The value of routine bone marrow biopsy in patients with diffuse large B-cell lymphoma staged with PET/CT: A Danish-Canadian study


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ABSTRACT

Background

The added diagnostic and prognostic value of routine bone marrow biopsy (BMB) in patients with diffuse large B-cell lymphoma (DLBCL) undergoing PET/CT staging is controversial.

Patients and Methods

Patients with newly-diagnosed DLBCL who underwent both staging PET/CT and BMB were retrospectively identified in British Columbia, Aalborg, and Copenhagen. Original written PET/CT and pathology reports were retrospectively reviewed to determine Ann Arbor stage and outcomes, with and without the contribution of BMB.
Results

A total of 530 patients were identified: 146 (28%) had focal bone marrow (BM) lesions on PET/CT and 87 (16%) had positive BMB. 52 of 146 patients (36%) with positive PET/CT had a positive BMB (39 DLBCL, 13 indolent non-Hodgkin lymphoma [iNHL]), while 35 of 384 patients (9%) with negative PET/CT had positive BMB (12 DLBCL, 23 iNHL). BMB upstaged 12/209 (6%) of stage I/II patients to stage IV, although this was the case for only 3 (1%) patients with DLBCL in the BMB. PET/CT identified bone marrow involvement by BMB with sensitivity 60%, specificity 79%, positive predictive value 36%, and negative predictive value 91%. Concordant histological involvement of the bone marrow by DLBCL was associated with worse OS and PFS than discordant or no involvement in univariate and multivariate analyses.

Conclusions

In patients with DLBCL, staging PET/CT can miss BM involvement with concordant DLBCL (less common) or discordant iNHL (more common). Routine BMB does not add relevant diagnostic or prognostic value over PET/CT alone in the majority of patients with DLBCL.

KEY WORDS

Diffuse large B-cell lymphoma, bone marrow biopsy, concordant, discordant, PET

KEY MESSAGE

In this cohort of 530 patients with DLBCL from Denmark and Canada who underwent staging with PET/CT and bone marrow biopsy, PET/CT accurately assessed bone marrow involvement in the majority. When bone marrow biopsy detected marrow involvement despite a negative PET/CT, the bone marrow biopsy generally did not alter treatment either because the biopsy only detected indolent NHL or other findings justified treatment appropriate for advanced stage disease.
INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin lymphoma.(1,2) Bone marrow (BM) involvement by DLBCL, assessed by random iliac crest bone marrow biopsy (BMB), can be identified in up to one quarter of newly diagnosed patients and is associated with poor outcomes.(3-6) For many decades, BMB has been the gold standard test to detect bone marrow involvement, and a mandatory component of the routine staging work-up for DLBCL.

18F-fluorodeoxyglucose (FDG) positron emission tomography combined with computed tomography (PET/CT) is now recommended as a standard staging investigation of FDG-avid aggressive lymphomas, including DLBCL.(7) In Hodgkin lymphoma (HL), routine BMB can erroneously miss BM involvement in patients with FDG-avid patchy lesions outside the biopsy area.(8) However, the occasionally missed BM involvement by PET/CT rarely alters HL prognosis or management since almost all of these patients already present with advanced stage according to PET/CT alone.(9,10) Therefore, BMB is no longer considered necessary in the routine staging of HL.(7)

In DLBCL, studies evaluating the role of BMB and PET/CT in the staging of the BM have been less consistent. In some studies, PET/CT accurately identified BM involvement,(11-13) while in others, it was often missed by PET/CT.(14,15) Furthermore, in contrast to HL, patients with DLBCL frequently have discordant involvement of the BM by an indolent non-Hodgkin lymphoma (iNHL).(3-6) For these reasons, recent guidelines recommending against routine
BMB for all PET/CT-staged patients with DLBCL(7) have been controversial.(16) Therefore, we undertook a large-scale study Danish-Canadian study to quantify the added diagnostic value of routine BMB in patients with newly diagnosed DLBCL undergoing PET/CT staging.

METHODS

Patient Identification

This is a retrospective study of patients with newly-diagnosed DLBCL from the academic centers of the British Columbia Cancer Agency (BCCA), Aalborg, and Copenhagen (Rigshospitalet). Patients with treatment-naïve DLBCL who underwent staging PET/CT and unilateral iliac crest BMB were identified through queries in the BCCA Lymphoid Cancer Database and the national Danish Lymphoma Registry (LYFO). These databases have been described in detail previously.(17,18)

Baseline clinical and pathological characteristics were retrieved from these databases and missing information was collected from individual medical records whenever possible. Original PET/CT and pathology reports were reviewed by a local investigator for disease stage, sites of extranodal involvement, diagnostic biopsy results, and BM histology. Patients with a previous diagnosis of iNHL and those with composite histology in the diagnostic biopsies were excluded.
PET/CT Imaging and Reporting

PET/CT scans were performed locally on dedicated machines and in accordance with manufacturer guidelines. The CT component of the PET/CT was diagnostic (e.g., contrast-enhanced) unless a diagnostic CT was performed separately in addition to the PET/CT. In the latter case, a low-dose CT was performed for attenuation correction.

Original staging PET/CT reports were reviewed for the presence of FDG-avid lesions in bone/bone marrow suggestive of lymphomatous infiltration and their number (unifocal=1, bifocal=2, multifocal>2). CT reports were reviewed for the presence of bone abnormalities (osteolytic and/or osteosclerotic) corresponding to the areas of abnormal skeletal FDG uptake. Areas of focally increased FDG-uptake in bone and/or BM were considered positive for BM involvement by lymphoma unless definitively explained by an alternate cause. A distinction between cortical bone and BM involvement was not made given the possibility of small spatial misalignments between CT and PET fusions, and the absence of cortical bone involvement in patients without increased FDG uptake in the marrow space, as previously reported.(12)

Bone Marrow Biopsy

In all three institutions, blind unilateral posterior iliac crest trephine biopsy and aspirate were performed routinely upon diagnosis of DLBCL. Cases were reported locally by dedicated hematopathologists. The diagnosis of lymphoma was based on standard morphology and
immunohistochemistry. Flow cytometry was available at all centers and was performed routinely or at the discretion of the hematopathologist. Fluorescent in-situ hybridization was performed according to institutional guidelines or if deemed necessary by the hematopathologist. All patients were considered to have an adequate BMB as determined by the hematopathologist. The length of the BM core was not collected.

**Statistical Analysis**

Baseline characteristics were described using summary statistics. Ann Arbor stage was determined using PET/CT with and without the contribution of BMB, and the proportion of stage IV cases by each method was calculated.(19,20) The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each approach were computed.

Overall survival (OS) was defined as the time from diagnosis of DLBCL to death from any cause or date of last follow-up alive. Progression-free survival (PFS) was defined as the time from diagnosis of DLBCL to first relapse or progression, death from any cause, or last follow-up. OS and PFS were estimated using the Kaplan-Meier method, and differences between groups were compared using the log-rank test.(21)

Cox proportional hazard models were constructed to evaluate the impact of PET/CT and BMB findings, adjusting for the individual risk factors of the International Prognostic Index (IPI): age (>60 vs. <60), LDH (high vs. normal), performance status (>1 vs. 0-1), number of extranodal sites (>1 vs. 0-1), stage (III-IV vs. I-II).(22) Statistical analyses were performed using SPSS
14.0 for Windows. The study was approved by the Danish Data Protection Agency, the Danish National Board of Health, and the University of British Columbia/BCCA Research Ethics Board.

RESULTS

Baseline Characteristics

A total of 551 patients were initially identified from all institutions, of which 21 with composite histology in the diagnostic tissue biopsy were excluded. The study base was composed of 530 patients: BCCA (n=149, 2011-2013), Aalborg University Hospital (n=179, 2007-2013), and Copenhagen University Hospital (n=202, 2009-2012). These time periods reflect the availability of PET/CT at each center. Table 1 shows their baseline characteristics and treatments. A total of 181 (34%) patients had bone/BM involvement as established by PET/CT alone (n=94), BMB alone (n=35), or both (n=52). Twenty patients underwent directed biopsies of FDG-avid focal BM lesions, and all were positive for lymphoma.

Figure 1 shows that 52 of the 146 patients (36%) with positive PET/CT had a positive BMB (39 DLBCL, 13 iNHL), while 35 of the 384 patients (9%) with negative PET/CT had a positive BMB (12 DLBCL, 23 iNHL). Of the 87 patients with positive BMB, histology was therefore concordant with the diagnostic tissue biopsy (e.g., both showed DLBCL) in 51 (59%) patients, while it was discordant (e.g., BMB showed iNHL) in 36 (41%) patients. Three patients with both DLBCL and FL in the BMB have been coded as having DLBCL for analysis purposes.
Performance of PET/CT with and without BMB

BMB upstaged 12/209 (6%) stage I/II patients to stage IV, including 3 patients with DLBCL and 9 with iNHL in the bone marrow. BMB upstaged 14/92 (16%) stage III patients to stage IV, including 5 patients with DLBCL and 9 with iNHL in the bone marrow, as shown in Supplementary Table S1. Despite this stage migration, the distribution of IPI risk groups did not significantly change with the inclusion of BMB results, as shown in Supplementary Table S2.

PET/CT identified focal skeletal lesions consistent with BM involvement by DLBCL or iNHL (n=87) with sensitivity 60% (95% confidence interval [CI] 49, 70), specificity 79% (95% CI 75, 83), positive predictive value (PPV) 36% (95% CI 28, 44) and negative predictive value (NPV) 91% (95% CI 88, 94). Supplementary Table S3 shows additional comparisons of the diagnostic performance of PET/CT and BMB for the assessment of bone/BM disease.

Outcomes

With a median follow up of 2 years (range 3 months – 6.5 years) in living patients, the 2-year OS and PFS were 79% (95% confidence interval [CI] 75%, 83%) and 74% (95% CI 70%, 78%), respectively. There was no difference in OS (p=0.319) or PFS (p=0.186) between the three treatment centers, enabling pooling of data for the present analysis.

In the 256 patients with stage IV, 2-year OS was 79% (95% CI 71%, 87%) for those with negative PET/CT for BM involvement (n=126) and 63% (95% CI 53%, 73%) for those with positive (n=130) PET/CT (p=0.001). The 2-year PFS was 74% (95% CI 64%, 84%) and 53%
(95% CI 43%, 63%), respectively ($p<0.001$). In stage IV ($n=256$) patients, 2-year OS was 82%
(95% CI 72%, 92%) for those with negative PET/CT and BMB for bone/bone marrow
involvement ($n=91$) and 65% (95% CI 57%, 73%) for those with positive ($n=165$) PET/CT or
BMB ($p=0.003$). The 2-year PFS was 75% (95% CI 65%, 85%) and 57% (95% CI 49%, 65%),
respectively ($p=0.003$). Kaplan-Meier curves are shown in Supplementary Figure S1.

The 51 patients with concordant involvement of the BM experienced significantly worse
outcomes than the 36 patients with discordant involvement or the 443 patients with no
histological involvement of the BM. In multivariate analysis, concordant involvement of the BM
was associated with worse OS and PFS compared to no histological BM involvement, while
discordant involvement had OS and PFS similar to those with no BM involvement.
Characteristics and outcomes are summarized in Supplementary Table S4 and Supplementary
Figure S2.

**DISCUSSION**

To our knowledge, this is the largest study to date examining the value of BMB in PET/CT-
staged patients with an initial diagnosis of DLBCL. BMB has been the gold standard for the
detection of BM infiltration by lymphoma. However, it is well recognized that BMB has several
limitations, including the possibility of missing a patchy pattern of marrow involvement, as
reflected in studies comparing bilateral vs. unilateral BMB.(23) Risks include pain, anxiety,
infection, and bleeding.(24-27) Thus, readdressing the value of this traditional staging
investigation is relevant with the advent of advanced imaging technologies, including PET/CT.
The recent 2014 Lugano classification proposes omitting routine BMB in DLBCL cases where PET/CT is unequivocally positive for BM involvement. On the other hand, if the PET/CT is negative for BM involvement, routine BMB is necessary to exclude concordant or discordant histology in the BM, which may only be relevant if it will affect patient management. (7)

In theory, BMB may upstage some patients from early stage to advanced stage, can distinguish between indolent and aggressive histology in the marrow, and can add prognostic value. In our study, BMB upstaged 12 (6%) of patients with stage I/II who had a negative PET/CT for BM involvement. However, the majority had iNHL in the BM, and only 3 patients (1%) were upstaged due to missed DLBCL in the BM. This means that 70 patients with stage I/II and negative PET/CT need to undergo BMB in order to identify 1 DLBCL, and 23 to identify one case of iNHL in the BM missed by PET/CT. Furthermore, in the 35 patients with negative PET/CT but positive BMB, 28 (80%) already had evidence of advanced disease by PET/CT alone, including 26 with stage III/IV and 2 with stage I/II with bulky masses ≥8cm.

Potential management changes when DLBCL is detected in the BM include a longer course of rituximab-containing chemotherapy and the addition of CNS prophylaxis. Consolidative radiotherapy may no longer be indicated in some of those initially assigned stage I/II DLBCL by PET/CT, avoiding its late toxicity. Potential management changes when iNHL is detected in the BM include longer/indefinite follow-up (although the clinical importance is debatable given that these patients and their general practitioners will have increased awareness of lymphoma in case of health related issues), and possibly the use of maintenance rituximab (although its role in the
Our data suggest that BMB is unnecessary in patients with a PET/CT that is positive for BM involvement. BMB was negative in 94/146 (64%) patients with positive PET/CT and, thus, missed disease that altered staging assessment and management. In 52/146 (36%) patients with positive PET/CT and BMB, 39 had DLBCL and 13 had iNHL in the BM. Thus, in 39 cases the BMB confirmed bone or bone marrow involvement but did not add information that would affect treatment planning. Even in the 13 cases with iNHL in the marrow it is unlikely that the BMB result would alter management since choice of treatment also includes consideration of other findings that may suggest the presence of advanced stage or poor prognosis disease. Additionally, 75/87 (86%) patients with positive BMB had already been assigned stage III/IV by PET/CT alone, suggesting that BMB would have been superfluous in designating them as having advanced stage disease.

There are many findings in common between our study and another large international collaboration by Cerci et al of 327 patients, of which 25/86 (29%) with positive PET/CT had positive BMB, while 10/241 (4%) with negative PET/CT had positive BMB, again reflecting the high NPV of PET/CT.(13) Consistent with our findings, PET/CT upstaged 12 stage I/II patients with negative BMB. In contrast to our study, Cerci et al found that patients with stage IV disease and BM involvement detected by PET/CT or BMB had similar outcomes to patients with no BM involvement. They suggested this was likely to be due to the extent of bone marrow disease, which is possibly supported by our findings that only patients with multifocal lesions on PET had worse OS and PFS when adjusted for IPI. Consistent with Cerci et al, we found that those...
with concordant BM involvement experienced worse outcomes compared to those with discordant or no BM involvement.

The results of the present study should be interpreted in light of the limitations inherent to its retrospective design. Immunohistochemistry for cell of origin determination and cytogenetics for MYC/BCL2 and other rearrangements were not available. There was no central review of PET/CT images or pathology, although all original imaging and pathology reports were reviewed. These reports constitute data upon which clinical decisions were made, and all were reported by experienced functional imaging specialists and pathologists, respectively. It is impossible to verify the extent to which these physicians were blinded to each others’ results at the time of reporting, although in general they analyze their material independently and often results of the other test are not necessarily available at that moment.

Even though we aimed to include all patients with DLBCL during the study period, a small proportion at each center may not have had both PET/CT and BMB at diagnosis; for example, critically ill patients in need of immediate therapy. The degree to which their exclusion may have altered the comparison of test performance or outcomes is likely low, while the degree to which their exclusion increases the relevance of results to real-world clinical practice is very high. PET/CT equipment, image acquisition, processing, and interpretation criteria evolved throughout the 6-year study period (2007-2013). Therefore, intra and inter-observer variation may have altered the reliability of PET/CT interpretation. We did not collect standardized uptake values (SUV), again introducing subjectivity in the interpretation of scans. However, using semi-quantitative measures like mean or maximum SUV requires a high degree of standardization,
which often hampers external validity.

In conclusion, PET/CT accurately assessed BM involvement in the majority of patients with DLBCL. It is difficult to identify a subgroup of patients with negative PET/CT in whom treatment and prognosis would have been altered by BMB, as even in those with stage I/II by PET/CT, the likelihood of upstaging with a meaningful change in treatment is small. Even though discordant iNHL is missed without routine BMB, the favorable outcome of patients with discordant iNHL supports that they are managed safely with standard DLBCL therapy. Diagnostically, BMB only minimally improved NPV after the findings on PET/CT scan were considered and did not lead to important changes in the distribution of IPI risk groups. Our data suggest routine BMB is not a critical element of disease staging in all patients with newly diagnosed DLBCL undergoing upfront PET/CT staging.

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REFERENCES


(6) Campbell J, Seymour JF, Matthews J, Wolf M, Stone J, Juneja S. The prognostic impact of bone marrow involvement in patients with diffuse large cell lymphoma varies according to the


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Figure 1. Patient distribution according to PET/CT and BMB assessments. PET/CT: positron emission tomography combined with computed tomography, BMB: bone marrow biopsy.
Figure 1

530 PET/CT and BMB staged DLBCL patients

PET/CT positive 146
- BMB negative 94
  - DLBCL + iNHL 3
  - DLBCL 36
  - iNHL 13
- BMB positive 52
  - DLBCL 12
  - iNHL 23

PET/CT negative 384
- BMB negative 349
- BMB positive 55
Table 1.

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