

# Pattern of Bone Marrow Infiltration in Non-Hodgkin's Lymphomas

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## Abstract

**Background:** Non-Hodgkin's lymphomas have a predilection for bone marrow involvement, and bone marrow biopsy is performed in an attempt to evaluate stage IV disease. This study was conducted to evaluate the pattern of bone marrow infiltration and morphology of atypical cells in freshly diagnosed patients of Non-Hodgkin's Lymphomas (NHL).

**Methods:** This prospective, descriptive study was conducted in the Department of Pathology, Pakistan Institute of Medical Sciences (PIMS) Islamabad from January 2003 to December 2004. A total of 50 cases of non-Hodgkin's lymphoma diagnosed on tissue biopsy, and found to have bone marrow infiltration were randomly selected. The H & E stained trephine sections were examined for evidence of infiltration and pattern of infiltration, viz. focal random, focal para-trabecular, diffuse interstitial and diffuse infiltration.

**Results:** In 52% patients, the infiltration was diffuse; of these patients, 34% showed a complete replacement of normal marrow cellular elements by lymphoma cells, and 18% manifested diffuse interstitial infiltration. An additional 10% cases manifested diffuse as well as diffuse interstitial infiltration. In 30% of patients diffuse or diffuse interstitial infiltration was accompanied with focal random or focal paratrabecular infiltration.

**Conclusion:** Diffuse bone marrow infiltration is much more common in our set-up probably because of a relatively late presentation by patients of non-Hodgkin's lymphoma

**Key Words:** Non-Hodgkin's Lymphoma; NHL; Bone marrow infiltration

## Introduction

Non-Hodgkin's lymphomas (NHLs) are characterized by neoplastic transformation of lymphoid cells, and have a high potential for spread to various tissues throughout the body especially bone marrow, liver, spleen, lungs, and brain etc. Bone marrow biopsy is an essential part of diagnostic workup in NHL, and the patients presenting in stage IV disease with infiltration of bone marrow and/or other tissues manifest poor prognosis and response to treatment.<sup>1,2</sup> It has been observed that patients in developing countries generally present at a relatively advanced stage in comparison with western patients.<sup>3-5</sup>

In patients with bone marrow involvement, the marrow smears and trephine sections are collectively interpreted for cytology, pattern of bone marrow infiltration, and sometimes immunophenotyping.<sup>6</sup> On marrow smears, the infiltrating cells can be recognized as atypical cells, and their number is usually determined on 500-cell myelogram as percentage. Trephine imprints are also very useful, especially if aspiration has manifested a blood/dry tap. Trephine biopsy permits an assessment of pattern and extent of infiltration, which is of both diagnostic and prognostic relevance.<sup>7</sup> Sometimes, trephine biopsy may demonstrate lymphoma when no abnormal cells have been detected in blood and bone marrow smears.

Four distinct patterns of infiltration by NHL are usually recognized<sup>1</sup>:

Interstitial pattern, in which lymphoid cells are loosely dispersed amidst haematopoietic and fat cells.

ii) Diffuse (packed marrow) pattern, in which lymphoid cells are densely packed within the marrow spaces; lymphoid nodules are not discernable.

iii) Nodular pattern, in which multiple, partly confluent nodular aggregates of lymphoid cells are recognized. The nodules vary in size from 0.5- 3 mm in diameter and are usually located in the central inter-trabecular areas.

iv) Para-trabecular pattern, in which the striking morphological feature is streaming of lymphoid infiltrate in apposition to osseous trabeculae.

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The objective of the present study was to evaluate the pattern of bone marrow infiltration and morphology of atypical cells in freshly diagnosed patients of NHL.

### Patients and Methods

This prospective, descriptive study was conducted in the Department of Pathology, Pakistan Institute of Medical Sciences (PIMS) Islamabad from January 2003 to December 2004. A total of 50 cases of non-Hodgkin’s lymphoma diagnosed on tissue biopsy, and found to have bone marrow infiltration were randomly selected. The patients who had already received cytotoxic therapy and those who were not found to have bone marrow infiltration were excluded from the study.

The Wright-stained bone marrow smears were examined for cellularity, number and maturation of normal cells and the number and morphology of atypical lymphoid (lymphoma) cells. The following features of atypical cells were noted: size; nuclear chromatin; nuclear cleavage; cell and nuclear outline and nucleoli

The H & E stained trephine sections were examined for evidence of infiltration and pattern of infiltration, viz. focal random, focal para-trabecular, diffuse interstitial and diffuse infiltration.

### Results

**Bone Marrow smears:** Bone marrow smears showed atypical lymphoid cells in 46 (92%) patients. As shown in table 1, the cell size was variable; about half of the patients showed large and about a quarter mixed small and large cells; 20 of patients manifested predominantly small-sized cells. Table 1 also gives the nuclear morphological details. Nuclear chromatin was coarsely stippled in majority of cases. Nuclear cleavage was a prominent feature in 28% of patients. Nucleoli were conspicuous in 20% and inconspicuous in 50% of cases

**Bone Marrow Trephine Biopsy:** The marrow fragments were hypercellular in 45 cases; in 5 of them hypocellular pockets were also observed. In 4 cases, marrow fragments were moderately cellular and in 1 hypocellular.

**Pattern of infiltration (Table 2):** In 26 (52%) patients, the infiltration was diffuse without any areas of focal infiltration. Of these patients, 17 (34%) showed a diffuse infiltration thereby replacing the normal bone

marrow cellular elements in the area of infiltration with complete effacement of normal architecture.

**Table 1: Features of cells on bone marrow smears**

| Features                               | No (%)  |
|--|---------|
| <b>Size</b>                            |         |
| Small                                  | 10 (20) |
| Large                                  | 23 (46) |
| Mixed small / large                    | 12(24)  |
| Medium to large size cells.            | 01 (02) |
| <b>Cell/nuclear outline</b>            |         |
| Regular cell/nuclear outline           | 33 (66) |
| Irregular cell/nuclear outline         | 14 (28) |
| Regular cell/irregular nuclear outline | 03 (06) |
| <b>Nuclear chromatin</b>               |         |
| Fine stippled                          | 19 (38) |
| Coarsely stippled                      | 28 (56) |
| Vesicular                              | 03 (06) |
| <b>Nuclear cleavage</b>                |         |
| Present                                | 14 (28) |
| <b>Nucleoli</b>                        |         |
| Conspicuous                            | 10 (20) |
| Inconspicuous                          | 30 (50) |

**Table 2: Pattern of infiltration (Trephine biopsy)**

| Type                              | No (%)  |
|-----------------------------------|---------|
| Diffuse                           | 9 (18)  |
| Diffuse interstitial              | 17 (34) |
| Focal random/diffuse interstitial | 6 (12)  |
| Diffuse/ diffuse interstitial     | 5 (10)  |
| Focal paratrabecular/interstitial | 4 (08)  |
| Focal paratrabecular/diffuse      | 2 (04)  |
| Focal random / diffuse            | 2 (04)  |
| Diffuse in leukemic phase         | 2 (04)  |
| Follicular/diffuse interstitial   | 1 (02)  |
| Focal random/ paratrabecular      | 1 (02)  |
| Focal random                      | 1 (02)  |

Thus, the fat spaces were completely encroached upon. Two additional patients manifesting similar diffuse infiltration presented in leukemic phase at the time of first diagnosis. In the remaining 9 cases (18%), the infiltrating atypical cells were admixed with the normal bone marrow cellular elements. Fat spaces were found to be preserved to a variable extent. These

patients were diagnosed as having diffuse interstitial infiltration.

**Table 3: Cell morphology on Trepine biopsy**

| Cell Morphology                    | No (%)  |
|------------------------------------|---------|
| Small non-cleaved                  | 25 (50) |
| Small cleaved                      | 4 (08)  |
| Large non-cleaved                  | 6 (12)  |
| Large cleaved                      | 7 (14)  |
| Mixed small and large non- cleaved | 5 (10)  |
| Mixed cells in leukemic phase      | 2 (04)  |
| Small cleaved in leukemic phase    | 1 (02)  |

In an additional 5 (10%) cases, areas manifesting diffuse as well as diffuse interstitial infiltration concomitantly were observed.

**Table 4: Pattern of bone marrow infiltration as compared to previous studies**

| Author (Year)         | Interstitial | Diff use | Para-trabecular | Focal Nodular | Mixed |
|-----------------------|--------------|----------|-----------------|---------------|-------|
| Zhongura et al (2006) | 11.6%        | 44.9%    | 0.0%            | 29.36%        | 0.0%  |
| Lim et al (2000)      | 0.0%         | 71.4%    | 0.0%            | 0.0%          | 0.0%  |
| Chen et al (2000)     | 23.0%        | 22.0%    | 0.0%            | 7.0%          | 18.0% |
| Hassan* et al (1995)  | 0.0%         | 77.5%    | 10%             | 12.5%         | 0.0%  |
| Lee et al (1994)      | 56%          | 31%      | .0%             | 0.0%          | 0.0%  |
| Malik* et al (1992)   | 0.0%         | 46.3%    | 19.5%           | 12.5%         | 0.0%  |
| Bartl R et al (1982)  | 26%          | 25%      | 8.0%            | 41%           | 0.0%  |

Amongst the remaining 17 cases, in 15 (30%) diffuse or diffuse interstitial infiltration was concomitantly associated with focal random or focal para-trabecular infiltration, or follicular pattern as detailed in table 2.

In one patient (2%) areas of focal random as well as focal para-trabecular infiltration were observed, without an evidence of diffuse or diffuse infiltration. In one patient, only focal random

infiltrates were observed

**Atypical cells:** As shown in table 3, half of the patients showed small non-cleaved cells. Large cleaved, large non-cleaved and mixed small / large non-cleaved cells were observed in 7 (14%), 6 (12%) and 5 (10%) cases each. Four (8%) patients exhibited small cleaved cells. Amongst the patients in leukemic phase, mixed cells were observed in 2 (4%) and small cleaved cells in 1 (2%) patient, respectively (Figs 1-6).

Fibrosis of a variable degree was observed in 33 cases. It was mild in 19 (38%), moderate in 8 (16%) and marked in 6 (12%) cases, respectively. Fibrosis was not observed on routine H & E stained section in the remaining 17(34%) cases and marked.

An evidence of scattered areas of necrosis was observed in histological trephine section in 3 patients

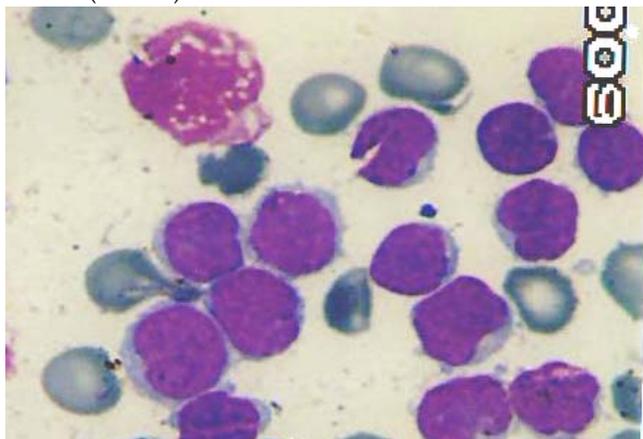
## Discussion

The non-Hodgkin’s lymphomas (NHLs) have a propensity to disseminate widely, and especially to bone marrow, liver and spleen.

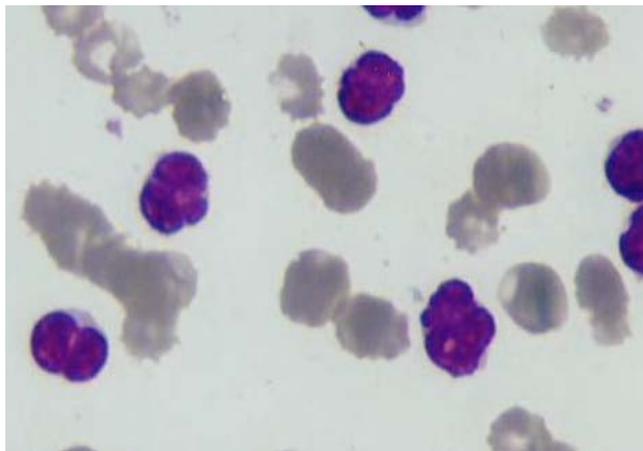
Literature searched from within the country has shown two published studies regarding bone marrow infiltration by Non- Hodgkin’s lymphoma. In the first study, the lymphoma tissue in marrow presented as diffuse infiltrate in 46.3% cases, focal aggregates in 12.5%, and paratrabecular distribution in 19.5% of all types of lymphoma.<sup>8</sup> In the second study the diffuse infiltrate was reported in 77.5% cases, focal random (non-paratrabecular) in 12.5 % and paratrabecular distribution in 10% of all types of lymphoma.<sup>9</sup>

The pattern of bone marrow infiltration by NHL cells in the present study, when compared with the two above mentioned series, shows almost similar findings. In the present study, the commonest pattern of bone marrow infiltration was that of diffuse type (66%), followed by focal (34%); amongst the latter, focal random or non paratrabecular pattern was observed in 20% cases, and focal paratrabecular in 14% cases. The percentage of diffuse infiltration in our series was lower than that in the study presented by Hassan et al, but higher than series published by Malik S et al<sup>8,9</sup> The frequency of focal paratrabecular pattern in the present series was lower than the series published by Malik S et al.<sup>8</sup> However, in both the previous local series, the percentage of focal random

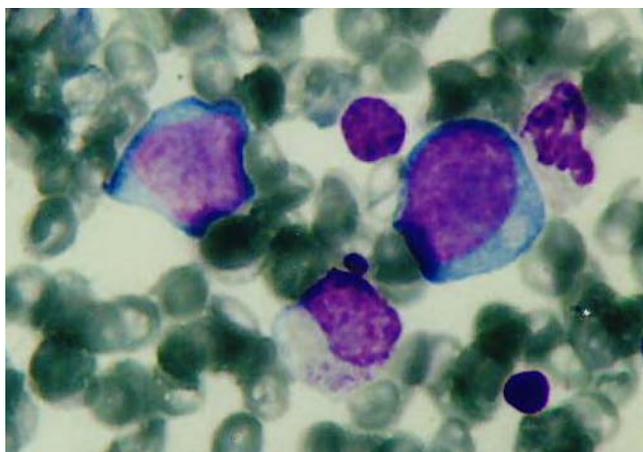
and non paratrabecular infiltration were similar i.e., 12.5%. (Table4)



**Figure 1 : Small non-cleaved cells in bone marrow smear**

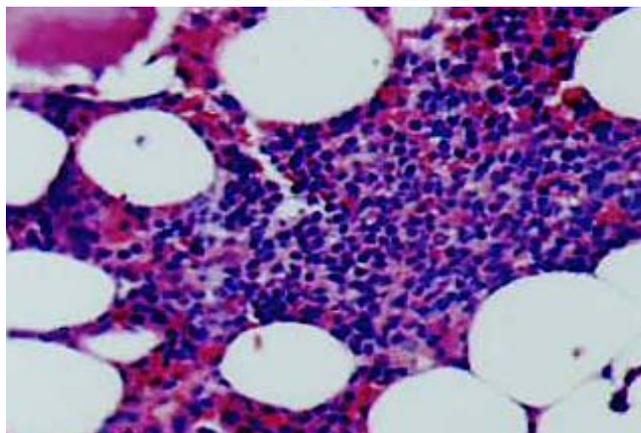


**Figure 2 : Small cleaved cells on bone marrow smear**

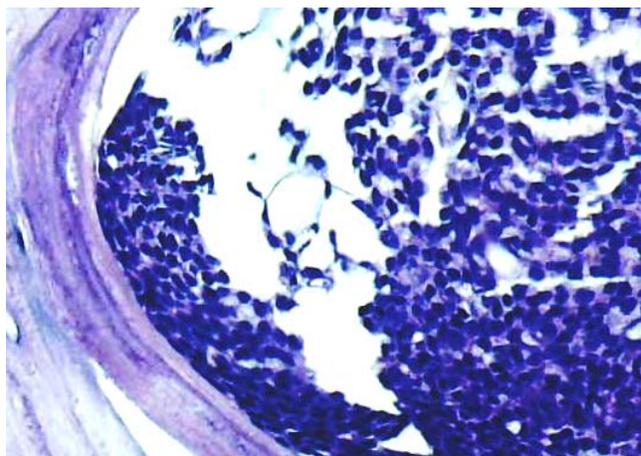


**Figure 3 : Large sized lymphoma cells on**

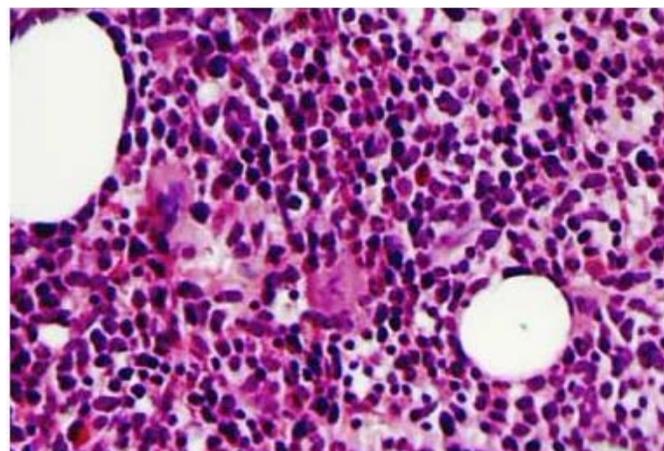
**bone marrow smear**



**Figure 4 : Focal random infiltration on trephine section**



**Figure 5 : Focal paratrabecular marrow infiltration by NHL on trephine section**



**Figure 6 : Diffuse infiltration on trephine**

### **section**

The western studies have shown patterns of bone marrow infiltration varying from mainly focal random or focal paratrabecular in some to mainly diffuse in others.<sup>8,10,11</sup> We found high rate of diffuse infiltration in our patients (66%) as also observed in previous two local studies, and this probably reflects that most of our patients present rather late for examination or are diagnosed rather late when disease has already advanced to stage IV.

In a study by Lai et al, bone marrow involvement by NHL was the most frequently found in follicular small cleaved cell (57%), diffuse mixed small and large cell (56%), and lymphoblastic (56%) lymphomas. A paratrabecular pattern of marrow involvement tended to occur more frequently in low or intermediate grade lymphomas. High grade lymphomas tended to show an interstitial or diffuse pattern of marrow involvement. The results of marrow aspiration and biopsy were complementary to each other.<sup>12</sup> In their study, in 43% of cases with marrow lymphoma there was peripheral blood involvement at the time of diagnosis. Blood involvement was most frequently found in lymphoblastic lymphoma (70%). In general, the degree of blood involvement was related to the extent of marrow involvement. Patients with marrow involvement often presented with 'B' symptoms and hepatomegaly. Abnormalities in at least one of the blood counts were quite common (93%). Patients with marrow involvement showed a significantly higher frequency of hepatomegaly and abnormal blood counts, as compared to those with negative marrows.<sup>12</sup>

In another study by Chen et al, a total of 70 patients with NHL (Male 52, Female 18; median age: 49 years) were studied. The extent of bone marrow involvement was minimal in 15 cases, moderate in 16 cases and severe in 39 cases. The bone marrow involvement was of interstitial type in 23 cases, nodular type in 7 cases, mixed type in 18 cases and diffuse type in 22 cases. The frequency of splenomegaly in nodular type NHL was significantly higher than that in any other type. Nodular type NHL occurred mainly in B-cell lymphoma.<sup>13</sup>

Staples WG et al noted that in their study focal involvement of the bone marrow by NHL was frequent. They also stated that non- Hodgkin's lymphoma and Hodgkin's disease can occur primarily in the bone marrow.<sup>14</sup>

In a previous study the most frequent patterns of marrow involvement in NHL were interstitial and diffuse (56% and 31%, respectively).<sup>15</sup> The

paratrabecular pattern was relatively uncommon (4%). Most lymphomas (42%) extensively involved marrow space thereby infiltrating greater than 76% of total marrow space. It was further stressed that bone marrow trephine biopsy was the best method for evaluation of bone marrow infiltration, especially when compared to bone marrow smear and clot sections. Study revealed that most common histologic type of marrow lymphoma was diffuse large cell type with frequent interstitial and diffuse pattern and extensive involvement of marrow space.<sup>15</sup>

All types of Non- Hodgkin's lymphomas do not invade the bone marrow with an equal frequency.<sup>16</sup> The pattern of marrow involvement also differs to some extent in various NHL subtypes, for example paratrabecular infiltration is strongly associated with follicular lymphoma. A bone marrow biopsy performed in patients with low grade lymphoma sometime shows unexpected high grade transformation, which necessitates a different therapeutic approach.<sup>17</sup>

The cells infiltrating the marrow usually show a higher degree of concordance with corresponding lymph node histology. However, occasionally the cell morphology at the site of bone marrow infiltration is found to be different from the primary site of lymphoma.<sup>118</sup> Conlan et al have reported a high incidence of morphologic discordance (40%) between lymph node and bone marrow (1990). We observed this type of discordance in four patients.<sup>19</sup>

In a similar study, Adewuji B et al have reported that low grade lymphoma manifested predominantly diffuse pattern of marrow involvement and high grade lymphomas had nearly equal proportion with diffuse and focal patterns.<sup>20</sup> A mixed pattern of infiltration was most common, followed by paratrabecular, nodular diffuse and interstitial patterns. They also found discordance between the bone marrow morphology and other tissue sites in 24.9% of cases.

Juneja SK et al published a study of 260 patients with NHL who underwent bilateral bone marrow biopsy. They observed a strong correlation between follicular lymphoma and paratrabecular pattern, which was observed in 40 of 45 positive cases. Discordant histology was seen in 6 of 20 positive cases of DLCL and 2 of 37 positive cases of follicular small cleaved cell lymphomas.<sup>16</sup>

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