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Patterns of bone marrow infiltration in lymphomas- A retrospective descriptive analysis from a tertiary care centre in Southern India

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ABSTRACT

Background: Bone marrow biopsy (BMB) examination is the gold standard for staging of lymphoma which is interpreted along with other clinical, laboratory and radiological investigations. This study aimed to evaluate the various patterns of BMB infiltration by lymphomas which include Hodgkin and Non Hodgkin lymphoma and the usefulness of BMB compared to bone marrow aspiration (BMA).

Materials and Methods: In a period of three years, there were 212 cases which showed lymphoma infiltration in bone marrow. We assessed the peripheral blood smear (PBS), BMA and BMB slides and concordance between each was evaluated. BMB slides were assessed for the pattern of involvement.

Results: The most frequent subtype was chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) (21.70%) followed by Hodgkin lymphoma (HL)(12.73%)and follicular lymphoma (FL)(11.32%). Predominant pattern of infiltration showed by CLL/SLL cases was mixed pattern, by FL was paratrabecular pattern, mantle cell lymphoma(MCL) showed either diffuse or nodular, diffuse large B cell lymphoma (DLBCL) and Burkitt lymphoma(BL) showed predominantly diffuse pattern. Hairy cell leukemia (HCL) and peripheral T cell lymphoma (PTCL) showed an interstitial pattern. In HL, the pattern was usually focal nodular however diffuse involvement was seen in a few. Of the 212 cases, 50.94% cases had atypical lymphocytes or lymphocytosis in PBS and 81.14% cases showed infiltration in BMA.

Conclusion: Atypical lymphoid cells may not be present at times in peripheral blood or bone marrow aspirate in spite of infiltration in the bone marrow. Different lymphomas show characteristic patterns of marrow involvement.

1. Introduction

Clinical and laboratory data along with imaging and evidence of BM infiltration on histopathological examination is needed for lymphoma staging. Histopathological examination of BMB remains the gold standard for lymphoma staging. BMB involvement upstages the case to stage IV. It is also required for evaluating the treatment response.[1] WHO classifies lymphoma on the basis of morphology, immunophenotyping and molecular genetics characteristics into clinicopathologically distinct entities.[2] Involvement of BM is seen in around 40% of cases of lymphomas. Presence or absence of BM infiltration, analysis of pattern of infiltration in BMB along with the morphology in BMA affects lymphoma diagnosis, prognosis and treatment. It also helps in selecting patients who may relapse early or fail to respond to treatment. It also rules out any other pathology in bone marrow.

This study evaluates various patterns of BMB infiltration by lymphomas which includes Hodgkin and non-Hodgkin

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lymphoma (NHL) and the usefulness of BMB is compared to BMA.[3]

2. Materials and Methods

This is a retrospective record based descriptive study of bone marrow biopsies done in patients with suspicion for diagnosis or staging of lymphomas. Cases in whom a previous BMA/BMB had been obtained showing bone marrow infiltration by lymphoma cells and came again for follow up were excluded.

A total of 375 cases were evaluated for lymphoma infiltration in bone marrow from January 2015 to December 2018 including diagnostic as well as staging bone marrows. Diagnostic marrows included BMA/BMB involved by lymphomatous infiltration in patients without any history of lymphoma at any other site. Primary site of involvement was searched and identified after BMA/BMB showed lymphoma infiltration. Staging marrows included cases having a prior diagnosis of lymphoma showing lymphomatous infiltration in BMA/BMB either at the time of diagnosis or follow up. Of these, 212 cases which showed lymphoma infiltration in bone marrow, were included in this study. For each case, age, clinical details and diagnosis, complete hemogram determined by automated analyzer (Sysmex 5 part) and Romanowsky stained PBS and BMA smears were also studied along with the BMB. Serial sections of 3- 4 μm thickness were stained by hematoxylin and eosin (H and E) and reticulin stains and evaluated for adequacy, cellularity, trilineage hematopoeisis, presence of lymphomatous infiltration, its histologic pattern, morphology, and secondary changes like reticulin fibrosis and necrosis. Reticulin was graded from 0 to 3.[2] Immunohistochemistry (IHC) was performed as and when indicated. In case of staging marrows, a basic panel was performed to confirm marrow infiltration. However in diagnostic cases, a complete panel was done.

Whether lymphoid nodules were neoplastic or reactive were differentiated on the basis of morphology and IHC. IHC for B- cell lymphoproliferative disorders (LPD) like CD20, CD79a, CD10, CD23, BCL2, BCL6, C-MYC along with CD3, CD5, CD7, CD4, CD8 for T- cell LPD were usually performed for typing and sub typing lymphomas. CD15, CD30, CD20 and PAX5 were used in cases of HL. Other IHC markers like CD34, terminal deoxy nucleotidase (TdT), leucocyte common antigen (LCA), CD99, CD38, CD138, kappa, lambda were performed wherever indicated.

2.1. Statistical analysis

The concordance between PBS, BMA and BMB slides was assessed in 209 cases in which PBS and BMA were available. Data were collected and a descriptive analysis was done. Quantitative results were expressed as mean (SD) and qualitative variables were expressed as frequency and percentages.

Patterns of involvement were classified as interstitial, nodular, mixed, paratrabecular, diffuse and intrasinusoidal. In interstitial infiltration neoplastic cells are interspersed between haematopoietic and fat elements. In spite of generalized BM involvement, trilineage hematopoeisis and architecture of BM is preserved. Nodular infiltration is oval or round lymphoid aggregates having a well-defined border in a non-paratrabecular location. Paratrabecular infiltration shows a band of lymphoid cells adjacent to the bony trabeculae or a broad based lymphoid aggregate adjoining the trabecula. In intrasinusoidal infiltration neoplastic cells are found within sinusoids. Diffuse or packed marrow pattern is effacement of BM architecture and absence of trilineage hematopoietic and fat cells which are replaced by lymphoid cells. Diffuse interstitial infiltration is seen in many lymphomas which indicates that there is widening of interstitium with loosely dispersed lymphoma infiltrate throughout the BM intertrabecular spaces. Mixed pattern denotes nodular- interstitial pattern (Figure 1).[4]

3. Results

The study included 212 cases which showed lymphoma infiltration in BM. In this study 75% (159) of cases were male and 25% (53) were female patients constituting 3:1 male:female ratio. The age ranged between 3 to 84 years with mean age of 49.5 years. Amongst all 212 cases, the most common pattern of BM infiltration was diffuse 32.08% (68) followed by nodular 21.23% (45), mixed 18.40% (39), interstitial 17.93 (38), paratrabecular 8.01% (17) and sinusoidal 2.35% (5).

Amongst all the cases 87.27% (185) cases were NHL and 12.73% (27) cases were HL. 82.16% (152) of all NHL cases were B cell lymphomas and 17.84 (33) were T cell lymphomas. The most frequent lymphoma subtype infiltrating the BM was CLL/SLL (21.70%) followed by HL(12.73%)and FL(11.32%). Out of 27 cases of BM infiltration by HL, 25 cases were of classic Hodgkins lymphoma (CHL) and only 2 cases were of nodular lymphocyte predominant Hodgkins lymphoma (NLPHL). MCL, MZL, DLBCL, PTCL, BL and LPL were other commonly occurring lymphomas invading BM (Table 1). Low occurrence lymphomas were seen in 13.67% (29) of cases of which 5 were diagnosed as anaplastic large cell lymphoma (ALCL), 4 as hepatosplenic T cell lymphoma (HSTCL), 4 as HCL, 3 of T lymphoblastic lymphoma (T-LBL), 3 of B lymphoblastic lymphoma (B-LBL), 2 of plasmablastic lymphoma (PBL) and 2 of T-prolymphocytic leukemia (T-PLL). One case each of T-large granular lymphocytic leukemia/ lymphoma (T-LGL), NK-T cell leukemia/ lymphoma (NK-TCL), T cell rich B cell lymphoma (TCRBCL), angioimmunoblastic T cell lymphoma (AITCL), B- prolymphocytic leukemia (B-PLL) and splenic lymphoma with villous lymphocytes (SLVL)

were studied.

CLL/SLL showed various types of patterns including interstitial, nodular, mixed and diffuse. However predominant pattern of infiltration was mixed followed by diffuse pattern. SLL or early CLL showed nodular pattern whereas late stage of CLL showed interstitial, mixed or diffuse patterns. Most common pattern of infiltration by FL was paratrabecular followed by few cases of nodular infiltration. One rare case of follicular lymphoma showed myelofibrosis along with paratrabecular and nodular pattern of infiltration. MCL showed either diffuse or nodular pattern. DLBCL and BL showed predominantly diffuse pattern. Predominant pattern of infiltration in HCL was interstitial. Most common pattern in PTCL was interstitial followed by diffuse pattern. Few cases of nodular pattern were also seen. The marrow was diffusely packed in LBL. MZLs of all types showed uniformly interstitial, nodular, paratrabecular and diffuse patterns. Most prominently mixed and interstitial followed by nodular, diffuse and sinusoidal. Sinusoidal pattern was seen only in SMZL and HSTCL. Out of five cases of ALCL two showed interstitial pattern and one case each showed nodular, diffuse and mixed pattern of infiltration. Most common pattern of infiltration in LPL was diffuse followed by interstitial. In HL, the pattern was usually focal nodular however diffuse involvement was seen in a few cases of nodular sclerosis and lymphocyte depleted types of HL. Diffuse involvement in HL included the Reed-Sternberg cells as well as the reactive population throughout the bone marrow (Table 1). IHC was used for differentiating reactive lymphoid aggregates from lymphoma infiltration and also to identify subtle foci of infiltration.

Out of 212 cases of BMB with infiltration, only 50.94%(108) cases had atypical lymphocytes or lymphocytosis in PBS and 81.14%(172) cases showed infiltration in BM. PBS and BMA slides were not available for three cases. PB involvement was infrequent in FL, DLBCL, T-cell lymphomas and absent in HL.

BMA showed no involvement in cases showing nodular infiltration in BMB as well as cases showing secondary stromal changes like fibrosis or necrosis. Some lymphomas like HL, HCL and few PTCL cases were non aspirable due to increased reticulin leading to dry tap so only BMB was required to assess marrow involvement. Stromal fibrosis was identified in 21.6% of cases commonest in HL, DLBCL, PTCL and HCL. One case of FL also showed stromal fibrosis (Figure 2). Necrosis was associated with 17.4% of cases comprising predominantly of DLBCL, BL and HL. One case of HL showed a dual lesion of lymphoma as well as tuberculosis confirmed by IHC and stain for Acid fast bacilli.

Out of all the positive BMB, 57.1% marrows were staging marrows and 42.9% were diagnostic marrows. Especially in HL, BL and MCL, 77.8%, 75% and 71.5% of all the positive marrows were staging marrows respectively (Table 2). Diagnostic marrows comprised of cases with unknown primary lymphoma which were predominantly small cell lymphomas. BMB along with immunophenotyping was very helpful for diagnosis of lymphoma, precluding the need for a lymph node biopsy especially if lymph nodes or the organ affected like spleen were not easily approachable. In 42.9% of our cases BMB was diagnostic of lymphoma and bone was the first site of tissue diagnosis. 66.7% of all positive cases of FL, 66.7% of LPL and 54.3% of CLL/SLL were diagnosed on BM examination.

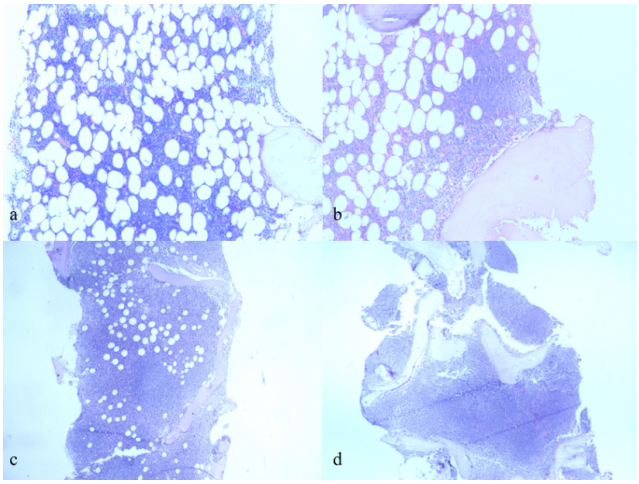

Fig. 1: Various patterns of lymphoma infiltration in bone marrow biopsy in H & E stain; **a:** Interstitial 4X; **b:** Nodular 10X; **c:** Mixed 4X; **d:** Diffuse 4X.

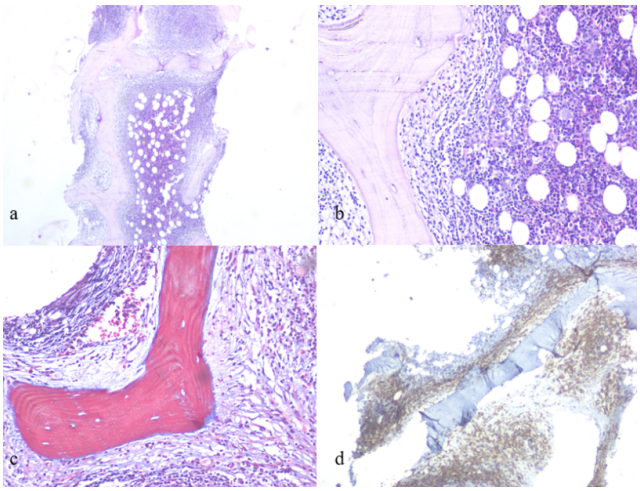

Fig. 2: Paratrabecular infiltration of FL along with Myelofibrosis; **a:** 4X (H&E stain); **b:** 10X (H&E stain); **c:** Masson's Trichrome showing myelofibrosis; **d:** IHC positivity for CD10 4X.

Table 1: Distribution of various lymphoproliferative disorders showing BM infiltration along with their common patterns of BM involvement.

Lymphoma type	Frequency% (Number of cases)	Predominant pattern observed
CLL/SLL	21.70 (46)	Mixed, diffuse
HL	12.73 (27)	Focal nodular
FL	11.32 (24)	Paratrabecular
MCL	9.90 (21)	Diffuse, nodular
MZL	8.50 (18)	Mixed, interstitial
DLBCL	8.02 (17)	Diffuse
PTCL	7.54 (16)	Interstitial
BL	3.78 (8)	Diffuse
LPL	2.83 (6)	Diffuse
Others	13.67 (29)	
ALCL	2.35 (5)	Interstitial
HSTCL	1.89 (4)	Sinusoidal
HCL	1.89 (4)	Interstitial
T-LBL	1.42 (3)	Diffuse
B-LBL	1.42 (3)	Diffuse
PBL	0.94 (2)	Diffuse, nodular
T-PLL	0.94 (2)	Mixed, interstitial
T-LGL	0.47 (1)	Interstitial
NK-TCL	0.47 (1)	Interstitial
TCRBCL	0.47 (1)	Diffuse
AITCL	0.47 (1)	Diffuse
B-PLL	0.47 (1)	Nodular
SMZL	0.47 (1)	Sinusoidal
Total	100 (212)	

Table 2: BMB negative vs positive and diagnostic vs staging for lymphoma infiltration

BMB sent for lymphoma infiltration	Total BMB received	P ositive for infiltration (%)	Positive for infiltration		Negative for infiltration
			Diagnostic (%)	Staging (%)	
CLL/SLL	53	46(86.8)	25(54.3)	21(45.7)	7(13.2)
HL	61	27(44.3)	6(22.2)	21(77.8)	34(55.7)
FL	34	24(70.6)	16(66.7)	8(33.3)	10(29.4)
MCL	25	21(84)	6(28.5)	15(71.5)	4(16)
MZL	38	18(47.4)	8(44.4)	10(55.6)	20(52.6)
DLBCL	60	17(28.3)	7(41.1)	10(58.9)	43(71.7)
PTCL	19	16(84.2)	1(6.3)	15(93.7)	3(15.8)
BL	16	8(50)	2(25)	6(75)	8(50)
LPL	8	6(75)	4(66.7)	2(33.3)	2(25)
Others	61	29(47.5)	16(55.2)	13(44.8)	32(52.5)
Total	375	212(56.6)	91(42.9)	121(57.1)	163(43.4)

Table 3: Comparison of infiltration patterns in various other studies.

Name of study	Year	Diffuse Pattern (%)	Nodular pattern (%)	Intersitial pattern (%)	Paratrabecular pattern (%)
Lim et al.	2000	71.4	0.0	0.0	0.0
Chen et al.	2000	22.0	7.0	23.0	0.0
Zhongura et al.	2006	44.9	29.3	11.6	0.0
Jamila et al.	2008	34	0.0	18	30
Lone et al.	2011	25	0.0	45	20
Cabezas-Quintario et al.	2016	6.3	6.5	7	4.4
Sultan et al.	2016	14.6	6.5	5.4	4.8
Present study	2018	32.08	21.23	17.93	8.01

4. Discussion

A similar study on 184 patients revealed that B cell lymphomas were more common than T cell lymphomas. However, unlike the present study, DLBCL constituted major subtype in 67.9% patients, followed by FL in 7.6% patients and MZL in 3.8% patients, MCL in 3.2%, ALCL in 2.7%, BL in 2.1% and TCRBCL in 2.1% patients. The lowest occurrence was of SLL in 1.6% patients. This was because they did not include cases of CLL in their study.[5]

FL infiltrates the BM with a predominantly paratrabecular pattern. This is due to presence of CXCL4 which binds with its ligand CXCL12 in the osteoblastic niches located in the paratrabecular areas of BM. SMZL shows a predominantly intrasinusoidal infiltration due to presence of CD44 and integrins which interact with hyaluronan and ICAM-1 in endothelial cells. SMZL, HSTCL and HCL, mostly involve the spleen and the liver sinusoidal compartment which expose abundant hyaluronan similar to vascular niches of BM.[6] The clonal neoplastic B-cells in DLBCL recruit T cells, histiocytes and CD14+ monocytes by releasing CCL-5. Reticulin fibrosis is usually seen in HCL or PTCL. Fibroblastic proliferation is also commonly seen in BM infiltration by classic HL.[7]

The predominant pattern of BM infiltration by lymphomas, as reported in literature, is usually diffuse followed by focal aggregates and paratrabecular infiltrates.[5,8] Unlike our findings, Arber and George, showed focal, paratrabecular, or interstitial to diffuse patterns of BM infiltration.[9] The high occurrence of diffuse infiltration, in the present study reflects the fact that patients here present at a much later stage of lymphoma progression. Table 3 shows the comparison of BMB pattern of infiltration in various studies.[5]

Two large case series of bone marrow involvement by lymphomas are by Sovani et al[3] and Arber and George,[9] on 511 and 450 cases, respectively. In both their series, FL was the commonest lymphoma subtype to involve marrow, which was 26% and 30% respectively. Paratrabecular and mixed pattern of infiltration was common. In the present study, intrasinusoidal infiltration was seen in only 2.35% of cases and that too only with SMZL and HTCL. Arber and George, included sinusoidal infiltration as a part of interstitial infiltration.[9] Various other studies also show a wide variation in estimating occurrence of intrasinusoidal pattern. However, most of them consider it as pathognomonic of SMZL.[3,10,11]

In 42.9% of our cases BMB was diagnostic of lymphoma and bone was the first site of tissue diagnosis, these included mainly the low grade lymphomas comprising of FL, LPL and CLL/SLL. In all of these, BM along with immunophenotyping was useful in subcategorizing the lymphoma, precluding the need for a lymph node biopsy. Recently PET/CT has developed as an important tool in the assessment of marrow infiltration by lymphomas. Studies on PET-CT conclude that although BMB remains the best method in patients with indolent lymphomas, it may not be performed in patients diagnosed with HL and DLBCL, where PET-CT is positive.[12,13] The study by Ozpolat et al suggested that lymphoma infiltration should not be decided solely based PET-CT or BMB findings. Both BMB and PET-CT along with clinical and laboratory findings should be used synergistically. PET-CT is useful for predicting lymphoma infiltration in BM in patients for whom BMB is contraindicated.[14]

5. Conclusion

Atypical lymphoid cells may not be present at times in peripheral blood or bone marrow aspirate inspite of infiltration in the bone marrow. BMB is more sensitive than BMA for lymphoma infiltration and is the gold standard for identification of lymphoma infiltration in BM. Different lymphomas often demonstrate characteristic patterns of marrow involvement and secondary changes which can help in differentiating them from each other.

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7. Conflicts of Interest

None to declare.

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