Published in final edited form as:

Appl Immunohistochem Mol Morphol. 2009 March ; 17(2): 93–95. doi:10.1097/PAI.0b013e318184cd3f.

Lack of Utility of CD20 Immunohistochemistry in Staging Bone Marrow Biopsies for Diffuse Large B-cell Lymphoma

Daniel Baiyee, M.D., Roger Warnke, M.D., and Yasodha Natkunam, M.D., Ph.D.

Department of Pathology, Stanford University School of Medicine, Stanford, California

Abstract

The utility of CD20 immunohistochemistry in the evaluation of staging bone marrow biopsies of newly diagnosed diffuse large B-cell lymphomas (DLBCL) patients has not been extensively studied. We used 113 routinely processed bone marrow biopsies to study the extent and pattern of involvement by lymphoma and CD20 staining. Twelve (10.6%) of 113 cases had involvement by morphology, and five (41.7%) of these showed histologic discordance between the primary site and the bone marrow. All cases with morphologic evidence of bone marrow involvement showed staining for CD20. Four (3.5%) of 113 cases had non-neoplastic aggregates that stained for CD20. One case (0.9%) showed a small benign lymphoid aggregate by immunohistochemistry that was not evident by morphology. Our results demonstrate that CD20 staining did not detect any examples of bone marrow involvement by DLBCL that were not evident by morphology. We conclude that immunohistochemistry for CD20 adds no increase in the sensitivity of detection of bone marrow infiltration by DLBCL.

Keywords

Diffuse large B-cell lymphoma; bone marrow; staging; immunohistochemistry; CD20

INTRODUCTION

Diffuse large B-cell lymphoma is a heterogeneous entity accounting for approximately 30% of non-Hodgkin lymphoma. The incidence of bone marrow involvement in patients with DLBCL ranges from 12 to 35%.^{1–3} Staging bone marrow biopsy has been a valuable diagnostic and prognostic tool to assess the extent of disease in patients with newly diagnosed DLBCL. The pattern and extent of bone marrow infiltration and the histologic discordance between the primary site and the bone marrow have been recognized as factors of valuable prognostic significance.^{2–6} The extent of bone marrow involvement and a high percentage of large cells have been associated with a worse clinical outcome.⁷

Although traditionally morphologic evaluation has been considered the gold standard for staging bone marrow evaluation, immunohistochemistry can be a useful adjunct for assessment of the extent of bone marrow involvement especially in cases with minimal infiltration or discordant morphology.^{8–10} Moreover, decalcification and processing may compromise optimal histologic preservation making morphologic interpretation challenging in some instances. In this study, we evaluated the usefulness of immunohistochemistry for CD20 in identifying bone marrow involvement by newly diagnosed DLBCL.

MATERIALS AND METHODS

Case selection

Staging bone marrow core biopsies of 113 patients with newly diagnosed DLBCL between 1995 and 2005 were identified from the surgical pathology files of Stanford University Medical Center. All cases with a prior history of lymphoma or treatment using anti-CD20 antibodies (Rituximab) were excluded from the study. The cases were selected without prior knowledge of CD20-staining results. Institutional Review Board approval was obtained for these studies.

Histologic studies

Hematoxylin and eosin (H&E)-stained sections of acid-decalcified, formalin-fixed, paraffin-embedded bone marrow core biopsies were reviewed. The extent of bone marrow involvement was assessed as the percentage of total cells in the medullary space by estimating the percentage of the neoplastic lymphoid cells at 40X magnification (an average of 10 different 40X fields assessed). In cases where less than 10 fields were available, an average of the estimated fields were used. Cases with less than five fields were excluded. The pattern of bone marrow involvement was subdivided into five categories: diffuse, nodular, interstitial, paratrabeular and mixed. The size, pattern of distribution and cytologic features of the lymphoid infiltrates were evaluated. The presence of benign and malignant lymphoid aggregates was assessed and classified according to previously described criteria.^{2,11-13} The bone marrow was considered not involved or negative if infiltrates were absent or if benign lymphoid aggregates were noted by morphologic evaluation alone. The histologic discordance between the primary site and the bone marrow was defined as the presence of a lymphoid infiltrate with large cells constituting less than 50% of the B-cell infiltrate (there were no examples of T-cell and histiocyte rich DLBCL among the cases we studied).

Immunohistochemical studies

Immunohistochemistry were performed on formalin-fixed, paraffin-embedded sections of 4 micrometers which were deparaffinized in xylene and hydrated in a series of graded alcohols. Staining for CD20 (clone L26, DAKO, Carpinteria, California) was performed using standard retrieval on an automated staining machine (Ventana Medical Systems, Tuscon, AZ). The pattern and extent of CD20 expression was compared to the morphologic features on the H&E slides. CD20 staining was scored positive if any CD20-positive large cells were present. A tissue microarray with cores of multiple tissue types including tonsil, thymus and spleen were used as positive and negative controls for staining. In addition, all cases had a few scattered normal small lymphocytes serving as internal positive controls.

RESULTS

Histologic features

Twelve (10.6%) of 113 cases showed morphologic evidence of bone marrow involvement by lymphoma. The pattern of involvement was as follows: 5 diffuse, 3 nodular, 2 mixed and 2 interstitial. The extent of bone marrow involvement ranged from 5 to 90%. Five (41.7%) of 12 cases showed histologic discordance between the primary site and the bone marrow. None of the infiltrates in this series of cases were found in a paratrabeular location. In all cases the marrow neoplastic cells were medium- to large-sized lymphocytes with moderate to abundant cytoplasm, ovoid to irregular nuclei, dispersed to slightly coarse chromatin and inconspicuous to small nucleoli. Rare to occasional immunoblasts were noted. No T-cell/histiocyte-rich large B-cell lymphomas were identified. In all five cases with discordant morphology in the bone marrow, the marrow infiltrates were predominantly composed of small lymphoid cells,

cytologically compatible with low-grade B-cell non-Hodgkin lymphoma. Only rare to few scattered intermediate to large-sized lymphocytes were present.

Immunohistochemical features

Table 1 summarizes the results of bone marrow involvement and CD20 staining. Twelve of 113 cases in which the bone marrow was considered positive by morphologic evaluation showed CD20 staining. In all cases the morphologic pattern of CD20 expression matched the pattern of bone marrow infiltration by the neoplastic cells (Figure 1). The extent of CD20 staining did not differ among the diffuse, nodular, interstitial and mixed patterns. One hundred and one (89.4%) cases showed no bone marrow involvement by morphology and the corresponding CD20 stain was negative. Discordant histology was found in 5 of 12 cases (41.7%) where a low-grade lymphoma was detected in the bone marrow. In cases with discordant histology CD20 staining was found in both small and large neoplastic cells. None of the cases showed a CD20-positive large cell population that was not identified by morphologic evaluation of H&E sections. In addition, the number (percentage) of CD20-positive large cells were similar to those identified by morphologic evaluation with the exception of one case where approximately 50% of the large cells stained for CD20. This partial expression is most likely secondary to plasmacytoid differentiation and loss of CD20 expression in neoplastic cells. Four cases (3.5%) had benign lymphoid aggregates where a subset of lymphoid cells ranging from 20–50% expressed CD20. One case (0.9%) showed a small benign lymphoid aggregate by immunohistochemistry that was not evident by morphologic evaluation.

DISCUSSION

Bone marrow involvement by neoplastic large cells in DLBCL ranges from 12–35%.^{1–3} In the current study, 11% of cases exhibited bone marrow involvement by morphologic evaluation at the time of diagnosis. The role of immunohistochemistry as an adjunct to the evaluation of bone marrow biopsies for purposes of staging patients with lymphoproliferative disorders has been previously studied.^{9,14,15} CD20 is an excellent pan B-cell marker and its usefulness on paraffin-embedded tissue to characterize lymphoid infiltrates in bone marrow biopsies in patients with non-Hodgkin lymphoma has been well documented. In our experience B-lymphoid infiltrates in the bone marrow are readily highlighted with a stain for CD20.

Our results demonstrate that there is an excellent correlation between morphology and CD20 staining in the detection of disease involvement of staging bone marrows in newly diagnosed DLBCL patients. In our study the stain for CD20 did not detect additional cases of DLBCL involving the bone marrow not identified by morphologic evaluation. Thus, a CD20 immunostain for the purpose of detecting bone marrow involvement of DLBCL is not advocated based on these findings. It must be emphasized that none of the patients in this study cohort had a history of prior treatment with the anti-CD20 antibody, rituximab.^{16,17} Hence our findings may not be applicable to detection of recurrent DLBCL involving the bone marrow in patients treated with a combination of rituximab and anthracyclin-containing chemotherapy. In addition, some plasmablastic and immunodeficiency-related DLBCL may also lack CD20 staining. In these instances if an immunohistologic stain is needed another pan B-cell marker such as PAX5, CD79a, CD19 or CD22 may prove to be more useful. Our results argue that a CD20 stain is an unnecessary test in the evaluation of staging bone marrow biopsies for DLBCL and adds no value above that provided by thorough morphologic evaluation.

Acknowledgments

Supported in part by NIH P01CA34322

References

1. Arber DA, George TI. Bone marrow biopsy involvement by non-Hodgkin's lymphoma: frequency of lymphoma types, patterns, blood involvement, and discordance with other sites in 450 specimens. *Am J Surg Pathol* 2005;29:1549–1557. [PubMed: 16327427]
2. Conlan MG, Bast M, Armitage JO, Weisenburger DD. Bone marrow involvement by non-Hodgkin's lymphoma: the clinical significance of morphologic discordance between the lymph node and bone marrow. Nebraska Lymphoma Study Group. *J Clin Oncol* 1990;8:1163–1172. [PubMed: 1694234]
3. Morra E, Lazzarino M, Castello A, et al. Bone marrow and blood involvement by non-Hodgkin's lymphoma: a study of clinicopathologic correlations and prognostic significance in relationship to the Working Formulation. *Eur J Haematol* 1989;42:445–453. [PubMed: 2471652]
4. Hodges GF, Lenhardt TM, Cotelingam JD. Bone marrow involvement in large-cell lymphoma. Prognostic implications of discordant disease. *Am J Clin Pathol* 1994;101:305–311. [PubMed: 8135186]
5. Yan Y, Chan WC, Weisenburger DD, et al. Clinical and prognostic significance of bone marrow involvement in patients with diffuse aggressive B-cell lymphoma. *J Clin Oncol* 1995;13:1336–1342. [PubMed: 7751877]
6. Chung R, Lai R, Wei P, et al. Concordant but not discordant bone marrow involvement in diffuse large B-cell lymphoma predicts a poor clinical outcome independent of the International Prognostic Index. *Blood* 2007;110:1278–1282. [PubMed: 17475910]
7. 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 degree of infiltration and presence of discordant marrow involvement. *Eur J Haematol* 2006;76:473–480. [PubMed: 16529599]
8. Kubic VL, Brunning RD. Immunohistochemical evaluation of neoplasms in bone marrow biopsies using monoclonal antibodies reactive in paraffin-embedded tissue. *Mod Pathol* 1989;2:618–629. [PubMed: 2587569]
9. Maes B, Achten R, Demunter A, Peeters B, Verhoef G, De Wolf-Peeters C. Evaluation of B cell lymphoid infiltrates in bone marrow biopsies by morphology, immunohistochemistry, and molecular analysis. *J Clin Pathol* 2000;53:835–840. [PubMed: 11127265]
10. van der Valk P, Mullink H, Huijgens PC, Tadema TM, Vos W, Meijer CJ. Immunohistochemistry in bone marrow diagnosis. Value of a panel of monoclonal antibodies on routinely processed bone marrow biopsies. *Am J Surg Pathol* 1989;13:97–106. [PubMed: 2464955]
11. Bartl R, Frisch B, Burkhardt R, Jager K, Pappenberger R, Hoffmann-Fezer G. Lymphoproliferations in the bone marrow: identification and evolution, classification and staging. *J Clin Pathol* 1984;37:233–254. [PubMed: 6699189]
12. Schmid C, Isaacson PG. Bone marrow trephine biopsy in lymphoproliferative disease. *J Clin Pathol* 1992;45:745–750. [PubMed: 1401199]
13. Thiele J, Zirbes TK, Kvasnicka HM, Fischer R. Focal lymphoid aggregates (nodules) in bone marrow biopsies: differentiation between benign hyperplasia and malignant lymphoma--a practical guideline. *J Clin Pathol* 1999;52:294–300. [PubMed: 10474523]
14. Kremer M, Quintanilla-Martinez L, Nahrig J, von Schilling C, Fend F. Immunohistochemistry in bone marrow pathology: a useful adjunct for morphologic diagnosis. *Virchows Arch* 2005;447:920–937. [PubMed: 16231177]
15. West RB, Warnke RA, Natkunam Y. The usefulness of immunohistochemistry in the diagnosis of follicular lymphoma in bone marrow biopsy specimens. *Am J Clin Pathol* 2002;117:636–643. [PubMed: 11939740]
16. Foran JM, Norton AJ, Micallef IN, et al. Loss of CD20 expression following treatment with rituximab (chimaeric monoclonal anti-CD20): a retrospective cohort analysis. *Br J Haematol* 2001;114:881–883. [PubMed: 11564080]
17. Seliem RM, Freeman JK, Steingart RH, Hasserjian RP. Immunophenotypic changes and clinical outcome in B-cell lymphomas treated with rituximab. *Appl Immunohistochem Mol Morphol* 2006;14:18–23. [PubMed: 16540725]

Figure 1.

Table 1

Bone marrow involvement and staining for CD20

Morphologic Evaluation	N	%	CD20 Expression	
			Positive	Negative
Bone marrow involvement	12	10.6	12/12 (100%)	0/12 (0%)
DLBCL	7	6.0	7/7 (100%)	0/7 (0%)
Discordant histology (indolent)	5	4	5/5 (100%)	0/5 (0%)
No bone marrow involvement	101	89.4	0/101(0%)	101/101(100%)
Non-neoplastic aggregates	4	3.5	0/4 (100%)	* 4/4(0%)
Total	113			

N, number of cases, %, percent,

* benign lymphoid aggregate