Bone marrow examination in staging of Lymphoma: Revisited

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ABSTRACT

Objective: The aim of this study was to establish the prevalence of bone marrow involvement in non-Hodgkin’s Lymphoma (NHL) and Hodgkin’s disease, to assess the agreement in bone marrow involvement between various modalities such as bone marrow aspiration, unilateral and bilateral trephine biopsies and bone marrow clot section, and to determine the association between the length of trephine biopsy and disease prediction.

Methods: The bone marrow specimens of 374 patients with established diagnosis of lymphoma based on morphologic lymph node examination and immunophenotyping by immunohistochemistry were reviewed retrospectively.

Results: The most frequently encountered lymphoma by working formulation classification regardless of age and site is diffuse large cell lymphoma (50%) with a minimal involvement of the bone marrow (14.3%). However, lymphoblastic lymphoma is the most common type in children with a bone marrow involvement reaching 38.7% excluding acute lymphoblastic leukemia cases.

Conclusion: In this group of patients, an additional bone marrow sampling showed no statistically significant increase in the detection rate of bone marrow involvement. High detection rate of involvement in bone marrow was noticed at a length of 2.0-2.5 cm. However, a lengthy biopsy of 2.5 cm does not significantly increase the yield of detection.

Keywords: Lymphoma, bone marrow, staging.


Bone marrow examination is routinely carried out during the evaluation and staging of patients with Hodgkin’s disease (HD) and non-Hodgkin’s lymphoma (NHL). Bone marrow involvement denotes stage IV disease in the presence of nodal lymphoma. Although occasional reports have shown concordance between bone marrow aspiration and biopsy for detecting lymphoma, most studies have found biopsies to be superior. Bilateral rather than unilateral iliac crest marrow biopsy is believed to minimize sampling errors, and increase the lymphoma detection rate. Few if any recent studies have correlated the length of the biopsy with lymphomatous involvement and determined the minimal length of trephine biopsy which could be accepted as adequate for diagnosis. This study is undertaken with the following aims: Firstly, to establish the prevalence of bone marrow involvement in HD and NHL and its subtypes at the time of diagnosis; secondly, to assess the agreement in bone marrow involvement between various parameters including bone marrow aspiration, unilateral and bilateral bone marrow biopsies and bone marrow clot section; and thirdly, to determine the association between the length of trephine biopsy and disease prediction.

Materials and methods. The bone marrow specimens of 374 patients with established diagnosis of lymphoma based on morphologic lymph node
examination and immunophenotyping by immunohistochemistry were reviewed retrospectively. The bone marrow specimens have been examined at the Department of Pathology and Laboratory Medicine at King Faisal Specialist Hospital and Research Centre, between January 1991 through December 1994. The bone marrow samples have been taken from a posterior iliac crest with a Jamshidi needle. The aspirates were stained with Wright-Giemsa stain. The trephine biopsy specimens were fixed in B-5 fixative, decalcified and cut into 2-3 μm sections. Two sections were stained with hematoxylin and eosin (H&E) stain.

The bone marrow blood clot material was fixed in B-5 fixative and two sections were cut at 2-3 μm and stained with H&E. Three more deeper sections of trephine biopsy and clot will be examined if available two are negative. The lymph node histopathology reports were reviewed to identify the type and site of lymphoma. Bone marrow reports were reviewed and data in relation to bone marrow involvement in each of the three preparations was collected. The site of bone marrow specimen collection “unilateral vs bilateral”, availability of trephine bone marrow biopsy, clot section and bone marrow aspirate were tabulated. The bone marrow trephine biopsy length was measured in all cases. The data were entered using microsoft Excel (PC-compaq 486) and converted to JMP Software (Power Mac 7500/100).

Univariate analysis was done for discrete variable such as sex, type of lymphoma, site of disease, bone marrow aspirate, clot section and trephine biopsy. Frequencies were calculated for categorical variables and the statistical parameters (mean, max, min, SD) were calculated for continuous variables. Kappa statistic was used to measure the agreement between the bilateral trephine biopsies, the trephine versus aspiration samples and clot section versus trephine biopsy. A student t-test was applied to compare the mean length of bone marrow biopsies for possible involvement.

Results. A total of 442 bone marrow specimens are included in this study. All were taken from patients with an established diagnosis of lymphoma (Hodgkin’s disease and non Hodgkin’s lymphoma). Sixty-eight bone marrows which were repeat examination were deleted from this analysis, therefore 374 bone marrows were evaluated for the possibility of lymphoma involvement. 231 patients were males (62%) and 143 were females (38%). The age range was 4 to 94 years, the mean was 47 years. Extranodal lymphoma represented 38% (142 cases) of the total cases, leaving 62% (232 cases) as nodal lymphoma. The most frequently encountered lymphoma by working formulation classification regardless of age and site was diffuse large cell lymphoma (50%). Lymphoblastic lymphoma was the most common type in children. Figure 1 illustrates the different histological types of lymphoma at the time of diagnosis, and the percentage of bone marrow involvement. Table 1 summarizes the findings using different preparations and the percentage of cases with positive bone marrow involvement.

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<th>Table 1 - Number of cases with bone marrow involvement using different modalities of preparation.</th>
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<td><strong>Trephine Biopsy</strong></td>
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trephine biopsy, denoting a moderate agreement (K=0.48) between these two variable. The biopsy size was analyzed and the following results were obtained; the size ranged from 0.2 to 3.2 cm in length. The mean of sizes was 1.20 cm, and the standard deviation 0.57 cm. Figure 2 shows the percent of positive involvement in different bone marrow sizes with the higher detection rate at the length of 2-2.5 cm. However, it is statistically significant (p value = 0.82).

**Discussion.** The value of bone marrow examination in the process of lymphoma staging is an old subject which has been thoroughly investigated for a period of time. The role of multiple sampling to achieve an accurate staging has been emphasized too.1-10,13 Due to the high incidence of lymphoma in Saudi Arabia,14,15 the unusual histologic pattern with more aggressive presentation and the improvement of radiological imaging techniques and its role in staging of tumors,16-18 we decided to revisit this subject aiming to establish the prevalence of bone marrow involvement in lymphoma subtypes, to evaluate the role of multiple sampling and the adequate length of bone marrow trephine biopsy required for staging lymphoma. Our results supported the previous study by Ali, et al19 which showed the diffuse large cell lymphoma to be the most common histological type among Saudi patients. This is in contrast with Western reports in which low-grade lymphomas and in particular follicular lymphoma are the most common histological types.20-22 Although the number of low grade lymphomas is small, the incidence of bone marrow involvement in comparison to diffuse large cell lymphoma (DLCL) is still high. Bone biopsies of 58 treated and untreated patients with Hodgkin’s disease (HD) were investigated. Marrow involvement was found in a high percentage (29.6%) of patients compared to reported figures from most large series of patients with untreated HD.23 The incidence of bone marrow involvement was in the range of 5% to 15%. This high incidence among our patients could be explained by the mixture of treated and untreated patients as some of those patients will be referred to King Faisal Specialist Hospital, Oncology Unit at an advanced stage of the disease. Positive biopsies are rare in clinical stages I and II (1% and 2%), but the incidence might rise to 255 and 455 in stages III and IV respectively.24 The statistical measure of agreement (Kappa) was calculated between several parameters including bilateral trephine biopsies, bone marrow aspirate versus trephine biopsy and trephine biopsy versus clot section. Disagreement in 6% of the cases whenever a bilateral trephine biopsy is performed suggests that it may be unnecessary to perform such multiple bone marrow sampling. This could be explained by the high number of cases with intermediate grade lymphoma (diffuse large cell lymphoma) which involves the bone marrow in less than 15% of the cases. The examination of bone marrow clot section increased the chance of detecting bone marrow involvement by lymphoma when used in conjunction with trephine biopsy. The Kappa value (K-0.48) denotes a moderate degree of agreement between the clot section and trephine biopsy. Few studies have addressed the importance of bone marrow trephine biopsy length in staging of lymphoma to determine the involvement by disease.11 On review of the slides, the actual length of bone marrow trephine biopsy present was measured and the presence or absence of lymphomatous involvement was recorded. The range of lengths of trephine biopsies was from 0.2 to 3.2 cm higher detection rates were shown in trephine biopsies measuring 2-2.5 cm in length. There was no statistical significance among different sizes of bone marrow trephine biopsies however, biopsies with the length of less than 0.5 cm are unacceptable because only very few cases with bone marrow involvement were detected.

A large study from the Christie Hospital, Manchester, demonstrate wide variation in the length of untraumatised valuable bone marrow adult patients. The analysis of the relation between length of trephine and the rate of positivity for neoplasia yielded in minimum adequate length of 1.2 cm in section.

In conclusion, this study confirms the high incidence of intermediate grade lymphomas (diffuse large cell lymphoma) and high grade lymphomas mainly of immunoblastic lymphoma, lymphoblastic lymphoma and to a lesser degree anaplastic large cell lymphoma in Saudi Arabia. This is in contrast with the Western studies reporting high incidence of low grade lymphomas mainly of the small lymphocytic lymphoma, follicular lymphomas and mantle zone lymphomas.20-22 This unusual pattern could be attributable to the delay in seeking medical advice.
and presenting at a later stage when the disease is more clinically advanced. It could also be explained by a different etiology and pathogenesis of malignant lymphoma in this part of the world. In this group of patients, bilateral bone marrow trephine biopsy showed no statistically significant increase in the detection rate of lymphoma involving the bone marrow. Reid et al found no practically important correlation between the numbers of cases submitted and success in obtaining adequate specimens.26 This could be attributed to the lesser tendency of diffuse large cell lymphoma to involve the bone marrow microenvironment.27-29 Therefore, additional sampling of bone marrow might not be recommended. A thorough examination of bone marrow clot section increases the rate of detection as well as facilitating the application of immunohistochemistry for further cell type classification. A generous bone marrow trephine biopsy sample is recommended but a very lengthy one reaching more than 2.5 cm does not significantly increase the yield of detection. However, short biopsies (<0.5 cm) might lead to missing of an occult bone marrow involvement and jeopardize proper staging and treatment.

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References