INTRODUCTION
Lymphoma is cancer that occurs due to the malignant transformation of lymphocytes in the lymphatic system. Incidence of Bone marrow infiltration in Non-Hodgkin Lymphoma (NHL) varies from 15% to 75% due to heterogeneous inclusion criteria used by different studies. Follicular lymphoma is most common to involve the marrow followed by diffuse large B-cell lymphoma. In the adult population, the estimated incidence of marrow infiltration by Hodgkin lymphoma (HL) is around 2-32% with an average of 10%, whereas the incidence in the pediatric population is low accounting for 1.8%. The involvement of the marrow in a patient with lymphoma represents stage IV disease. The clinical significance of bone marrow involvement can vary based on the type of lymphoma.

Trephine biopsy is more useful in detecting bone marrow infiltration. Trephine biopsy helps in assessing the presence or absence of infiltration, the pattern of infiltration and any morphological discordance with primary lymphoma. It has an important role in the routine staging of Hodgkin and Non-Hodgkin lymphoma.
Bone marrow involvement by lymphoma confirms advanced-stage disease and may affect both treatment and prognosis. Therefore bone marrow examination has an important role in staging and predicting prognosis in lymphoma.

**MATERIALS AND METHODS**

This is a 4 year retrospective study of 86 diagnosed cases of lymphoma with marrow infiltration conducted in the Department of Pathology, Kasturba Medical College and Kasturba Hospital, Manipal.

The clinical data were retrieved from the medical records and the bone marrow (aspiration and biopsy) slides, lymph node biopsy slides were reviewed. Peripheral smears and all bone marrow aspirates were stained with Leishman stain as per the standard procedure. All trephine biopsy sections and lymph node biopsies were stained with Hematoxylin and Eosin stain as per the standard procedure.

**INCLUSION CRITERIA:**

All cases of Lymphoma show bone marrow involvement.

**EXCLUSION CRITERIA:**

- Diagnosed cases of lymphoma for which bone marrow aspiration or biopsy was not done.
- Cases of lymphoma with inadequate bone marrow biopsies.

**RESULTS**

During the study period, a total of 135 cases of lymphoma were diagnosed on lymph node biopsy, out of which 106 cases (78.5%) were of Non-Hodgkin lymphoma and 29 cases (21.4%) were Hodgkin lymphoma. Out of the 106 Non-Hodgkin lymphoma cases, 76 cases (71.7%) showed marrow infiltration and out of 29 cases of Hodgkin lymphoma, 10 cases (34.5%) showed marrow infiltration. Overall marrow infiltration by Non-Hodgkin lymphoma is higher compared to Hodgkin lymphoma. The Overall incidence of marrow infiltration in NHL and HL was 63.7% (86/135). Only 86 cases that had marrow infiltration by lymphoma were analyzed in the study. The results of various variables of the study are as follows:

In Non-Hodgkin lymphoma - Follicular lymphoma (36.8%) was the most common, followed by Diffuse large B-cell lymphoma (27.6%). Among 10 cases of Hodgkin lymphoma with marrow infiltration, there were 3 cases of Mixed cellularity, 3 cases of Lymphocyte depleted classical Hodgkin lymphoma and 1 case of Nodular sclerosis. Further, 2 Cases of Non-Hodgkin lymphoma and 3 cases of Classical Hodgkin lymphoma were not subclassified, as IHC was not done.

The age of patients with marrow involvement ranged from 19-84 years, with the median age being 51 years, with a peak age incidence observed between 55 to 64 years. Male preponderance was seen with a male to female ratio of 2.45:1.

The most common presenting symptom was cervical or inguinal lymphadenopathy (100%), the next most common presenting symptom was giddiness due to anaemia (34.8%), followed by fever (33.7%). Splenomegaly was found in the majority of cases (67%), followed by hepatomegaly (45.3%).

Haemoglobin values in males ranged from 4.5-15.6g/dl, with the median value being 10.5 g/dl. In females, the haemoglobin values ranged from 5.9-12.1g/dl with the median value being 10.5 g/dl. WBC count ranged from 1.4 - 60.3 x 10^3/µL with the median value being 6.5 x 10^3/µL. Platelet count ranged from 11 – 913 x 10^3/µL with the median value being 174 x 10^3/µL.

Among cytopenias, anaemia (78%) was most common, followed by thrombocytopenia (45%) and Leucopenia (20%). Peripheral blood involvement by abnormal lymphoid cells accounted for 15% and was seen only in NHL cases. The most common lymphoma with peripheral blood involvement was Follicular lymphoma and DLBCL, each accounting for 4.6%.

Aspirate studies of only 43 (50%) cases showed abnormal lymphoid cells. Follicular lymphoma was the most common NHL picked up on aspiration studies, followed by DLBCL.

The predominant pattern of marrow infiltration on bone marrow biopsy in different subtypes of 76 Non-Hodgkin lymphoma cases was-Mixed type (32.8%), followed by diffuse pattern (23.6%). Among the Mixed type: Para trabecular and Interstitial (15.7%) was the most common. The obtained results for infiltration patterns in different types of lymphoma are tabulated in Table-1

In Hodgkin lymphoma, the most common pattern of marrow infiltration was diffuse-type accounting for 60%.

Common secondary changes seen in the bone marrow were fibrosis, necrosis and granuloma accounting for 11.6%, 5.8% and 2.3% of cases, respectively.

When bone marrow aspiration and bone marrow biopsy were compared, bone marrow biopsy was found to be more accurate for assessment of marrow involvement by lymphoma, with a sensitivity rate of 100% (Table-2).

Morphological discordance between lymph node and trephine biopsy was seen only in 13 cases (15.1%), all of which were Non-Hodgkin Lymphoma. No discordance was found in Hodgkin Lymphoma. Follicular lymphoma was the most common subtype with a discordant rate of 25%, followed by DLBCL (23.8%).

During treatment 51(59.3%) patients expired, 15 patients (17.4%) were lost for follow up, remission was seen only
in 20 patients (23.2%). The commonest cause of death was septic shock accounting for 72.5%. The most common pattern associated with poor prognosis was a diffuse pattern, accounting for 45%, and the majority of diffuse large B-cell lymphoma patients who expired had a diffuse pattern of marrow infiltration.

**DISCUSSION**

The diagnosis and classification of lymphoma are made on tissue biopsy i.e. lymph node and bone marrow biopsy. In the present study, we discuss the incidence and pattern of marrow infiltration in Non-Hodgkin Lymphoma and Hodgkin Lymphoma and also correlate lymph node biopsy findings with the bone marrow biopsy morphology.

The overall incidence of marrow infiltration by lymphoma in our study was 63%, which is very high when compared to studies conducted by Bartl et al.4 (69%) and Georgii et al.7 (75%). The difference may have been exaggerated as our study population is much smaller as compared to the other two studies. Another reason could be due to different spectrums of lymphoma occurring in different countries.

**NON – HODGKIN LYMPHOMA**

The incidence of Bone marrow infiltration varies from 15% to 75% due to heterogeneous inclusion criteria used by different studies.1 The incidence of marrow infiltration by NHL in our study was 71%, which is similar to studies conducted by Bartl et al.4 (69%) and Georgii et al.7 (75%). In contrast, studies carried out by Shi et al.3 (18.1%), Bonadonna et al.8 (16%), Jones et al.9 (18%) and Conlan et al.10 (32%), showed a lower incidence of marrow infiltration. The difference in incidence could be attributed due to different types of lymphoma occurring at different places. Hassan K et al.11 in his studies found anaemia to be the most common cytopenia, followed by thrombocytopenia and neutropenia, similar results were obtained in our study. The cause of cytopenia could be due to the replacement of marrow elements by lymphomatous cells at a later stage and due to the release of cytokines by malignant cells. This could be the reason why patients in our setup, who generally present late in the course of disease with the higher frequency of marrow infiltration, manifest cytopenias more frequently.

Peripheral smear involvement accounted for 17.1% of our study. Similarly, Kumar et al.12 reported a frequency of 7.4%. However, Studies conducted by Hassan K et al.11, Arber etal.13, and Jeong et al.14 showcased a higher frequency of peripheral blood involvement. The presence of abnormal/atypical lymphoid cells in peripheral blood was found to be associated with an increase in total leucocyte count, similar observation was also found in other studies.

In our study, follicular lymphoma was the most common type of NHL with marrow infiltration accounting for 36.8%, followed by DLBCL - 27.6%, these findings are comparable to studies done by Arber et al.13 as they also found follicular lymphoma to be the most common accounting for 39.8%, followed by DLBCL -16% (Table-3).

In contrast, studies carried out by Kumar et al.12 and Shi et al.3 found Lymphoblastic lymphoma (44.8%) and Mantle cell lymphoma (18.3%) respectively, to be the most common subtype of NHL to infiltrate the marrow. Variations in the incidence of marrow infiltration could be due to the inclusion of different proportions of various histologic subtypes of lymphoma in different studies, as the incidence of marrow pathology varies according to histologic subtypes.

Predominant histopathological patterns of marrow infiltration encountered in different subtypes of 76 NHL cases in our studies were -Mixed (32.8%), followed by diffuse (23.6%), interstitial (18.4%) and para-tra trabecular (14.4%) - Figure 1.

Arber et al.13 also reported a higher incidence of marrow infiltration with a mixed pattern consistent with our study, as both our studies had a higher incidence of Follicular lymphoma cases which is associated with a mixed pattern of marrow involvement. These findings differ from studies conducted by Bolkainy et al.15 and Hassan et al.11 who found marrow infiltration to be predominantly Paratrabecular & Diffuse type respectively and in these the frequently occurring NHL was SLL.

Whereas Chen et al.16 in his studies found interstitial to be most common pattern followed by diffuse pattern. Studies conducted by Foucar et al.17 had Focal involvement as the predominant pattern.

**HODGKIN LYMPHOMA**

In the present study, the incidence of marrow infiltration by Hodgkin lymphoma accounted for 34.5%, in concordance with the study conducted by Ikram et al.18 (35%), Sharma et al.19 (36.2%) and Ayaz et al.20 (38%). In contrast, a lower incidence of marrow infiltration was seen in studies conducted by Howell et al.21 (5.2%), Franco et al.22 (10%), and Lamberti et al.4 (11.6%). Variations in incidence occur due to heterogeneous inclusion criteria used by different studies.

Out of 10 cases of Hodgkin lymphoma, all patients were anaemic accounting for 100%, whereas a study conducted by Ikram et al.18 found 66% of patients to be anaemic with marrow infiltration, he also observed leukocytosis and thrombocytosis accounting for 19% and 23% respectively, which they have attributed to reactive changes in the bone marrow of Hodgkin lymphoma cases. In contrast, the present study had no case with thrombocytosis or leukocytosis in Hodgkin lymphoma patients with marrow infiltration. In our study,
Peripheral blood involvement in Hodgkin lymphoma was not observed.

The most common pattern of infiltration in Hodgkin lymphoma observed in our study was the diffuse type, followed by focal pattern, in contrast to studies conducted by Ayaz et al. and Ikram et al. where they found the interstitial pattern to be more common and in the study conducted by Sultan et al. focal pattern were more common followed by the diffuse type. This is attributed to the fact that the patients in our country seek medical help much later in the course of the disease by the time the patients will be in the more advanced stage of the disease.

In our study, among 86 cases with marrow infiltration only in 43 cases abnormal cells were detected on Bone Marrow Aspiration (BMA) giving a sensitivity rate of 50%. Whereas the sensitivity of detection of marrow infiltration on Bone Marrow Biopsy (BMB) was 100%. Hence it is essential to perform BMB for all cases of lymphoma so that cases with marrow involvement are not missed.

The detection rate of marrow infiltration on aspirate is low, the cause for this could be - dilution of bone marrow, the difference in a procedure performed or distortion of normal marrow architecture.

The discordant rate between lymph node and trephine biopsy in our study was 15.1%. Most of the other studies have shown a higher percentage of morphological discordance between bone marrow biopsy and Lymph node biopsy compared to the present study.

In the present study, morphological discordance between lymph node and trephine biopsy was seen frequently in follicular lymphomas and diffuse large B-cell lymphomas, similar observations were made by Arber et al and Kumar et al. in their studies.

Present studies and other studies have also shown fibrosis and necrosis as the most common secondary change in bone marrow involved by lymphoma. A study done by Sovani et al. has shown a higher incidence of granulomas, probably due to the higher number of Hodgkin lymphoma cases in their study.

Out of 51 patients - 37 patients (72%) died due to septic shock and due to chemotherapy induced febrile neutropenia, which was the most common cause of death, followed by respiratory failure in 6 patients (11.7%), cardiac failure in 5 patients (9.8%), and rest 3 with renal failure (5.8%). Similarly, in study done by Ostrow et al. septic shock and respiratory failure was found to be most common cause of death.

**CONCLUSION**

Overall marrow infiltration by Non-Hodgkin Lymphoma is higher when compared with Hodgkin Lymphoma. Variations in incidence occur, due to heterogeneous inclusion criteria used by different studies and due to different types of lymphoma occurring at different places.

Bone marrow aspiration is of mere importance in the diagnosis of marrow infiltration by lymphoma when compared to Bone marrow biopsy. As the sensitivity of Bone Marrow Aspiration is 50%, whereas the detection of lymphoma on Bone Marrow Biopsy is 100%. The causes for inadequate bone marrow aspiration could be fibrosis and technical errors during the procedure. Marrow infiltration by lymphoma is definite evidence of disseminated disease. Therefore knowledge about the presence or absence of marrow involvement is vital. As the specific pattern of bone marrow infiltration is associated with specific subtypes of lymphoma, hence study of patterns of marrow infiltration in lymphoma aids in accurate diagnosis when correlated with lymph node biopsy. A small proportion of lymphoma cases can show morphological disparity on bone marrow biopsy when compared to lymph node biopsy. Hence IHC is recommended for accurate subtyping of lymphomas.

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Table 1: Infiltration Patterns in Different Types of Lymphoma

| Lymphoma                          | Primary Pattern | Mixed Pattern |
|-----------------------------------|-----------------|---------------|
|                                   | D   | I   | P   | N   | F   | P&I  | P&D  | I&D  |
| Non-Hodgkin lymphoma              |     |     |     |     |     |      |      |      |
| Follicular lymphoma               | 2   | 3   | 10  | -   | -   | 7    | 6    | -    |
| Diffuse large B-cell lymphoma     | 8   | 4   | 1   | 1   | -   | 3    | 3    | 1    |
| Angioimmunoblastic T-cell lymphoma| 2   | 1   | -   | -   | -   | -    | 2    |      |
| Mantle cell lymphoma              | -   | 1   | -   | 1   | -   | 2    | 1    |      |
| Peripheral T-cell lymphoma        | 1   | 3   | -   | -   | -   | -    | -    |      |
| T-Lymphoblastic lymphoma          | 2   | -   | -   | 1   | -   | -    | -    |      |
| Anaplastic large cell lymphoma    | -   | 1   | -   | 1   | 1   | -    | -    |      |
| Small lymphocytic lymphoma        | -   | -   | -   | 1   | 1   | -    | -    |      |
| Nodal marginal zone lymphoma      | -   | -   | -   | 1   | -   | -    | -    |      |
| B-Lymphoblastic lymphoma          | 1   | -   | -   | -   | -   | -    | -    |      |
| Not Subclassified                 | 1   | 1   | -   | -   | -   | -    | -    |      |

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### Table 1: (Continued)

| LYMPHOMA                        | PRIMARY PATTERN | MIXED PATTERN |
|---------------------------------|-----------------|---------------|
|                                 | D   | I   | P   | N   | F   | P&I | P&D | I&D |
| T-cell lymphoma /ALPD           | 1   | -   | -   | -   | -   | -   | -   | -   |
| Classical Hodgkin lymphoma      |     |     |     |     |     |     |     |     |
| Nodular sclerosis               | 1   | -   | -   | -   | -   | -   | -   | -   |
| Mixed cellularity               | 1   | -   | -   | -   | 2   | -   | -   | -   |
| Lymphocyte-depleted             | 1   | 1   | -   | -   | -   | -   | 1   | -   |
| Not subclassified               | 3   | -   | -   | -   | -   | -   | -   | -   |
| Sub Total                       | 24  | 15  | 11  | 6   | 4   | 12  | 11  | 3   |

D - Diffuse, I- Interstitial, P- Paratrabecular, N- Nodular, F- Focal, Mixed [P&I- Paratrabecular and Interstitial, P&D - Paratrabecular and Diffuse, I & d - Interstitial and Diffuse]

### Table 2: Comparison of Marrow Involvement B/W BMA & BMB

| Lymphoma                           | Positive Biopsy | BMA Consistent | BMA not consistent | Concordance (%) |
|------------------------------------|-----------------|----------------|-------------------|-----------------|
| Follicular lymphoma                | 28              | 17             | 11                | 60.7            |
| Diffuse large B-cell lymphoma      | 21              | 8              | 13                | 38              |
| Angioimmunoblastic T-cell lymphoma | 5               | 1              | 4                 | 20              |
| Mantle cell lymphoma               | 5               | 4              | 1                 | 80              |
| Peripheral T-Cell lymphoma         | 4               | 3              | 1                 | 75              |
| T- Lymphoblastic lymphoma          | 3               | 2              | 1                 | 66.6            |
| Anaplastic large cell lymphoma     | 3               | 2              | 1                 | 66.6            |
| Small lymphocytic lymphoma         | 2               | 2              | 0                 | 100             |
| Nodal marginal zone lymphoma       | 1               | 0              | 1                 | 0               |
| B- Lymphoblastic lymphoma          | 1               | 1              | 0                 | 100             |
| Not Subclassified                  | 2               | 1              | 1                 | 50              |
| T- Cell lymphoma /ALPD             | 1               | 1              | 0                 | 100             |
| Classical Hodgkin lymphoma         | 10              | 1              | 9                 | 10              |
| Total                              | 86              | 43             | 43                | 50              |

### Table 3: Subtypes of Non-Hodgkin Lymphoma with Marrow Infiltration

| Subtypes                          | Present study | Arber et al 14 | Kumar et al 15 | Shi et al 6 |
|-----------------------------------|---------------|----------------|----------------|------------|
| Follicular lymphoma               | 36.8%         | 39.8%          | 10.2%          | 14.3%      |
| Diffuse large B-cell lymphoma     | 27.6%         | 16%            | 18.3%          | 14.4%      |
| Angioimmunoblastic T-Cell lymphoma| 6.5%          | 0.6%           | -              | 2%         |
| Mantle cell lymphoma              | 6.5%          | 9.3%           | 2%             | 18.3%      |
| Peripheral T-Cell lymphoma        | 5.2%          | 3.5%           | 12.2%          | 1.3%       |
| Anaplastic large cell lymphoma    | 4%            | 0.8%           | -              | 0.7%       |
| Small lymphocytic lymphoma        | 2.6%          | 4.4%           | 6.1%           | 14.4%      |
| Nodal marginal zone lymphoma      | 1.3%          | 0.22%          | -              | 6.5%       |
| Lymphoblastic lymphoma            | 5.2%          | 8.4%           | 44.8%          | 11.8%      |
| NHL-Not Subclassified             | 2.6%          | -              | -              | -          |
| T- cell lymphoma /ALPD            | 1.3%          | -              | -              | -          |
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**Figure 1:** Diffuse Pattern of Infiltration in Case of Classical Hodgkin Lymphoma.

**Figure 2:** Interstitial Pattern of Infiltration in Case of Diffuse Large B-Cell Lymphoma.

**Figure 3:** Nodular Pattern of Infiltration in Case of Small Lymphocytic Lymphoma.

**Figure 4:** Paratrabecular & Interstitial Pattern of Infiltration in case of Follicular Lymphoma.