidine (DAB) chromogen and hematoxylin counterstaining. Marker panels included B-cell, T-cell, and Hodgkin-specific antibodies, with additional markers applied as indicated. Lymphoid aggregates were interpreted as reactive or neoplastic based on morphology and IHC staining pattern. Ethical clearance was obtained from the Institutional Ethical Committee (Ref No: AIIMS/IEC/21/726).

RESULTS

A total of 96 cases of lymphoma were studied, of which 20 (20.8%) were HL and 76 (79.2%) were NHL as shown in

Table 1. Among HL cases, classical HL (CHL) accounted for 90% and nodular lymphocyte predominant HL (NLPHL) for 10%. In NHL cases, B-cell lymphomas were predominant (88.2%), while T-cell lymphomas constituted 11.8% as shown in Table 2. The age of patients ranged from 6-80 years. The highest number of cases was observed in the 41-60 years of

Table 1: Incidence of bone marrow aspiration infiltration in different lymphoma histological subtypes.

Group	Infiltration (bone marrow aspiration)		
	Present (%)	Absent (%)	Total (%)
Hodgkin lymphoma	1 (3.0)	19 (30.2)	20 (20.8)
Non-Hodgkin lymphoma	32 (97.0)	44 (69.8)	76 (79.2)
Total	33 (100.0)	63 (100.0)	96 (100.0)

Table 2: Incidence of BMB infiltration in different lymphoma histological subtypes.

Lymphoma subtype	Infiltration BMB		
	Present (no. of cases)	Absent (no. of cases)	Total (%)
DLBCL	3	17	20 (26.3)
MCL	9	4	13 (17.1)
FL	5	1	6 (7.9)
LPL	5	1	6 (7.9)
SLL	6	0	6 (7.9)
MZL	5	2	7 (9.2)
PTCL	0	4	4 (5.3)
SMZL	4	0	4 (5.3)
BL	1	2	3 (3.9)
T-Lymphoblastic Lymphoma	1	1	2 (2.6)
ALCL	0	1	1 (1.3)
B-Lymphoblastic lymphoma	1	0	1 (1.3)
Enteropathy-associated T-cell lymphoma	0	1	1 (1.3)
Intravascular LBCL	1	0	1 (1.3)
Lennert lymphoma	0	1	1 (1.3)
CHL	7	11	18 (90)
NLPHL	1	1	2 (10)
Total	49	47	96 (100.0)

BMB: Bone marrow biopsy, DLBCL: Diffuse large B-cell lymphoma, MCL: Mantle cell lymphoma, FL: Follicular lymphoma, LPL: Lymphoplasmacytic lymphoma, SLL: Small lymphocytic lymphoma, MZL: Marginal zone lymphoma, PTCL: Peripheral T-cell lymphoma, SMZL: Splenic Marginal zone lymphoma, BL: Burkitt lymphoma, ALCL: Anaplastic large cell lymphoma, Intravascular LBCL: Intravascular large B-cell lymphoma, CHL: Classic Hodgkin's Lymphoma, NLPHL: Nodular lymphocytic predominant Hodgkin's Lymphoma

age group. Age distribution showed statistically significant differences between HL and NHL ($p < 0.001$). The overall male-to-female ratio was 2.2:1. Bone marrow aspiration (BMA) infiltration was noted in 33 cases (34.4%) and BMB infiltration in 49 cases (51.0%), as shown in Table 3. BMA infiltration was significantly higher in NHL than HL ($P = 0.002$), while BMB infiltration showed no statistically significant difference between HL and NHL ($P = 0.267$). Among HL subtypes, BMB infiltration was seen in 7 CHL and 1 NLPHL case. In NHL, BMB infiltration was most frequent in mantle cell lymphoma (9 cases), followed by small lymphocytic lymphoma (SLL) (6), and other subtypes, including follicular lymphoma (FL), lymphoplasmacytic lymphoma (LPL), marginal zone lymphoma (MZL), and splenic marginal zone lymphoma. Comparison of BMB and BMA showed that BMA had a sensitivity of 65.3% in detecting bone marrow infiltration. The patterns of infiltration on BMB included diffuse (42.9%), nodular (36.7%), interstitial (14.3%), intrasinusoidal (4.1%), and paratrabeular (2.0%) [Figure 1a-l]. Diffuse and nodular patterns were more common in B-cell NHL, while interstitial was the primary pattern in the single T-cell NHL case, with infiltration, as shown in Table 4. The extent of marrow involvement in positive cases showed >50% infiltration in 57.1%, 10-50% in 36.7%, and <10% in 6.1% of cases, as shown in Table 5. Secondary changes in marrow included necrosis in 6.2% and fibrosis in 6.2% of cases. No granulomas were observed, as shown in Table 6.

DISCUSSION

According to global cancer observatory (GLOBOCAN) 2020, NHL accounts for 544,000 new cases and 260,000 deaths annually.^[14] Globally, HL comprises ~10% of all lymphomas, with NHL constituting the remaining 90%. In India, the incidence of NHL is 2.2/100,000, with 23,801 new cases reported.^[1-3] The incidence is nearly twice as high in developed nations compared to those undergoing epidemiological transition.^[14]

In our study of 96 lymphoma cases, 76 NHL and 20 HL. NHLs were further sub-classified into B-cell and T-cell types using IHC. Consistent with prior literature, a male predominance was observed.^[1] Bone marrow infiltration (BMI) was detected in 51% of cases, a rate comparable to that reported by Kaur K *et al.* (55%),^[3] yet significantly higher than studies by Shi

Table 3: Association between “Infiltration (bone marrow biopsy)” and “Infiltration (bone marrow aspiration).”

Infiltration (bone marrow aspiration)	Infiltration (Bone marrow biopsy)		
	Present (%)	Absent (%)	Total (%)
Present	32 (65.3)	1 (2.1)	33 (34.4)
Absent	17 (34.7)	46 (97.9)	63 (65.6)
Total	49 (100.0)	47 (100.0)	96 (100.0)

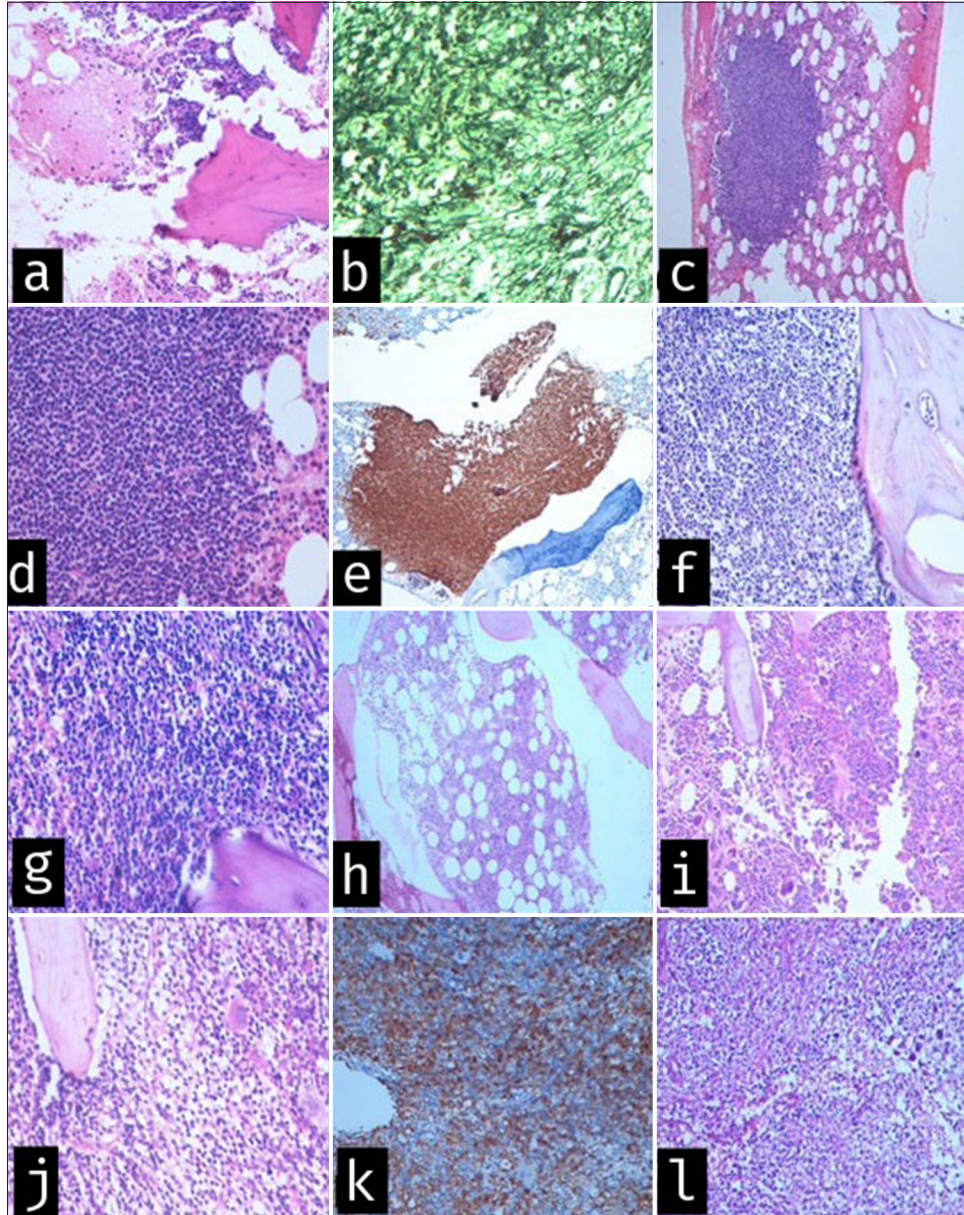


Figure 1: Bone marrow biopsy showing, (a) Areas of necrosis [Hematoxylin and eosin (H&E), $\times 200$] and (b) Fibrosis WHO Grade 2 (Reticulin stain), (c) Nodular pattern of involvement in MCL (H&E, $\times 100$), (d) $\times 400$, (e) Tumor cells are positive for CD20 $\times 100$, (f) Diffuse pattern of involvement in MCL (H&E, $\times 400$), (g) Diffuse pattern in SMZL (H&E, $\times 40$), (h) Interstitial pattern $\times 100$ (H&E stain), (i) Intrasinusoidal pattern in intravascular large B cell lymphoma (H&E), $\times 200$, (j) Diffuse pattern of involvement in FL (H&E, $\times 200$), (k) tumor cells are positive for CD10 $\times 200$, (l) CHL, Bone marrow biopsy show nodular polymorphic infiltrate comprising of histiocytes, lymphocytes, eosinophils, few plasma cells and scattered large mononuclear variant of RS cell (H&E, $\times 200$). WHO: World Health Organization, MCL: Mantle cell lymphoma, SMZL: Splenic Marginal zone lymphoma, FL: Follicular lymphoma, CHL: Classic Hodgkin's lymphoma, RS: Reed–Sternberg.

YF *et al.*, which reported 16%.^[5] Variations in infiltration rates could be attributed to sample size differences and the geographical distribution of lymphoma subtypes.

Histologically, diffuse infiltration was the most prevalent pattern (42.9%), followed by nodular (36.7%), interstitial (14.3%), intrasinusoidal (4.1%), and paratrabeular (2.0%) patterns. These

Table 4: Distribution of primary patterns of marrow involvement by subtypes of NHL.

Types of cases	No of cases	Biopsy involved cases	Diffuse	Nodular	Interstitial	Intra sinusoidal	Para trabecular
DLBCL	20	3	2	0	1	0	0
MCL	13	9	5	4	0	0	0
FL	6	5	2	2	0	0	1
LPL	6	5	3	1	1	0	0
SLL	6	6	4	2	0	0	0
MZL	7	5	2	0	3	0	0
SMZL	4	4	1	2	0	1	0
BL	3	1	1	0	0	0	0
T-LBL	2	1	0	0	1	0	0
B-LBL	1	1	1	0	0	0	0
Intravascular Large B-cell lymphoma	1	1	0	0	0	1	0
PTCL	4	0	0	0	0	0	0
ALCL	1	0	0	0	0	0	0
Lennert lymphoma	1	0	0	0	0	0	0
Enteropathy-associated T-cell lymphoma	1	0	0	0	0	0	0
Total	76	41	21	11	6	2	1

NHL: non-Hodgkin lymphoma, DLBCL: Diffuse large B-cell lymphoma, MCL: Mantle cell lymphoma, FL: Follicular lymphoma, LPL: Lymphoplasmacytic lymphoma, SLL: Small lymphocytic lymphoma, MZL: Marginal zone lymphoma, SMZL: Splenic marginal zone lymphoma, BL: Burkitt lymphoma, T-LBL: T-cell lymphoblastic lymphoma, B-LBL: B-cell lymphoblastic lymphoma, PTCL: Peripheral T-cell lymphoma, ALCL: Anaplastic large cell lymphoma

Table 5: Association between lymphoma subtype and extent of bone marrow involvement.

Subtypes	<10 (%)	10–50 (%)	>50 (%)
Diffuse large B-cell lymphoma	0 (0)	0 (0)	3 (100)
Mantle cell lymphoma	1 (11.1)	3 (33.3)	5 (55.6)
Follicular Lymphoma	1 (20)	1 (20)	3 (60)
Lymphoplasmacytic lymphoma	0 (0)	2 (40)	3 (60)
Small lymphocytic lymphoma	0 (0)	2 (33.3)	4 (66.7)
Marginal zone lymphoma	0 (0)	3 (60)	2 (40)
Splenic marginal zone lymphoma	0 (0)	2 (50)	2 (50)
Burkitt lymphoma	0 (0)	0 (0)	1 (100)
T- Lymphoblastic lymphoma	0 (0)	1 (100)	0 (0)
B-Lymphoblastic lymphoma	0 (0)	0 (0)	1 (100)
Intravascular large B-cell lymphoma	0 (0)	1 (100)	0 (0)
Classic Hodgkin's lymphoma	1 (14.3)	2 (28.6)	4 (57.1)
Nodular lymphocytic predominant Hodgkin's lymphoma	0 (0)	1 (100)	0 (0)

findings align with those of Sultan S *et al.*, who reported diffuse (46.3%) and nodular (20.6%) as the dominant patterns.^[2] The wide range in reported BMI incidence (16-75%) likely reflects heterogeneity in study designs and inclusion criteria.

Table 6: Secondary changes seen in lymphoma

Secondary changes	Lymphoma cases (96) (%)
Necrosis (yes)	6 (6.2)
Fibrosis (yes)	6 (6.2)
Granuloma (yes)	0 (0.0)

Among the 76 NHL cases, B-cell lymphomas were predominant (88.2%), with T-cell lymphomas accounting for 11.8%, consistent with Arber DA *et al.*, who found B-cell and T-cell incidences of 84% and 16%, respectively.^[29] A separate study on 659 cancer cases similarly identified B-cell NHL as the most common, followed by HL and T-cell NHL.^[21]

The BMI incidence in NHL was 53.9%, aligning with literature that reports rates between 29% and 69%.^[16] In our cohort, DLBCL was the most common subtype (26.3%), yet only 15% (3/20) showed marrow infiltration. Mantle cell lymphoma (MCL) constituted 17.1%, with a higher infiltration rate (22%). Other subtypes included MZL (9.2%), SLL (SLL, 7.9%), follicular and LPL (7.9% each), splenic MZL (5.3%), Burkitt's lymphoma (3.9%), and rare T-cell types including T-LBL, ALCL, EATL, and Lennert's lymphoma. MCL exhibited the highest marrow involvement, similar to findings by Shi YF *et al.* (18.3%).^[5]

Kumar S *et al.* identified FL (30%) as the most frequent subtype with marrow involvement, followed by DLBCL

(20%), MCL (15%), and others.^[21] The lower frequency of FL in our study may explain the divergence in findings. Shi YF *et al.*, however, found mantle cell lymphoma (18.3%) to be the most common NHL subtype infiltrating marrow.^[5] These discrepancies likely stem from variations in histologic subtype prevalence across study populations.

In terms of marrow infiltration patterns in NHL, diffuse was most common (51.2%), followed by nodular (26.8%), interstitial (14.6%), intrasinusoidal (4.9%), and paratrabeular (2.4%). These findings are consistent with those of Sultan *et al.* and Ishtiaq *et al.*, who also reported diffuse as the predominant pattern.^[2,16] However, Kumar S *et al.* reported a predominance of mixed patterns (51.8%).^[21] Secondary patterns were also observed, primarily interstitial in follicular and LPLs, with nodular in MZL.

In HL, the overall incidence of BMI was 40%, comparable to Lone and Naeem (38%),^[24] but higher rates were reported by Belukar (8.3%), Kirthi (18.4%), and Muthu (12.3%).^[25] Sample size differences may account for this variability. CHL was the most common subtype (18/20 cases), followed by NLPHL (2/20). Among those with marrow infiltration, CHL predominated (38.9%).

Histologically, nodular infiltration was most prevalent in HL (87.5%), followed by interstitial (12.5%), differing from Franco V *et al.*, who found diffuse and focal patterns.^[17] BMB proved superior to aspiration (BMA) for detecting HL involvement, as Reed-Sternberg cells were often missed on aspiration due to fibrosis and dry taps.^[25] In our study, seven of eight HL cases with marrow infiltration were diagnosed solely on biopsy, confirmed by CD30 and CD15 markers. Fibrosis was observed in five cases.

Out of 49 total infiltrated cases (HL + NHL), 32 (65.3%) showed concordance between BMA and BMB. However, 17 cases were detected only on biopsy. The low sensitivity of BMA could be attributed to hemodilution, clotting, or poor smear preparation.

Necrosis was noted in six lymphoma cases (one HL, five NHL), and fibrosis in another six (five HL, one NHL). No granulomas were identified, contrasting with Sovani *et al.*, who reported a higher granuloma incidence due to greater HL representation.^[8] Anandani GM *et al.* also observed necrosis and fibrosis as common findings in HL marrow infiltration.^[20]

To date, limited literature exists exploring correlations between secondary marrow changes and histological subtypes. Our study highlights the critical role of BMB in accurate staging, especially in HL, and underscores the heterogeneity of infiltration patterns across lymphoma subtypes.

Despite advances in imaging modalities, our findings demonstrate that BMA alone missed 34.7% of infiltrated cases, reinforcing the continued relevance of trephine biopsy, particularly in HL and low-grade NHL.

CONCLUSIONS

BMI is a critical factor in the diagnosis and prognosis of lymphoma. In this 5-year study of 96 cases, 51% showed marrow infiltration, predominantly among low-grade B-cell NHL subtypes such as MCL and SLL. Diffuse infiltration was the most common pattern. BMB outperformed aspiration, which missed 35% of positive cases, highlighting its role as the diagnostic gold standard. Integrating biopsy with IHC enhances detection accuracy. Although limited by sample size, the findings reinforce the value of marrow evaluation in lymphoma staging and management. Larger multi-center studies are warranted.

Ethical approval: The research/study was approved by the Institutional Review Board at AIIMS RISHIKESH, approval number AIIMS/IEC/21/726, dated 21st September, 2021.

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