

# Different expression of MIB1 in primary site of non-Hodgkin lymphoma and its bone marrow deposits, a pilot study

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**Abstract:** Evaluation of mindbomb E3 ubiquitin protein ligase 1 (MIB1) (Ki67) proliferation index (PI) in B-cell non-Hodgkin lymphomas is increasingly a common addition to classification of lymphoma and staging procedures. Clinicians rely on PI as a surrogate marker of biologic activity; however, no established guidelines have been published whether PI at the primary site of the tumor gives the same answer as evaluation of tumor in staging marrow. In our study, dual immunohistochemical staining for MIB1 and CD20 was performed on tissue from primary site and bone marrow involved by B-cell non-Hodgkin lymphoma to compare PI for each individual patient. For all patients, MIB1 expression was higher at primary tumor site as compared to staging marrow. Additional analysis was performed to investigate the degree of difference depending on lymphoma morphology. Patients with large cell lymphoma at the primary site and large cell morphology in the marrow (LCL-L), those with large cell morphology at the primary site and small cell morphology in the marrow (LCL-S), and those with small cell morphology at the primary site and small cells in the marrow (SCL-S) were compared. As expected, LCL cases had a higher mean PI at the primary site when compared to SCL cases (28.5% vs 2.8%,  $P=0.0001$ ). In addition, the most significant difference between medullary and extramedullary PI was observed in cases with discordant morphology (LCL-S) (21% vs 1.1%,  $P=0.009$ ). Our results indicate that PI of lymphoma within the bone marrow should not be used as a surrogate prognostic indicator of lymphoma biology in its primary site.

**Keywords:** proliferation index, biologic behavior, prognosis

## Introduction

Bone marrow (BM) biopsies are routinely performed for the staging of patients with most types of B-cell non-Hodgkin lymphomas (NHLs). On occasion, the hematologist/oncologist may request to determine the proliferation index (PI) of a lymphoma present within the staging marrow. Mindbomb E3 ubiquitin protein ligase 1 (MIB1) is a protein coding gene which has been regarded as a sensitive antibody useful in the determination of PI in paraffin-embedded tissues.<sup>1</sup>

Many previous studies have shown that an increased PI in certain types of B-cell NHLs is associated with a more aggressive disease course.<sup>2–8</sup> These studies have been performed on diagnostic lymph nodes and other non-marrow tissues. We have encountered many requests for PI determination of the lymphoma on marrow specimens in cases where the marrow was the only diagnostic specimen. At other times, a diagnostic lymph node or other soft tissue-based lymphoma specimens are available. In either situation, the accuracy and significance of PI of lymphoma in staging marrow have not been clearly studied.

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Our intent was to explore and characterize PI of B-cell NHLs in staging BMs and compare it to PI of the same patient at the primary, extramedullary tumor site in order to evaluate whether the MIB1 expression in BM involved by lymphoma can be used as an equivalent to that determined by staining of extramedullary primary tumor.

## Materials and methods

In this retrospective study, the anatomic pathology database at Penn State Hershey Medical Center was searched for all patients diagnosed with B-cell NHL for whom a subsequent staging BM biopsies positive for involvement by lymphoma was also examined. A standard research protocol was reviewed and approved by Penn State Hershey Medical Center Institutional Review Board. Obtaining patient consent was deemed unnecessary due to the retrospective nature of this study. All patients meeting these criteria and evaluated within a 7-year period (2005–2012) at Hershey Medical Center were initially selected; however, patients with relapsed disease submitted for restaging and those treated prior to staging were excluded. Also, those for whom tissue samples either from primary or BM site was insufficient for additional staining were excluded. All slides were stained with hematoxylin and eosin and immunohistochemical stains were reviewed by two hematopathologists and hematopathology fellows for confirmation of the diagnosis and determination of percentage of MIB1-positive tumor cells. Commercially available immunohistochemical stains for MIB1 (permanent red chromogen with ethylenediaminetetraacetic acid [EDTA] antigen retrieval, 1:25 dilution, 30 minutes at room temperature) and CD20 (Diaminobenzidine [DAB] chromogen with EDTA antigen retrieval, 1:500 dilution, 30 minutes at room temperature) were obtained and used in a dual immunohistochemistry process that was performed on each specimen. Dual immunohistochemistry was performed using Dako's Envision G/2 Doublestain system on a Dako Autostainer Plus (Dako North America, Inc., CA, USA) following manufacturer's protocol.<sup>9,10</sup> The PI for each site was determined by using MIB1 expression in 100 CD20-positive lymphoma cells in a representative section. Clinical data, including patient demographics, sites of involvement, International Prognostic Index, and survival, were collected. The *P*-values were calculated using two-sample Student's *t*-test when comparing quantitative variables or by Chi-square test when comparing proportions, with significance set at a *P*<0.05.

In addition, a comparative study of MIB1 expression was performed using non-decalcified, formalin-fixed, paraffin-embedded tissue and formalin-fixed, paraffin-embedded tissue after rapid decalcification (Decalcifier II [hydrochloric

acid <15%; EDTA, disodium salt <5%], decalcification time 45 minutes), the same as used for decalcification of our BM samples. No significant difference of staining intensity, pattern, or percentage was identified in this comparison.

## Results

Ample tissue at both sites of involvement to perform immunohistochemistry was present in 24 patients. The types of B-cell NHL included in this study, based on 2008 World Health Organization classification of the extramedullary site of involvement, were diffuse large B-cell lymphoma (eleven cases), follicular lymphoma (six cases), chronic lymphocytic leukemia/small lymphocytic lymphoma (four cases), extranodal marginal zone lymphoma (one case), and small B-cell lymphoma, not otherwise specified (two cases).

All cases were consolidated into three morphologic groups to increase statistical power of this analysis: large cell lymphoma at the extramedullary site with large cells in the BM (LCL-L), large cell lymphoma at the extramedullary site with discordant small cells in the BM (LCL-S), and small cell lymphoma at the extramedullary site with small cells in the BM (SCL-S) (refer to Table 1 for results).

In all cases, PI evaluated by MIB1 staining was higher at the extramedullary tumor site than that in the BM. Patients with LCL had a higher mean PI at the extramedullary site when compared to all SCL cases (28.5% vs 2.8%, *P*=0.0001). The difference was statistically most significant for LCL-S (21% vs 1.1%, *P*=0.009), but the trend was observed in all subgroups. Figures 1–3 illustrate the common patterns of PI seen within the three morphologic subdivisions. Patients with large cell morphology at the extramedullary site had a lower mean survival than those with small cell morphology (18.1 vs 49.8 months, *P*=0.002). Similarly, patients with large cell morphology in the BM also had a lower mean survival than those with small cell morphology (9.3 vs 40.6 months,

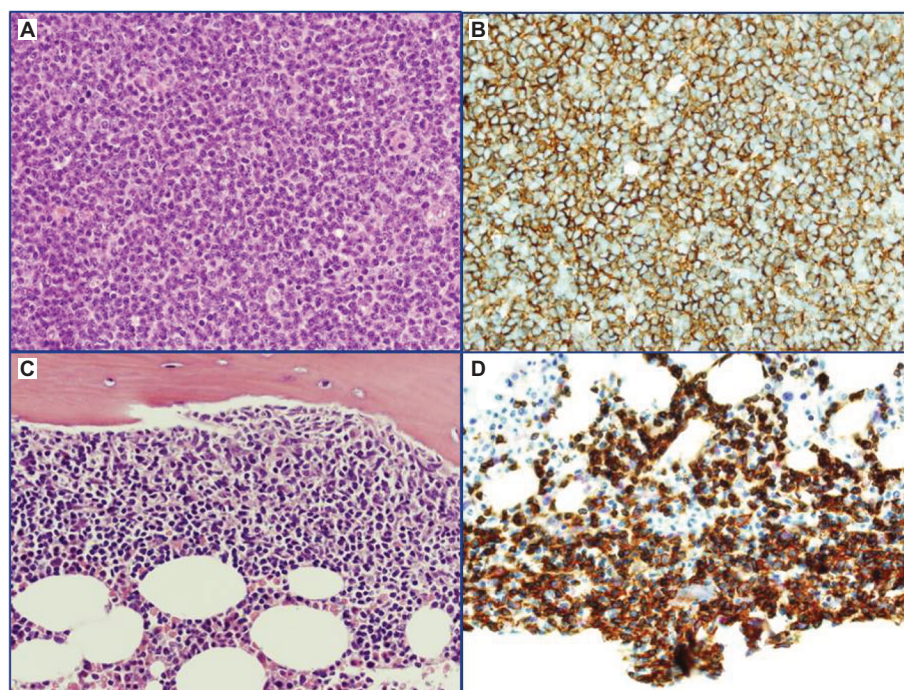
**Table 1** Mean and *P*-value of PI comparison at extramedullary tumor site versus BM sites in B-cell non-Hodgkin lymphomas

Group	No of cases	Extramedullary site mean PI	BM site mean PI	<i>P</i> -value
All cases	24	14.6	3.6	0.009
All LCL	11	28.5	6.5	0.005
LCL-L	4	41.5	16	0.073
LCL-S	7	21	1.1	0.009
All SCL-S	13	2.8	1.1	0.057

**Note:** The *P*-values were calculated using two-sample Student's *t*-test when comparing quantitative variables or by Chi-square test when comparing proportions, with significance set at a *P*-value <0.05.

**Abbreviations:** BM, bone marrow; LCL-L, large cell lymphoma at the primary site and large cell morphology in the marrow; LCL-S, large cell lymphoma at the primary site and small cell morphology in the marrow; PI, proliferation index; SCL-S, small cell morphology at the primary site and small cells in the marrow.

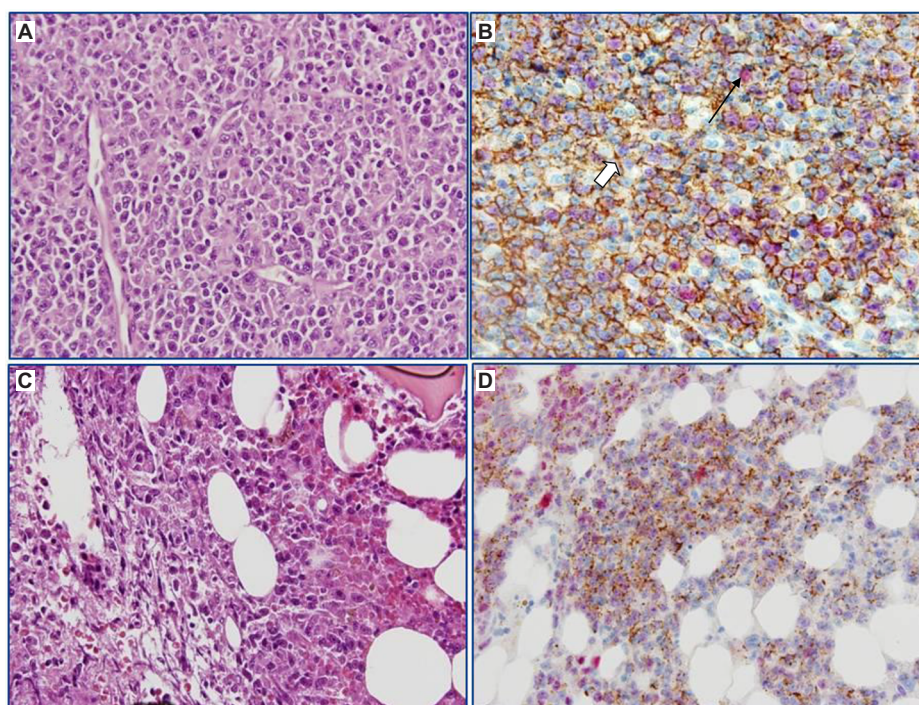




**Figure 1** SCL-S.

**Notes:** (A) Low grade follicular lymphoma involving the lymph node (H&E). (B) The same tissue as (A); dual stain: brown CD20 and red nuclear MIB1 staining demonstrated low MIB1 expression of 2%–3% (mean 2.8%). (C) Follicular lymphoma of the same patient, involving the bone marrow (H&E). (D) The same tissue as (C); dual stain demonstrated low MIB1 expression of 1%–2% (mean 1.1%); all images original, magnification  $\times 500$ .

**Abbreviations:** H&E, hematoxylin and eosin; MIB1, mindbomb E3 ubiquitin protein ligase 1; SCL-S, small cell morphology at the primary site and small cells in the marrow.

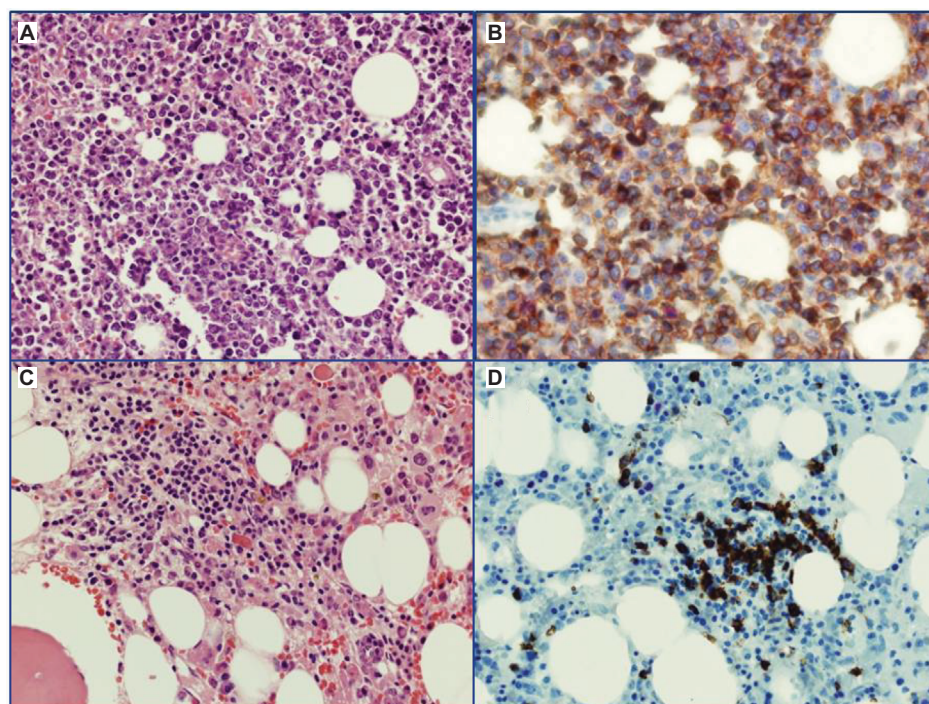


**Figure 2** LCL-L.

**Notes:** (A) Diffuse large B-cell lymphoma (H&E). (B) The same tissue as (A); dual stain demonstrated significantly higher MIB1 expression (mean 41.5%); black arrow points to red nuclear staining of MIB1, white arrow points to brown cytoplasmic, and membranous staining of CD20. (C) Bone marrow of the same patient demonstrated predominance of large cells. (D) The same marrow with MIB1 expression significantly lower than at its primary site (mean 16%); all images original, magnification  $\times 500$ .

**Abbreviations:** H&E, hematoxylin and eosin; LCL-L, large cell lymphoma at the primary site and large cell morphology in the marrow; MIB1, mindbomb E3 ubiquitin protein ligase 1.





**Figure 3** LCL-S.

**Notes:** (A) Diffuse large B-cell lymphoma (H&E). (B) CD20 and MIB1 dual stain demonstrated high MIB1 expression (mean 21%). (C) Marrow of the same patient involved by lymphoma with predominantly small cells morphology (H&E). (D) CD20 and MIB1 dual stain demonstrated low MIB1 expression (mean 1.1%); all images original, magnification  $\times 500$ .

**Abbreviations:** H&E, hematoxylin and eosin; LCL-S, large cell lymphoma at the primary site and small cell morphology in the marrow; MIB1, mindbomb E3 ubiquitin protein ligase 1.

$P=0.021$ ). Higher mean PI at the extramedullary site was observed in all cases. When considering survival, patients surviving 1 year or less had a significantly higher PI at the primary site than those surviving longer than a year (22.1% vs 10.8%,  $P=0.089$ ). A similar trend was present with mean PI at the BM site in patients stratified by survival of 1 year or less versus greater than a year (8.1% vs 1.3%,  $P=0.075$ ).

## Discussion

In our study, expression of MIB1 in lymphoma present in staging marrows was lower than the MIB1 expression at the extramedullary site in 100% of cases regardless of the morphology. The exact reason for this difference is unknown. It is possible that the BM has a different stromal and cytokine milieu than that of an extramedullary site, which may influence active proliferation of neoplastic cells. Discordance of morphology may also play a role. It is commonly accepted that discordant morphology between diffuse large B-cell lymphoma in its extramedullary location and its presence in the marrow can be due to diffuse large B-cell lymphoma arising from a preexisting low grade lymphoma or due to the presence of two clonally related or unrelated neoplasms.<sup>11</sup> Our study documented higher PI between primary tumor site and that in the staging marrow in all cases studied.

We conclude similar to other investigators that morphology (large vs small cells) helps to predict biologic activity of the disease. PI in BM specimens consistently lower than that of a primary tumor site strongly suggests that BM specimens are not equivalent to biopsy of primary tumor site and should not be used interchangeably with primary biopsy site in the determination of tumor PI or disease biology. Thus, BM biopsy as a sole diagnostic tissue should not be regarded equivalent to the biopsy of primary tumor site for prognostic determination.

It is noteworthy that mean PI in the extramedullary sites of involvement is lower in our study than reported in literature.<sup>2,4,12</sup> This finding is likely due to the use of a novel dual immunohistochemical staining technique optimized for staining in both BM and extramedullary sites. It must be recognized that native marrow cells can show up to 10% MIB1 expression in proliferating hematopoietic cells,<sup>13</sup> and thus spuriously elevating PI in BM, which is not truly reflective of proliferation of neoplastic lymphocytes, but rather creates subjective impression of higher proliferation than that which can be attributed purely to lymphoma. Even though we did not specifically analyze the data with reference to degree of BM involvement or specific lymphoma subtype, dual immunohistochemistry for CD20 and MIB1 performed allowed more accurate assessment of PI of the neoplastic

lymphocytes allowing separation of MIB1 staining in neoplastic B-cells from that of background hematopoietic cells. In fact, previous studies have observed wide interlaboratory and interobserver variability in MIB1 (Ki67) expression and suggested establishment of a laboratory-specific reference range for each immunohistochemical stain.<sup>2,8</sup> Our technique significantly decreases the degree of bias attributable to this interference between proliferation of neoplastic cells and background marrow cells.

## Conclusion

We conclude that for patients with BM involvement by B-cell NHL, the PI derived from BM should not be used as a surrogate prognostic indicator of PI of the lymphoma at its extramedullary site. Such inquiry may be treated as an additional source of clinically useful information, but if used inappropriately as an indicator of probable biologic behavior of lymphoma apart from the same determination at the primary tumor site, it may create a false impression of lower proliferative activity of the disease, which would be clinically misleading. We humbly recognize that our study is limited by the small number of studied cases and additional studies of this subject would be beneficial to further confirm or dispute our findings.

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## Disclosure

The authors report no conflicts of interest in this work.

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