Optimum trephine length in the assessment of bone marrow involvement in patients with diffuse large cell lymphoma

On behalf of the Australasian Leukaemia Lymphoma Group

Abstract

Background: The National Cancer Institute has recommended a bone marrow biopsy length of ≥20 mm for the staging and surveillance of patients with non-Hodgkin’s lymphoma. However, there are few published data to support this recommendation, particularly the role of examining multiple levels.

Patients and methods: Bone marrow biopsies from 172 patients with newly diagnosed diffuse large cell lymphoma (DLCL) entered in two consecutive trials of the Australasian Leukaemia and Lymphoma Group were analysed. The original haematoxylin and eosin-stained trephine biopsy and two or more deeper sections cut at 0.1–0.2 mm intervals were assessed with respect to the morphology, extent and pattern of lymphomatous involvement. The rate of positive diagnosis was correlated with the length of the biopsy specimen and the number of sections examined.

Results: Forty-seven biopsies (27%) demonstrated marrow involvement on examination of a mean of four trephine biopsy sections. The rate of positivity increased with the examination of multiple levels and correlated with increasing trephine length but was not dependent on the number of sites sampled. Twenty per cent of biopsies <20 mm in length were positive for lymphoma; this increased to 35% for biopsies ≥20 mm (P = 0.023).

Conclusions: Morphological bone marrow involvement in DLCL is optimally demonstrated by a 20-mm long trephine biopsy from a single site which is examined at multiple levels (four or more). This obviates the need for bilateral sampling, thereby reducing patient morbidity from the procedure. This study provides evidence to support the National Cancer Institute recommendations regarding trephine biopsy in the staging of DLCL, providing multiple levels are examined.

Key words: bone marrow involvement, bone marrow trephine, diffuse large cell lymphoma

Introduction

Assessment of bone marrow involvement in patients with diffuse large cell lymphoma (DLCL) provides important information regarding prognosis and is critical for the planning of optimal therapeutic strategies. Bone marrow involvement in this patient group portends a poorer prognosis [1, 2]. Morphological features that have been reported to influence prognosis in patients with DLCL with marrow involvement include the pattern and extent of marrow infiltration [3–5] and the presence of histological discordance between the primary site of involvement and bone marrow [4–7]; these findings emphasise the importance of adequate sampling of the bone marrow in determining the stage of disease and subsequent therapy.

Although bone marrow biopsy is accepted as a mandatory procedure and a valuable tool for the staging of patients with DLCL, there are few data regarding the amount of marrow that should be examined to assess accurately the presence or absence of lymphomatous involvement. In particular, discordant histological involvement (fewer than 50% large cells) is often limited to patchily distributed small foci of disease which may be missed if the area of the trephine biopsy examined is insufficient. This problem was addressed at a recent international working party convened by the National Cancer Institute (NCI) to develop standardised methods of assessment of staging and response in patients with non-Hodgkin’s lymphoma (NHL), at which a trephine length of at least 20 mm was recommended [8]. However, no evidence was provided to support this recommendation. Bain [9] has recommended a minimum trephine length of 16 mm. This was based on the findings of Bishop et al. [10], where a plateau in the rate of detection of metastatic tumour was observed once the trephine length exceeded 16 mm. Debate has existed for many years over the value of unilateral versus bilateral bone marrow aspirates in DLCL, with recent publications [8, 9] suggesting that overall trephine length may be more important than the number of sites sampled. Examination of serial sections of the trephine
biopsy would allow a greater area of bone marrow to be assessed with no extra morbidity to the patient, while potentially increasing the likelihood that any marrow involvement would be detected. We have analysed serial sections of the trephine biopsy in patients with DLCL to determine the incidence, pattern and proportion of marrow involvement, the rate of discordant histology and the effect of serial sectioning on the frequency of recognition of marrow involvement.

Patients and methods
A retrospective analysis of patients with DLCL according to International Working Formulation criteria [11] entered into the Australasian Leukaemia and Lymphoma Group NHL05 MACOP-B versus CHOP and NHL07 CEOP versus high-dose CEOP studies [12–14] was undertaken. Bone marrow trephine biopsies obtained at diagnosis were reviewed by two haematopathologists (J. K. C. and S. K. J.). Trephine biopsies 55 mm in length were considered inadequate for staging purposes and were therefore excluded from further analysis. The marrow aspirates performed at the same time were not analysed because of their recognised insensitivity in the detection of marrow involvement in DLCL [15]. The original haematoxylin and eosin-stained trephine biopsy and two or more deeper sections cut at 0.1–0.2 mm intervals were examined. Where bilateral biopsies were available, both were examined. Following review, the trephine biopsies were classified as positive, negative or indeterminate (equivocal) for involvement by lymphoma using the criteria of Cheson et al. [8]. Benign lymphoid aggregates were diagnosed according to previously described criteria [4, 16–18] and were typically small, well-circumscribed collections of mature lymphoid cells admixed with plasma cells, eosinophils and/or histiocytes with a prominent vascular component in an intertrabecular location. Features of malignant lymphoid aggregates included paratrabecular location, larger size, poor demarcation, infiltration into surrounding haemopoietic tissue, cytological atypia and the presence of large cells [18, 19]. The pattern of lymphoma involvement was defined as focal (nodular, paratrabecular) or non-focal (diffuse, interstitial). The extent of marrow involvement and the percentage of large cells in the infiltrate were estimated. Discordant histology was considered to be present if the bone marrow infiltrate contained <50% large cells. Where bilateral biopsies were available, the extent of marrow involvement and percentage of large cells were based on the side with the greatest degree of involvement. The length of core examined for bilateral biopsies was taken to be the sum of individual lengths of 32 and 41 mm. The number of positive cases following review of deeper sections was not strongly dependent on the length of core sampled (P = 0.16, Cochran–Armitage test for trend, two-sided test), although similar rates were observed for sampling between 22 and 30 mm and >30 mm (Table 2).

Results
Examination of serial sections of the trephine biopsy
The original trephine biopsy and two or more deeper sections were reviewed in 172 patients, including 28 patients who had bilateral biopsies with two or more deeper sections available for review on each side. The number of levels examined was not recorded for 17 biopsies. For the remaining 183 biopsies, an average of 4.0 levels were examined per biopsy. There were only two biopsies with two levels examined. Both of these were bilateral biopsies and there were three and four levels, respectively, examined on the other side. For bilateral biopsies, where data were available for both sides, a mean of 7.9 levels was examined in total. Benign lymphoid aggregates were present in 23 cases (13%). Lymphoma was unequivocally present in the original section of the trephine in 29 of the 172 patients (17%). A further 19 cases (11%) had lymphoid aggregates of indeterminate histology on the original section of trephine biopsy and 124 cases (72%) were negative. Following review of the additional deeper sections, 18 additional positive cases were identified, increasing the number of positive cases to 47, a rate of 27% [95% confidence interval (CI) 21% to 35%] (Table 1). There were 12 (7%) equivocal and 113 (66%) negative cases.

Eleven cases which were originally classified as being negative became positive on review. Ten patients had minimal marrow infiltration (<5%) and discordant histology (data not shown). Within the group of 19 cases with lymphoid aggregates of indeterminate histology on the original trephine biopsy, examination of deeper sections enabled seven (37%) to be classified as positive on the basis of histological findings, and allowed a negative diagnosis in a further seven patients (37%), as it was believed that the absence of lymphoid infiltrates in the deeper sections made the diagnosis of lymphoma highly unlikely. In all seven initially equivocal cases reclassified as positive, there was minimal marrow infiltration (<5%) and discordant histology (data not shown).

Impact of length of trephine core sampled
The median length of trephine core sampled was 19 mm. The length ranged from 6 to 73 mm, the latter being a bilateral biopsy with individual lengths of 32 and 41 mm. The number of positive cases increased significantly with increasing length of core (P = 0.042, Cochran–Armitage test for trend, one-sided test), although similar rates were observed for sampling between 22 and 30 mm and >30 mm (Table 2). The number of new positive cases following review of deeper sections was not strongly dependent on the length of core sampled (P = 0.16, Cochran–Armitage test for trend, two-sided test). The NCI criteria for the staging of patients with NHL recommend a trephine biopsy length of at least 20 mm [8]. In our series, 89 patients had <20 mm of trephine core examined. Following examination of the original section and the deeper levels, these patients had a bone marrow

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<th>Diagnosis after review of original section</th>
<th>Diagnosis after review of serial sections</th>
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<tr>
<td>Positive</td>
<td>Positive</td>
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<tr>
<td>Positive</td>
<td>29</td>
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<tr>
<td>Negative</td>
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<tr>
<td>Total (%)</td>
<td>47 (27)</td>
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positivity rate of 20%, compared with a rate of 35% in the 83 patients from whom ≥20 mm of bone marrow were obtained (P = 0.023, Fisher’s exact test, one-sided test).

**Bilateral biopsies**

Twenty-eight patients had bilateral trephines. Before review of the deeper sections, only four cases were positive and four equivocal. Following review, two equivocal and two negative cases became positive, an equivocal case became negative, a negative case became equivocal and the remaining results were unchanged. Thus overall there were eight positive cases (of which four were positive bilaterally and four positive on one side only), two equivocal cases (of which one was equivocal bilaterally and one equivocal on one side only) and 18 negative cases. The overall length of the core sampled was more important in determining whether a positive case would be detected than whether the sampling was done bilaterally or unilaterally. This was true for both the original results and the results following review of the deeper sections (Table 3). In a logistic regression analysis of the probability of a result being positive, after adjusting for the length sampled, the estimated odds of a positive result with a bilateral trephine relative to a unilateral trephine was 0.50 (95% CI 0.13–1.9, P = 0.31) for the original results and 0.72 (95% CI 0.25–2.1, P = 0.56) for the results following review of multiple sections.

**Discussion**

Previous studies of bone marrow involvement in patients with DLCL according to the Working Formulation criteria have detected disease in 12–31% of patients [3, 4, 20, 21]. Although not all of these reports state the number of trephine sections examined, bone marrow involvement was detected in 19% of patients in whom a single section of 3 × 18 mm was assessed [3], whereas this figure was increased to 26% of patients in a separate study where three sections of similar or greater length were examined [20]. These figures are remarkably consistent with our own findings. Inspection of a single trephine biopsy section (mean length 19 mm) was positive in 17% of cases; following review of at least two deeper sections, the number of positive results increased to a total of 27%, a relative increase of 62%. In addition to the increase in positive diagnoses, examination of serial sections reduced the proportion of equivocal results from 11% to 7%, thereby further improving the diagnostic certainty of the procedure.

The international working party convened by the NCI to standardise response criteria for NHL recommended that a minimum of 20 mm of trephine biopsy be examined for bone marrow involvement [8]. More recently, Bain [9] suggested a minimum trephine length of 16 mm, but stated that more focal lesions would be detected with larger biopsies. Other authors have recommended examination of at least five well-preserved marrow spaces [17], more than five high-power microscope fields [19], or evaluation of a total marrow area of 150 mm² [20]. However, there is little direct evidence in the literature to support any of these recommendations. In an audit of bone marrow biopsy in 767 patients with malignant tumours conducted at a single institution, the rate of bone marrow involvement was related to trephine length, with a plateau occurring at 16 mm [10]. These results were unchanged when the analysis was restricted to the 295 patients with unspecified subtypes of NHL. In our series of
patients with DLCL, the rate of positive diagnoses was also related to trephine length. Trephine biopsies <20 mm in length were positive in 20% of cases, compared with 35% of those >20 mm. Interestingly, these results only became statistically significant after the deeper trephine sections were examined (data not shown).

Because bone marrow involvement in DLCL is often focal, a number of authors have emphasised their preference for bilateral bone marrow biopsy in order to optimise the rate of positive results [7, 15]. Despite the high incidence of small, focal deposits in our patients, the likelihood of a positive diagnosis was clearly related to the trephine length, but no additional benefit was noted for bilateral biopsies once the trephine length was taken into account.

These data demonstrate that it is the overall trephine length that is critical, rather than whether the sample is taken from one or both sides of the pelvis and that the practice of examining serial biopsy sections from a single side can provide sufficient diagnostic information without the additional patient morbidity associated with bilateral biopsies. These findings provide evidence for the NCI recommendations regarding the optimum trephine length for the diagnosis of bone marrow lymphoma [8] providing that multiple sections are examined.

References