# COMPARATIVE STUDY OF THE EFFICACY OF BONE MARROW ASPIRATION, TREPHINE BIOPSY AND IMPRINT CYTOLOGY IN THE DIAGNOSIS OF BONE MARROW INVOLVEMENT IN LYMPHOMA

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**ABSTRACT**

**BACKGROUND**

Lymphoma involving bone marrow is considered as stage-IV irrespective of its primary presentation. The treatment and prognosis depend upon the stage of lymphoma. Bone marrow involvement can be confirmed by immunohistochemistry (IHC), cytogenetics and molecular analysis, and scanning. But, due to limited resources in developing countries like India, these procedures are not available in most of the institutions and laboratories, where morphological examination of bone marrow is the only way to diagnose bone marrow involvement in lymphoma. The purpose of the study is to compare the sensitivity and specificity of bone marrow aspiration, trephine biopsy, and imprint, to diagnose marrow involvement in lymphoma.

**METHODS**

The study was conducted on 51 lymphoma patients in BMCH for 18 months. In each case, bone marrow aspiration, trephine biopsy, and imprint, were taken. Bone marrow involvements were diagnosed, and confirmed, by trephine biopsy with IHC. Sensitivity and specificity of each of the procedures of morphological studies were calculated.

**RESULTS**

Bone marrow involvement was found in 14 cases (27.45%) with frequency in non-Hodgkin lymphoma being 32.5% and in Hodgkin lymphoma 9.09%. Small lymphocytic lymphoma involved the bone marrow most frequently (50%) followed by follicular lymphoma and diffuse large B cell lymphoma. Biopsy was most sensitive method, and imprint was most specific.

**CONCLUSIONS**

Trephine biopsy along with imprint smear should be the procedure of choice with IHC as an adjunctive procedure.

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## BACKGROUND

Bone marrow examination has an important role in the investigation, and diagnosis of haematological as well as non-haematological malignancies and various other diseases. In lymphoma bone marrow examination may be required for primary diagnosis and in most of the lymphoma cases bone marrow examination is done for staging purpose. Once histological diagnosis of malignant lymphoma has been established, the next is to determine the extent of disease so that proper treatment protocol may be decided. [1] For staging of lymphoma many systems have been described. The Ann Arbor staging originally described for HD is often used for NHL also. According to Ann-Arbor staging any lymphoma involving bone marrow is considered as stage-IV irrespective of its primary presentation. Bone marrow involvement not only changes the treatment protocol of lymphoma patient but also alters the prognosis and patient's survival. Marrow involvement in lymphoma portends a worse prognosis.[2] Furthermore, the pattern of involvement and the tumour cell burdens in bone marrow have prognostic significance. Diffuse involvement is found to have poorest prognosis and the tumour load is inversely proportional to survival.

Bone marrow involvement can be confirmed by immunohistochemistry (IHC), cytogenetics and molecular analysis [3] and can be suggested by MRI. [4] But, due to limited resources in developing countries like India, these procedures are not available in most of the institution and laboratories, where morphological examination of bone marrow is the only way to diagnose bone marrow involvement in lymphoma. Even there are many controversies in different literatures regarding the role of different bone marrow examination procedures. Thus the purpose of our study was to find out the frequency of bone marrow involvement in lymphoma on presentation diagnosed by bone marrow biopsy and taking IHC as gold

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standard and to evaluate relative contribution of bone marrow aspiration, trephine biopsy and imprint smear to find out the most sensitive and specific procedure in this context.

**METHODS**

The present cross-sectional and observational study was conducted for eighteen months in Burdwan Medical College and included 51 lymphoma patients. The patients having coagulation disorder or pregnancy or plasma cell disorder, and precursor lymphoid neoplasm were excluded from the study.

Written consent and detailed clinical history of the patients were taken, and all reports of prior investigation were reviewed. Coagulation profile and HIV serology were done as precautionary measures. In each patient peripheral blood was taken and bone marrow aspiration was done followed by bone marrow trephine biopsy from posterior superior iliac spine with a Jamshidi's needle under local anaesthesia and proper aseptic precaution. Imprint was taken on slide from biopsy material. Leishman's and May Grünwald Giemsa stains were done on aspiration and imprint smears. Trephine biopsy was fixed in 10% formal saline, decalcified in formal-EDTA and processed to make paraffin embedded blocks from which 4 µm sections were made by microtome. Haematoxylin and eosin stain was done in those slides. Reticulin stain was done whenever needed. Immunohistochemistry was done in every biopsy to confirm the bone marrow involvement by using following antibodies- CD19, CD3 (in NHL), and CD20, CD45, CD15, CD30 (in HD). Whenever diagnostic difficulty aroused CD5, CD23, CD10 were done for further confirmation. Morphological examination of each of the procedures was done to determine bone marrow involvement and their sensitivity and specificity were calculated in respect to biopsy with IHC result.

**RESULTS**

Out of 51 cases bone marrow involvement was found in 14 cases (27.45%). Among 40 cases of NHL 13 cases of bone marrow involvement was found (32.5%) and out of 11 HL cases only one case of nodular sclerosis was found to have bone marrow involvement (9.09%). Small lymphocytic lymphoma (SLL) was found to involve the bone marrow most frequently (50%) followed by follicular lymphoma (41.67%) and diffuse large B cell Lymphoma (DLBCL) (25%). No involvement was found in MALToma, mantle cell lymphoma and T-cell lymphoma. (Table 1)

Predominant histological pattern of bone marrow involvement by lymphomatous infiltrate was diffuse (61.54%) followed by mixed (15.38%), paratrabecular (15.38%) and nodular (7.69%).

Out of total 14 bone marrow involved cases diagnosed and confirmed by bone marrow biopsy along with IHC, only three cases could be diagnosed by bone marrow aspiration. Aspiration smears showed three cases of false positivity.

Thus, the sensitivity of bone marrow aspiration was 21.43% and the specificity was 91.89%. (Table 2a)

Bone marrow biopsy alone diagnosed all 14 cases of bone marrow involvement; but another two cases of nodular involvement were found falsely positive as they were diagnosed later as reactive lymphoid aggregate by IHC.

The sensitivity of bone marrow biopsy was 100%, but specificity was 94.59%. (Table 2b)

In imprint smear 12 cases of bone marrow involvement could be diagnosed and one false positive was found. Thus, the sensitivity of imprint was 85.71% and specificity was 97.3%. (Table 2c)

Bone marrow cellular morphology was better understood in bone marrow aspiration and imprint than the biopsy.

| Subtypes of Lymphoma | Total No. of Cases | Bone Marrow Involved Cases | Frequency of Bone Marrow Involvement | Pattern of Involvement |
|---|---|---|---|---|
| SLL | 8 | 4 | 50% | Diffuse-4 |
| Follicular Lymphoma | 12 | 5 | 41.67% | Nodular-1, Diffuse- 2, Mixed-1, Paratrabecular-1 |
| DLBCL | 16 | 4 | 25% | Mixed-1, Diffuse-2, Paratrabecular-1 |
| MALToma | 1 | 0 | 0 | - |
| Mantle Cell Lymphoma | 1 | 0 | 0 | - |
| T Cell Lymphoma | 2 | 0 | 0 | - |
| HL (Nodular Sclerosis) | 11 | 1 | 9.09% | Focal |

***Table 1. Distribution of Bone Marrow Involvement in Different Subtypes of Lymphoma***

| | BMB & IHC | | Total |
|---|---|---|---|
| | Uninvolved | Involved | |
| Aspiration Uninvolved | 34 | 11 | 45 |
| Involved | 3 | 3 | 6 |
| Total | 37 | 14 | 51 |

***Table 2a. Cross Tabulation of Bone Marrow Aspiration with Bone Marrow Biopsy (BMB) and IHC***

| | BMB & IHC | | Total |
|---|---|---|---|
| | Uninvolved | Involved | |
| Imprint Uninvolved | 36 | 2 | 38 |
| Involved | 1 | 12 | 13 |
| Total | 37 | 14 | 51 |

***Table 2b. Cross Tabulation of Bone Marrow Biopsy (Routine Stain) with Bone Marrow Biopsy (BMB) and IHC***

| | BMB & IHC | | Total |
|---|---|---|---|
| | Uninvolved | Involved | |
| BMB Uninvolved | 35 | 0 | 35 |
| (H & E) Involved | 2 | 14 | 16 |
| Total | 37 | 14 | 51 |

***Table 2c. Cross Tabulation of Bone Marrow Imprint with Bone Marrow Biopsy (BMB) and IHC***

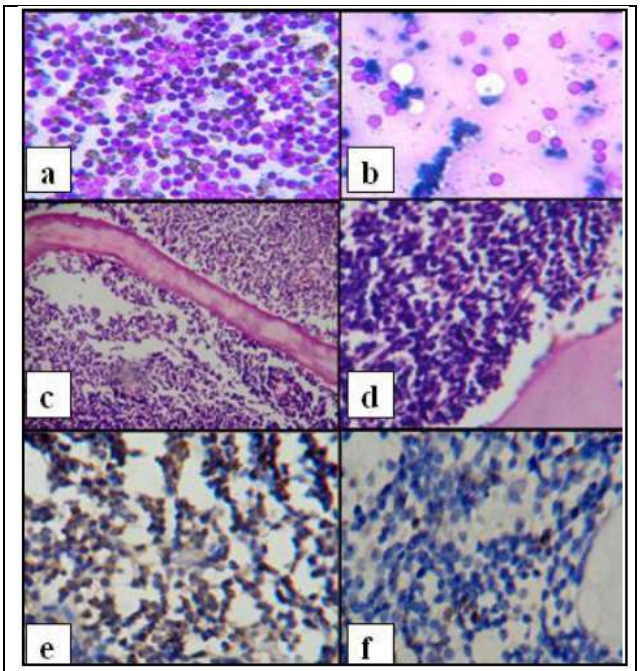


***Figure 1***

**Figure 1: (a)** F.N.A.C. of lymph node showing NHL (Leishman's stain;400x); **(b)** Imprint smear of bone marrow biopsy showing marrow involvement (Leishman's stain;400x); **(c)** Bone marrow biopsy showing diffuse involvement ( H and E;100x); **(d)** Bone marrow biopsy showing diffuse involvement ( H and E;400x); **(e)** Lymphoma cells in bone marrow showing CD 19 positivity (IHC,400x); **(f)** Lymphoma cells in bone marrow showing CD 3 negativity (IHC,400x).

## DISCUSSION

Considerable variation in bone marrow involvement in lympho-proliferative disorder has been reported ranging from 27.6% to 53%.[5-9] In our study out of 51 cases, bone marrow involvement was found in 14 cases (27.45%).

However the bone marrow involvement varies greatly according to the histological subtypes of lymphoma.[10] We found HL to involve the marrow in 9.09% which corresponds to other studies where it ranged between 2-32% and in another reported series of Howell SJ et al., it was 5.2% in adult patients.[11] In case of Hodgkin Lymphoma frequency of bone marrow involvement is reported highest in lymphocyte depleted (75%), followed by mixed cellularity (20-25%), and Nodular sclerosis (5-10%).[9] We found only one case of Nodular sclerosis among 11 cases of HL to involve bone marrow, which may be an incidental finding as the total number of Hodgkin lymphoma cases were only 11. In our study percentage of marrow involvement in NHL was found to be 32.5%. Kathy Foucar found that fifty-three percent (93 cases) of the 176 cases of NHL had bone marrow involvement by lymphoma at diagnosis [12] and in the study of Suneet kumar et al. the overall incidence of marrow involvement by NHL was 55.1% (27/49).[11] In another study 32% (102/317) had lymphoma in their bone marrow, and 9% had benign lymphoid aggregates.[13]

In our study, among the subtypes of NHL, SLL involved marrow most frequently (50%), followed by Follicular lymphoma (41.67%), and DLBCL (25%). It correlates with other studies where frequency of marrow involvement in SLL was 72% and Follicular lymphoma was 42%.[14] Compared to aggressive lymphoma, relatively high frequencies have been reported in indolent lymphomas, such as mantle cell lymphoma (MCL), follicular lymphoma (FL), and marginal-zone lymphoma. We did not find bone marrow involvement in MALToma, Mantle cell lymphoma and T cell Lymphoma. It is contradictory to other studies where bone marrow involvement in these lymphomas is found in significant number of cases. Even in some study T cell lymphoma involved bone marrow more frequently than B cell lymphoma.[15] This variation from previous study may be due to the fact that they studied large number of patients and the relative proportions of different subtypes of lymphoma were different from our study. Further the total number of T-cell lymphoma in our study was itself very less.

According to some author the bone marrow aspiration being a simple minimally invasive technique, is considered complimentary to bone marrow biopsy.[16,17] Use of biopsy with aspirate has been advocated by some;[18,19] others suggest core biopsy alone.[20,21] In another study sensitivity and specificity of bone marrow aspiration in diagnosing marrow involvement were 69% and 86% respectively and it was concluded in the study that bone marrow aspiration may be considered as an additional advantageous procedure to core biopsy.[22] But in our study the sensitivity of bone marrow aspiration was 21.43% as it could diagnose only three cases among 14 cases of marrow involvement. This sensitivity was very less in comparison to biopsy (100%) and imprint (85.71%). Aspiration could not diagnose marrow involvement in cases of paratrabecular, nodular and focal involvements. So focal involvement and low tumour burden in marrow may not be aspirated and seems to be normal. In cases of mixed pattern of involvement both nodular and interstitial involvements were present. The aspirate from interstitial involvement contains both normal hematopoietic cells and lymphoma cells and it is very difficult to diagnose marrow involvement if the lymphoma cells are small. This is because of the fact that lymphocytes normally represent 10% to 15% of cells on bone marrow aspirate smears in adults, and lymphoid precursor cells (hematogones) and mature lymphocytes may be normally increased in children and the elderly, respectively. Also, in some cases of diffuse involvement of marrow, bone marrow aspiration was negative. This was because the peripheral blood was uninvolved in those cases, and recurrent dry tap occurred followed by obtainment of scanty marrow material which

was diluted with peripheral blood. Because of the fibrosis associated with the lymphoma infiltrate, aspirate smears may not demonstrate an obvious abnormality.[23] The specificity of bone marrow aspiration was 91.89%. Three cases of reactive lymphocytosis in marrow were diagnosed falsely as lymphoma infiltration in aspiration. Among three false positive cases of aspiration two were also found falsely positive in biopsy but later these were proved by IHC to be reactive lymphoid aggregates. According to Bartl R et al., bone marrow aspiration rarely provides any information not available by biopsy.[24] There are very few reported cases of true positive cases of bone marrow aspiration with negative biopsy in non-haematological neoplasm.[25] It is also reported that aspirates are never positive with a negative biopsy in Hodgkin disease.[7] The single positive case of aspiration in our study which was found negative in biopsy was considered false positive after confirmation by IHC.

Bone marrow imprint was found very sensitive (85.71%) and specific (97.3%) procedure; even its specificity was found more than routine biopsy (94.59%). Imprint was proved to be very convenient as it was prepared without any additional cost and effort. Furthermore, the cellular morphology was better understood in imprint than biopsy. It also provided impression about marrow involvement in very less time than biopsy as it does not require any processing. So, imprint should always be considered along with biopsy.

Biopsy with routine stain was 100% sensitive to diagnose bone marrow involvement in lymphoma and its morphological diagnosis can be considered enough in most of the conditions. But its specificity was 94.59%. In two cases of reactive nodular lymphoid aggregates in marrow, biopsy was falsely interpreted as nodular involvement by lymphoma cells. In diagnosed cases of lymphoma in lymph nodes any lymphoid aggregates in bone marrow raises the suspicion because of frequent presence of discordant morphology between a diagnostic lymph node specimen and the cellular composition of the involved bone (Robertson et al., 1991).[26] The reactive aggregates were well circumscribed, small, non-paratrabecular in location, composed of predominantly small lymphocytes admixed with medium and large lymphocytes, as well as histiocytes. Germinal centre may be present in these aggregates. The true nodular lymphoid aggregate in bone marrow was unusually large having irregular or infiltrating borders. But in our study, it was located in non paratrabecular location. Thus, IHC was required to confirm the diagnosis in cases of non-paratrabecular nodular infiltrate. In lymphoma infiltrate there was monotonous population of B cells, whereas in reactive lymphoid aggregates were composed of predominantly CD3-positive T lymphocytes with smaller numbers of CD20-positive B cells. According to Hartsock RJ et al., a predominance of B lymphocytes in an aggregate of any pattern is generally an indication of bone marrow involvement by B-cell lymphoma, with a few exceptions.[27] The nodular pattern of infiltration is commonly encountered in Follicular lymphoma. This is to remember that immunohistochemical studies may also be misleading in evaluation of lymphoid aggregates of patients with follicular lymphoma, as this lymphoma type characteristically has numerous reactive T lymphocytes admixed with the lymphoma cells. Therefore, the detection of a mixed T and B cell population may incorrectly be interpreted as evidence of a reactive population. In the Bone marrow, follicular lymphoma cells are characteristically localized in the paratrabecular region, but may infiltrate interstitially with significant fibrosis and be virtually invisible in an ordinary morphological examination. However, the B cells predominate in those aggregates and they do not create confusion if located in paratrabecular location. Otherwise presence of CD10 expression in the atypical lymphoid proliferation can be useful in detecting follicular lymphoma in bone marrow. Again, CD10 expression is often focally or completely lost in cases with marrow involvement, and the absence of this marker should not be used to rule out disease involvement.[23]

Interpretation is not difficult in cases of diffuse involvement of bone marrow if lymphoid cells almost replace the marrow (very high tumour burden) or the lymphoid cells are large (congruent morphology in DLBCL). When DLBCL involves the bone marrow, it is usually extensive and fairly easy to identify. The lymphoma cells most commonly show chromatin clearing and multiple small nucleoli and sometimes display immunoblastic morphology, with over 90% of cells showing a central prominent nucleolus and more abundant, basophilic cytoplasm. Thus, demonstration of CD20 expression is sufficient for the diagnosis in most cases. But problem may arise when there is discordance of morphology, such as bone marrow involvement by low-grade, small cleaved cell lymphoma, which is relatively common in patients with DLBCL.[26] Therefore, small lymphocyte proliferations in the bone marrow should be considered with suspicion in patients DLBCL and in those cases further immune marker study such as BCL6 can be done.

We found diffuse marrow involvement in four cases out of eight patients of SLL in lymph node. The morphological interpretations of bone marrow biopsy in those cases positively correlated with IHC interpretations. Bone marrow involved cases showed membrane positivity of CD20, CD5, and CD23, and negative staining with CD10 and CD3. Surface CD20 expression may be weak characteristically, but the co-expression of CD5 and CD23 confirms the diagnosis (Jaffe et al., 2001; DiGiuseppe & Borowitz, 1998).[28] IHC may be needed in SLL also if the tumour burden is low and involvement is non-paratrabecular.

During diagnosis of marrow involvement in HD we used CD15, CD30 and CD45. Biopsy was suggestive of marrow involvement as there was focal collection of lymphocytes, histiocytes and grade III reticulin fibrosis. Presence of RS cell demonstrated by IHC confirmed the diagnosis. Although it was a case of Nodular Sclerosis the RS cell in bone marrow was binucleated and classical type rather than being lacunar cell. The RS cell showed membrane positivity for CD15 and CD30; but CD45 was negative. So, presence of atypical histiocytes and characteristic cellular background in bone

marrow and/or fibrosis or necrosis in bone marrow biopsy suggests marrow involvement in HD and further evaluation should be done.

**CONCLUSIONS**

Bone marrow examination should be done in all lymphoma patients**.** The frequency and pattern of marrow involvement depends upon lymphoma types. Among the procedures of bone marrow examination, trephine biopsy is still the gold standard with imprint aiding the marrow interpretation. So, trephine biopsy along with imprint smear should be the procedure of choice. IHC is adjunctive procedure to biopsy to differentiate reactive lymphoid aggregates from non-paratrabecular lymphoma involvement with nodular or interstitial pattern. IHC may be required also in diffuse marrow involvement if the infiltration in marrow consists of small lymphocytes with low tumour burden.

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