Lymphoid Nodules of Bone Marrow: Normal and Abnormal

By Arkadi M. Rywlin, Rolando S. Ortega, and Carlos J. Dominguez

A study of consecutive bone marrow aspirates from 365 patients without lymphoproliferative disorder, ten patients with chronic lymphocytic leukemia (CLL), and 25 patients with malignant lymphoma disclosed a clear separation of normal from abnormal lymphoid nodules (LN). Normal LN were found in 47% of patients and were classified into lymphoid follicles and lymphoid infiltrates. A new entity, nodular lymphoid hyperplasia (NLH), was diagnosed on ten bone marrows which contained a low-power field displaying four or more lymphoid nodules or showed a lymphoid nodule larger than 0.6 mm. The clinical significance of nodular lymphoid hyperplasia remains unknown; in certain cases it represents a precursor state of a mature lymphocytic lymphoproliferative disorder. Nodular aggregates in CLL are of the infiltrate type and exhibit a tendency to confluence. Eleven of the 25 patients with malignant lymphoma displayed lymphoreticular nodules which were cytologically similar to the original lymphoma and different from normal LN. Two patients, one with reticulum cell sarcoma and one with Hodgkin’s disease, showed NLH of the bone marrow. Bone marrow LN in patients with an established diagnosis of mature lymphocytic lymphoma have to be interpreted with the utmost caution. Confluence of LN, irregular shapes, and the presence of prolymphocytes and lymphoblasts speak for lymphomatous nodules. Additional clinicopathologic studies are necessary to sharpen the distinction between NLH and well-differentiated lymphoproliferative disorders.

LYMPHOID NODULES (LN) have been the subject of very few clinical studies performed on aspirated or biopsied bone marrow. They have been found in 1\(^o\) of specimens, a much lower incidence than the 21\(^o\) reported in autopsy material (Table 1). Lymphoid nodules have been encountered in the bone marrow with approximately the same frequency in autopsies of healthy accident victims and of hospital patients.\textsuperscript{5,10} Based on this and on clinicopathologic correlation studies, there is general agreement that bone marrow LN constitute a normal finding without any known clinical significance.\textsuperscript{2,3,5,11}

We have frequently observed LN in histologic sections of aspirated bone
Table 1. Incidence of Lymphoid Nodules in Bone Marrow; Review of Literature

<table>
<thead>
<tr>
<th>Year</th>
<th>Study</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1915</td>
<td>Askonazy</td>
<td>34%</td>
</tr>
<tr>
<td>1917</td>
<td>Fischer</td>
<td>62%</td>
</tr>
<tr>
<td>1939</td>
<td>Williams</td>
<td>24% (age &lt; 40 yr)</td>
</tr>
<tr>
<td>1957</td>
<td>Hashimoto et al.</td>
<td>34%</td>
</tr>
<tr>
<td>1960</td>
<td>Werner</td>
<td>21%</td>
</tr>
<tr>
<td>1962</td>
<td>Lennert and Nagai</td>
<td>36%</td>
</tr>
<tr>
<td>1967</td>
<td>Chomette et al.</td>
<td>43%</td>
</tr>
</tbody>
</table>

In Vivo Studies

<table>
<thead>
<tr>
<th>Year</th>
<th>Study</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1954</td>
<td>Johnstone (B)</td>
<td>9%</td>
</tr>
<tr>
<td>1954</td>
<td>Pettet et al. (A)</td>
<td>3%</td>
</tr>
<tr>
<td>1960</td>
<td>Rohr (A)</td>
<td>4%</td>
</tr>
<tr>
<td>1964</td>
<td>Duhamel (B)</td>
<td>1%</td>
</tr>
<tr>
<td>1968</td>
<td>Duhamel (B)</td>
<td>3%</td>
</tr>
<tr>
<td>1973</td>
<td>Rywlin et al. (A)</td>
<td>47%</td>
</tr>
</tbody>
</table>

Note: (A), aspiration, (B), biopsy.

marrow particles. At times, we have found it difficult to distinguish normal from abnormal LN and to decide whether the bone marrow was involved by a lymphoproliferative disorder.

In this study, we determine the incidence, size, density, cytology, and structure of normal LN occurring as an incidental finding in patients without a lymphoproliferative disorder. We describe a new entity—nodular lymphoid hyperplasia of the bone marrow. We study lymphoreticular nodules in chronic lymphocytic leukemia and malignant lymphoma and contrast them with normal LN.

MATERIALS AND METHODS

We reviewed consecutive bone marrow aspirates from 365 different patients without a lymphoproliferative disorder, ten patients with chronic lymphocytic leukemia, and 25 patients with an established tissue diagnosis of malignant lymphoma. The cytology of the malignant lymphoma in lymph nodes was compared with the lymphoreticular nodules found in the bone marrow.

The aspirations were performed in the posterior superior iliac spines or in the sternum. The aspirated material was processed by a simple, previously described technique that yields smears as well as sections of concentrated, aggregated, paraffin-embedded bone marrow particles. Three smears and at least ten embedded step sections were studied in every case. The smears were stained with a Wright-Leishman mixture. The following stains were performed on the sections: hematoxylin-eosin, Giemsa, Gomori's Prussian blue for hemosiderin, Gordon-Sweets' method for reticulum fibers, and the naphthol AS-D chloroacetate stain of Leder. The size of the lymphoid nodules was measured with a screw micrometer eyepiece. The highest number of lymphoid nodules that could be found in any low-power field (eyepiece 10 x, objective 4 x, approximate area 80 sq mm)was determined for each specimen.

The terminology used for malignant lymphomas is essentially that of Rappaport. A lymphoblastic lymphoma (stem-cell lymphoma) was diagnosed when the tumor cells were of the size of reticulum cells (histiocytes) and showed a narrow rim of dark blue cytoplasm and prominent nucleoli with the Giemsa stain. A negative Leder stain and the absence of eosinophilic myelocytes served to distinguish lymphoblastic lymphoma from granulocytic sarcoma, a tumor made up of myeloblasts. In prolymphocytic lymphoma, the predominant tumor cells were intermediate in size between mature lymphocytes and lymphoblasts, and their nuclei showed more detail than mature lymphocytes, particularly with the Giemsa stain. A few lymphoblasts were invariably present. Reticulum cell sarcoma is synonymous with histiocytic lymphoma.
RESULTS

A review of our material allowed a clear division of lymphoid nodules into normal and abnormal (Table 2).

Normal Lymphoid Nodules (LN)

Of the 365 bone marrows from patients without a lymphoproliferative disorder, 173 (47%) contained LN. Statistical data concerning average age and sex of patients with and without LN are shown in Table 3.

The number of LN varied from one LN per slide to as many as 13 per slide. The majority of patients showed two LN per slide. Since the amount of bone marrow varied from slide to slide, we determined the highest density of LN per low-power field. This density varied from 1 to 5 LN per low-power field. The LN measured from 0.08 to 1.2 mm in greatest dimension, with an average of about 0.3 mm. The normal LN could be subdivided into two distinct types: Lymphoid follicles (LF) and lymphoid infiltrates (LI). Lymphoid follicles constituted 84% of the LN and resembled Malpighian follicles of the spleen. They were round to oval, often sharply circumscribed with serrated edges created by an extension of lymphocytes into spaces between fat cells (Fig. 1). The LF were mostly solid structures, but a few fat cells at the periphery were found in 10% of cases. Lymphoid follicles displayed from one to four cross sections of small blood vessels, considered to represent precapillary arterioles. Cytologically, LF were made up of mature lymphocytes admixed with a few reticulum cells and some plasma cells and mast cells towards the periphery. Increased numbers of eosinophils surrounding the LF were noted in 10% of cases. Lipid granulomas were associated with LF in 10% of cases. Five percent of LF exhibited well-developed germinal centers (Fig. 2). Occasionally, centrofollicular hemorrhages and hyaline deposits were noted. The reticulum framework of the LF was increased when compared to the surrounding bone

<table>
<thead>
<tr>
<th>Table 2. Lymphoid Nodules of Bone Marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Normal</td>
</tr>
<tr>
<td>1. lymphoid follicles (LF)</td>
</tr>
<tr>
<td>2. lymphoid infiltrates (LI)</td>
</tr>
<tr>
<td>B. Abnormal</td>
</tr>
<tr>
<td>1. Cytologically normal but increased number* or size† of lymphoid nodules—nodular lymphoid hyperplasia</td>
</tr>
<tr>
<td>a. predominantly LF</td>
</tr>
<tr>
<td>b. predominantly LI</td>
</tr>
<tr>
<td>(pre-or chronic lymphocytic leukemia, lymphosarcoma, mature cell type)</td>
</tr>
<tr>
<td>2. Cytologically abnormal</td>
</tr>
<tr>
<td>a. malignant lymphoma†</td>
</tr>
<tr>
<td>1. Hodgkin’s disease</td>
</tr>
<tr>
<td>2. prelymphocytic or lymphoblastic</td>
</tr>
<tr>
<td>3. histiocytic (reticulum cell sarcoma)</td>
</tr>
<tr>
<td>4. lymphohistiocytic</td>
</tr>
<tr>
<td>3. Granulomas</td>
</tr>
</tbody>
</table>

*Four or more LN per any low-power field (80 sq mm).
†LN larger than 0.6 mm.
††May present with diffuse rather than nodular infiltrates.
Table 3. Statistical Data

A. Patients Without Lymphoproliferative Disorder 365 (100%)
   a. with lymphoid nodules 173 (47%)
      females 105 (61%)
      males 68 (39%)
      average age: 71.9
   b. without lymphoid nodules 192 (53%)
      females 92 (48%)
      males 100 (52%)
      average age: 63.5

B. Patients With Nodular Lymphoid Hyperplasia 10
   females 8
   males 2
   average age: 74.5

C. Patients With Lymphoproliferative Disorder 35
   a. chronic lymphocytic leukemia 10
      females 4
      males 6
      average age: 73.7
   b. malignant lymphoma 25
      females 15
      males 10
      average age: 62.6

D. Hospital Admissions (1972) 24,376
   females 13,107 (54%)
   males 11,269 (46%)
   average age: 63.8

Fig. 1. Well-circumscribed lymphoid follicle with serrated edges. Hematoxylin and eosin. x 308.
marrow and displayed a characteristic pattern. The centers of the follicles contained a few radially oriented reticulum fibers, while the periphery of the follicles showed a mesh or netlike arrangement of reticulum fibers created by the intersection of radial fibers with concentrically oriented peripheral fibers.

Lymphoid infiltrates were irregular in shape and frequently contained centrally located fat cells (Fig. 3). When they were solid, the lymphocytes were loosely arranged. The reticulum framework of the LI was normal or slightly

Fig. 2. Lymphoid follicle with germinal center. Hematoxylin and eosin. × 109.

Fig. 3. Lymphoid infiltrate located between four arrows. It is irregular in shape, contains numerous fat cells and is poorly circumscribed. Hematoxylin and eosin. × 143.
accentuated when compared to the normal marrow. Cytologically, LI were composed of mature lymphocytes with occasional reticulum cells, much fewer in number than in LF. Eosinophils were sometimes seen. Germinal centers were never seen in LI.

**Nodular Lymphoid Hyperplasia (NLH)**

Among the 173 bone marrows which contained LN, there were ten which were diagnosed as nodular lymphoid hyperplasia (NLH). Nodular lymphoid hyperplasia was arbitrarily considered to be present when we could demonstrate a low-power field (80 sq mm) containing four or more LN, or if any LN exceeded 0.6 mm in greatest dimension (Fig. 4). The LF and LI in NLH were similar in all other respects to the normal ones described above. In this group of patients, five LN per low-power field was the highest number seen, and the largest LN measured 1.2 mm. All ten marrows showed LF as well as LI. In eight of the marrows, the majority of the LN were LF, whereas in two, LI predominated. None of these ten patients had any clinical evidence of a lymphoproliferative disorder. The highest peripheral lymphocyte count recorded was 3290/cu mm. None of the five patients in whom a 2 2½ yr follow-up was available has developed a lymphoproliferative disorder. One patient died of congestive heart failure, and no follow-up information was available in four patients.

**Chronic Lymphocytic Leukemia (CLL)**

Three distinct patterns of bone marrow infiltration with lymphocytes were noted in the ten patients with CLL: Diffuse, nodular, and a combination of these two. Eight patients showed a diffuse infiltration of the marrow with formation of LN of the infiltrate type. The bone marrow fat cells were decreased in all these cases. In six patients, the diffuse infiltration was the more striking.

![Fig. 4. Nodular lymphoid hyperplasia. Note several lymphoid nodules (arrows) in very-low-power field. Hematoxylin and eosin. x 19.](image-url)
morphologic appearance, while in two, the nodular pattern was predominant (Fig. 5). One bone marrow contained numerous small, partially confluent LN of the infiltrate type with good preservation of the fat cells (Fig. 6). Another patient showed a diffuse infiltration of the marrow with mature lymphocytes without nodule formation and with almost total disappearance of fat (Fig. 7). Eight patients had an increased number of reticulum fibers in their marrow. The lymphocytes in all ten cases were mature, though compared to the nodular lymphoid hyperplasia, an increased number of lymphoblasts and prolymphocytes were present.

**Malignant Lymphoma**

The bone marrow showed involvement with lymphoma in 11 out of 25 cases. The involvement was nodular in all cases. In the non-Hodgkin lymphomas, the lymphomatous bone marrow nodules were cytologically identical with the original lymphoma (Figs. 8 and 9). The size and density of the nodules varied...
from case to case. Their reticulum framework was often accentuated and strikingly similar to that of normal LF. Five of the 11 cases of Hodgkin's disease showed bone marrow involvement. In all five cases, the bone marrow nodules were totally different from normal lymphoid nodules. These nodules were made up of atypical histiocytes admixed with eosinophils, plasma cells, lymphocytes, and fibroblasts (Fig. 10). In only one case did we find a Reed-Sternberg cell. In the other four cases, the marrow findings were considered to represent involvement with Hodgkin's disease because of the atypical histiocytes, even though there were no Reed-Sternberg cells.

Six of the 25 patients with malignant lymphomas displayed normal lymphoid nodules in their marrow. These nodules measured less than 0.6 mm, and their density did not exceed three per low-power field. Two cases showed nodular lymphoid hyperplasia. One was a case of reticulum cell sarcoma and the other
Fig. 9. Marrow involvement by lymphoblastic lymphoma. Note dark rim of cytoplasm and prominent nucleoli in many of the cells. Giemsa. ×594.

Fig. 10. Marrow involvement in a patient with Hodgkin’s disease. The nodule is composed of histiocytes and lymphocytes, quite different from a normal lymphoid nodule. Hematoxylin and eosin. ×308.

Fig. 11. Nodular lymphoid hyperplasia of bone marrow in patient with Hodgkin’s disease. Hematoxylin and eosin. ×21.
a patient with Hodgkin's disease (Fig. 11). The sex and average age of the patients are shown in Table 3.

DISCUSSION

Our study has revealed normal LN in 47% of the bone marrows. This incidence is much higher than the 9% reported by Johnstone,5 the highest published figure for in vivo studies (Table 1). Our incidence is also high when compared to autopsy studies and is only exceeded by the 62% reported by Fischer (Table 1).2 The high incidence of normal LN in our material is partly explained by the high average age—71.9 yr for our population. The discrepancy between our data and those reported in other in vivo studies is too high to be explained by age alone. The other in vivo studies show an incidence far below that found at autopsy, even in patients under 40 yr of age (Table 1).3 We suspect that, in addition to the high age of our population, the higher incidence in our material is due to our technique of aspirating more marrow and of concentrating the marrow particles by filtration prior to embedding in paraplast.13 It is possible that the aspiration of marrow by our technique at two or three sites might give more accurate staging of lymphomas than open surgical biopsy of marrow at one site.

Sixty-one per cent of our patients with LN were women. This higher incidence of LN in women has been previously noted by some observers4'7 but has been denied by Werner.5 The higher average age of patients with LN, 71.9 yr as compared to 63.5 yr for patients without LN, is statistically significant ($p = 0.02$).

The subdivision of normal LN into LF and LI is essentially a simplification of the more elaborate classification of LN by Hashimoto et al.4 Transition stages seem to exist between LF and LI, and occasionally a sharp separation between the two cannot be accomplished. It is possible that some LI represent tangential sections of LF.

Nodular lymphoid hyperplasia of the bone marrow has been separated from normal LN by the arbitrary criteria of size and number of LN. There is no doubt that some cases of NLH evolve into CLL or a malignant lymphoma after incubation periods which may be as long as 7 yr.17 The NLH of the marrow, which we have observed in some patients with reticulum cell sarcoma and Hodgkin's disease, does not represent involvement of the marrow with lymphoma, because the cytology of the nodules is quite different from the original lymphomas. It also is not likely to be a precursor of a well-differentiated lymphoproliferative disorder, because we have seen it too frequently, and it has been observed by others.19,20 Nodular lymphoid hyperplasia in Hodgkin’s disease is best interpreted as a nonspecific marrow reaction similar to granulocytic, megakaryocytic, or eosinophilic hyperplasia.

If NLH of the bone marrow is encountered in a malignant lymphoma of the mature lymphocytic type, it must be interpreted with the utmost caution. Confluence of LN, irregular shapes, markedly increased size, and, above all, the presence of prolymphocytes and lymphoblasts would be convincing arguments in favor of a malignant lymphoma. At the present time, when faced with a case
LYMPHOID NODULES OF BONE MARROW

of NLH, it is best to recommend periodic follow-up studies rather than to offer a definite diagnosis.

The number of lymphocytes in the bone marrow smears does not accurately reflect the presence of LN of the follicular type and may be misleading in terms of diagnosis. Occasionally, we have seen a smeared out LN which mimicked CLL. Lymphoid infiltrates correlate somewhat better with smears than lymphoid follicles. This may be due to a lesser development of the reticulum framework in LI.

The nodules in CLL were always of the infiltrate type and invariably showed some confluence. In one case, the marrow showed innumerable small, confluent lymphoid infiltrates with conservation of the fat cells, an unusual appearance for CLL (Fig. 6).

It is of interest that all three lymphoblastic lymphomas showed nodular bone marrow involvement. One of the cases was associated with a monoclonal macroglobulinemia. Though none of the mixed malignant lymphomas in this series showed bone marrow involvement, we have several such examples in our files.

Four of the patients with Hodgkin’s disease showed nodular lesions of the bone marrow consistent with Hodgkin’s disease. Histologically, in the absence of Reed-Sternberg cells, the differential diagnosis of these lesions lies between mixed lymphohistiocytic lymphomas, Hodgkin’s disease, and certain granulomas. Abundant plasma cells, eosinophils, and a tendency to fibrosis speak in favor of Hodgkin’s disease.

The recently described eosinophilic fibrohistiocytic lesion of the marrow occurs in close relation to LF and differs from Hodgkin’s disease by the absence of atypia in the histiocytes.22

ACKNOWLEDGMENT

We are grateful to Mr. Paul Marvan for technical assistance and to Mr. William H. Atkinson for photography.

REFERENCES
