Diagnostic Usefulness of Aberrant CD22 Expression in Differentiating Neoplastic Cells of B-Cell Chronic Lymphoproliferative Disorders From Admixed Benign B Cells in Four-Color Multiparameter Flow Cytometry

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Abstract

The diagnosis of B-cell chronic lymphoproliferative disorders is a great challenge when made in a background of polyclonal B cells. We studied the diagnostic usefulness of aberrant CD22 expression for differentiating neoplastic from benign B cells by 4-color flow cytometry. Of 56 cases of B-cell chronic lymphoproliferative disorders, we found that neoplastic cells showed aberrant CD22 expression in 39 (70%) of 56 cases, including chronic lymphocytic leukemia, mantle cell lymphoma, marginal zone lymphoma, hairy cell leukemia, and follicular lymphoma. In 4 cases, monoclonality was detected definitively only by evaluating the immunoglobulin light chain restriction in B cells with aberrant CD22 expression because numerous polyclonal B cells were present. Aberrant CD22 expression is a useful marker for detection of monoclonal B cells admixed with numerous benign polyclonal B cells.

Normal lymphoid tissue always contains 2 subsets of mature B cells; one subset expresses κ and the other expresses λ immunoglobulin light chain. The normal proportion of κ to λ B cells, often referred to as a κ/λ ratio, is within the range of 0.5 to 3.0 in the peripheral blood or bone marrow of healthy people1,2 and 1.2 to 2.7 in lymph nodes.3 In the lymph node, germinal center cells have slightly higher κ/λ ratios (1.1-3.1) than do mantle cells (1.0-2.2).3

Most mature neoplastic B cells express immunoglobulin light chains restricted to the κ or λ light chain. If neoplastic B cells in the lymphoid tissue are homogeneous, the neoplastic B cells will be 100% κ+ or 100% λ+ if immunoglobulin light chains are expressed. If, however, B cells are heterogeneous with an admixture of monoclonal (neoplastic) and polyclonal (benign) B cells, the κ/λ ratio will be altered to a degree depending on the relative ratio of monoclonal and polyclonal B cells. Therefore, the presence of a monoclonal population of light chain–expressing cells will not result in a κ/λ ratio out of the normal range in all cases.

The presence of a clonal population in a polyclonal background explains the difficulty in establishing a satisfactory cutoff for the κ/λ ratio that would permit differentiation of polyclonal reactive B-cell hyperplasia from a monoclonal B-cell lymphoproliferative disorder. Studies have shown that more than 20% of cases with monoclonal B cells would be missed if monoclonality were defined solely on numeric κ/λ cutoffs with specificity and positive predictive value set at 90% or greater.4,5

The diagnostic sensitivity for detection of neoplastic B cells is improved when B-cell subsets can be differentiated during the evaluation of light chain restriction by multiparameter flow cytometry. Surface markers that are expressed differently
in benign and neoplastic B cells are extremely useful for this purpose. Fukushima et al\(^6\) reported the diagnostic usefulness of abnormal expression of CD19 or CD20 in the detection of a small monoclonal population among admixed polyclonal B cells. However, many B-cell chronic lymphoproliferative disorders (B-CLPDs) do not have aberrant expression of CD19, and they sometimes do not have detectable CD20.

In our clinical practice, we have observed that the intensity of CD22 staining frequently was different in neoplastic B cells and admixed benign B cells. We hypothesized that CD22 is an additional diagnostic marker for identification of neoplastic B cells in the background of benign B cells. Our results showed that aberrant expression of CD22 was present in 39 (70\%) of 56 cases of B-CLPD, allowing good separation of neoplastic B cells and residual benign B cells for selective evaluation of \(\kappa\) and \(\lambda\) light chain expression. We concluded that CD22 is a useful marker for the identification of neoplastic B cells in a background of significant numbers of benign polyclonal B cells.

### Materials and Methods

#### Case Selection

We retrospectively identified, at Oregon Health & Science University, Portland, 56 cases of B-CLPD (January 2002–January 2004) using the criterion that flow cytometry showed a population of polyclonal B cells in addition to clonal B cells. These cases included small lymphocytic lymphoma/chronic lymphocytic leukemia (CLL, \(n = 27\)), follicular lymphoma (FL, \(n = 17\)), marginal zone lymphoma (MZL, \(n = 7\)), mantle cell lymphoma (MCL, \(n = 2\)), hairy cell leukemia (HCL, \(n = 2\)), and lymphoplasmacytic lymphoma (LPL, \(n = 1\)). For normal control samples, we also randomly selected 15 cases with polyclonal reactive B cells, including normal peripheral blood (\(n = 5\)), bone marrow (\(n = 5\)), and reactive lymph nodes or tonsils (\(n = 5\)). Pathology slides were reviewed for morphologic correlation and confirmation of diagnosis.

#### Flow Cytometric Immunophenotyping

Four-color flow cytometric analysis was performed in the clinical flow cytometry laboratory of the Oregon Health & Science University. Antibodies used in the study are listed in Table 1. Specimens were stained and analyzed within 24 hours of collection. For specimens of peripheral blood or bone marrow aspirate, heparinized samples were processed and RBCs were removed by density gradient centrifugation. For lymph node specimens, fresh tissue was minced and a single-cell suspension was prepared. Each monoclonal antibody combination was added to a cellular suspension containing 5 \(\times 10^5\) cells in 50 \(\mu\)L. After incubation for 30 minutes in the dark on ice, cells were washed once with phosphate-buffered saline containing 0.1% sodium azide and fixed in 1% formaldehyde before acquisition on a flow cytometer (FACSCalibur, Becton Dickinson, San Jose, CA). Data were analyzed using CELLQuest Pro software (Becton Dickinson). Lymphocytes were gated based on their forward- and side-scatter properties. B-cell subsets were gated based on their surface marker expression.

### Results

#### CD22 Expression Frequently Is Aberrant on Neoplastic Cells of B-CLPDs

To examine the frequency and pattern of CD22 expression in neoplastic cells, monoclonal B cells were compared with polyclonal B cells in the same specimens. With an antibody combination of \(\kappa/\lambda/CD20/CD22\), we initially compared \(\kappa^+\) B cells with \(\lambda^+\) B cells for CD20 and CD22 expression. If B-cell subsets with different expression of CD22 were identified, each subset was evaluated separately for light chain restriction. We considered CD22 expression as aberrant if CD22 staining intensity was altered in monoclonal B cells compared with polyclonal B cells in the same samples. With an anti-CD22 antibody combination of \(\kappa/\lambda/CD19/CD10\) or \(\kappa/\lambda/CD19/CD5\) combinations also were used.

Representative patterns of aberrant CD22 expression are shown in Image 1. Neoplastic B cells and residual normal B cells were visualized readily as different clusters on the dot plots, although some overlap existed. Of 56 cases of B-CLPD with neoplastic B cells admixed with benign B cells, aberrant CD22 expression was present in 39 (70\%) of 56 cases. Abnormal CD22 expression was seen much more frequently than abnormal expression of CD19 (5/56 [9\%]) and slightly more frequently than abnormal CD20 expression (37/56 [66\%]). However, abnormal CD22 expression and abnormal

Table 2
Alteration of Pan–B-Cell Antigen Intensity in B-Cell Lymphoproliferative Disorders

<table>
<thead>
<tr>
<th>Disease</th>
<th>CD22</th>
<th>CD20</th>
<th>CD19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>CLL (n = 27)</td>
<td>0</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>FL (n = 17)</td>
<td>13</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>MZL (n = 7)</td>
<td>4</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>MCL (n = 2)</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>LPL (n = 1)</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HCL (n = 2)</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total (%)</td>
<td>17 (30)</td>
<td>19 (34)</td>
<td>51 (91)</td>
</tr>
</tbody>
</table>

CLL, small lymphocytic lymphoma/chronic lymphocytic leukemia; FL, follicular lymphoma; HCL, hairy cell leukemia; LPL, lymphoplasmacytic lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; ↓, decreased; ↑, increased.

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CD20 expression did not always occur in the same cases. Complete loss of CD22 was not identified, whereas CD20 was undetectable in some cases. Underexpression of CD22 was much more common (35/56 [63%]) than overexpression (4/56 [7%]). Underexpression of CD22 was found in all 27 CLLs, 2 FLs, 3 MZLs, 2 MCLs, and 1 LPL. Overexpression of CD22 was seen in 2 HCLs and 2 FLs.

**Aberrant CD22 Expression Is Useful for Identification of Monoclonal B Cells**

A majority of cases studied did not present diagnostic difficulties because the neoplastic B cells greatly outnumbered residual normal B cells. However, in 4 cases, monoclonal B cells were not obvious based on κ/λ ratios of total B cells. When B-cell subsets with differential CD22 expression were examined for κ and λ light chain expression, aberrant CD22 expression clearly identified monoclonality. These cases included a peripheral blood sample in a case of CLL, a lymph node in a case of FL, an orbital mass in a case of MZL, and a bone marrow sample in a case of LPL.

In the CLL case **Image 2**, the numbers of monoclonal and polyclonal B cells were about equal, with similar CD19 antigen intensity. CD22 normal B cells were polyclonal and negative for CD5 and CD23. CD22 dim B cells were monoclonal and positive for CD5, CD19, and CD23. CD20 was detectable in normal B cells but not in neoplastic B cells.

In the FL case **Image 3**, flow cytometric analysis showed 2 B-cell subsets with differential CD22 expression. B cells with overexpression of CD22 expression showed monoclonal expression of very dim λ light chain. They also were positive for CD10 and overexpressed CD20. The monoclonal B cells would have been overlooked if light chain expression had not been evaluated separately in different B-cell subsets. CD22 normal B cells were polyclonal B cells with admixed CD10+ and CD10– cells. Sections of the biopsy specimen confirmed that the lymph node was involved partially by FL with residual reactive lymphoid follicles. The polyclonal B cells were the residual reactive germinal center and mantle B cells.

In the MZL case **Image 4**, B-cell subsets with differential CD22 expression also were detected by flow cytometry. B cells with underexpression of CD22 had CD20 overexpression. However, CD19 expression did not differentiate these B-cell subsets. The κ/λ ratio of all B cells was suggestive of the presence of monoclonal B cells. When light chain expression was examined among different B-cell subsets, the presence of monoclonal B cells was evident. B cells with underexpression of CD22 were CD10– with monoclonal expression of κ light chain, whereas other B cells were CD10+ and polyclonal. Tissue sections of the biopsy specimen showed MZL with follicular colonization. CD10+ B cells represented residual benign germinal center B cells.

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**Image 2** Underexpression of CD22 in a peripheral blood sample in a case of chronic lymphocytic leukemia. **A** and **B**, B cells consist of 2 subsets, CD22 normal (green, CD5–CD19+) and CD22 dim (pink, CD5+CD19+). **C**, These 2 populations show no difference in light scatter. **D**, CD20 is undetectable in CD22 dim B cells. **E**, B cells with normal CD22 are mainly polyclonal. **F**, B cells with underexpression of CD22 show monoclonal λ expression. FSC, forward scatter; SSC, side scatter.
In the case of LPL, flow cytometric analysis of the bone marrow revealed 3 B-cell populations based on CD22 expression: hematogones, polyclonal B1 B cells, and monoclonal B cells. Hematogones were positive for CD19, CD22, and CD10, variably positive for CD34 and CD20, and negative for surface κ and λ. B1 B cells were positive for CD5, CD19, and CD20 with polyclonal expression of surface light chains. Monoclonal B cells were negative for CD5 and CD10. CD20 intensity was the same in monoclonal B cells and polyclonal B1 B cells. The bone marrow revealed lymphoid infiltrates in paratrabecular and interstitial patterns. Although plasmacytoid lymphocytes were not apparent in this bone marrow specimen, other bone marrow biopsy specimens obtained from the same patient at different times showed some plasmacytoid lymphocytes. Serum immunoprotein studies revealed IgM monoclonal gammopathy. There was no evidence of other lymphoproliferative orders. The overall clinical and pathologic features were consistent with Waldenström macroglobulinemia.

**Discussion**

In the present study, we compared CD22 expression between neoplastic monoclonal B cells and admixed benign polyclonal B cells in the same specimen by using 4-color multiparameter flow cytometry. Our findings showed that CD22 frequently is aberrant in the neoplastic cells of B-CLPDs. More importantly, we demonstrated that aberrant CD22 expression was a very useful surface marker for elucidating
the clonality of neoplastic B cells in the background of polyclonal B cells when the antibody against CD22 was used with antibodies against κ and λ in multicolor flow cytometry.

Our findings are concordant with those of previous studies. CD22 frequently is overexpressed in HCL\(^7,8\) and splenic MZL\(^5\) but underexpressed in almost all other B-CLPD groups, including CLL\(^7-10\), prolymphocytic leukemia,\(^8\) MCL,\(^8\) and FL.\(^8\) However, these earlier studies did not address the difference of CD22 expression in neoplastic B cells and benign B cells in the same tissue samples from the same patients. In our study, by comparing CD22 antigen intensity between benign and neoplastic B cells within the same tissue samples, we showed overexpression of CD22 in HCL and underexpression of CD22 in CLL and MCL. In addition, we found that CD22 could be overexpressed, normal, or underexpressed in FL. In nodal or mucosal tissue samples from MZL cases, CD22 expression was normal or underexpressed, but overexpression was not observed.

The CD22 expression level in neoplastic cells might reflect the activation status or differentiation stage of neoplastic cells. It also is possible that altered CD22 cell signaling might relate to neoplastic transformation. CD22, a 135-kd sialoglycoprotein, is involved in the activation and adhesion of normal B cells.\(^11-13\) It augments calcium efflux after B-cell receptor cross-linking, subsequently regulating B-cell proliferation and antibody production.\(^14\) It is present in the cytoplasm of virtually all B-lineage cells and weakly expressed in hematogones but fully expressed on the B-cell surface only at mature stages of differentiation. CD22-directed antibody therapy is under active investigation in clinical trials,\(^12,15\) and it will be important to study the relationship between the CD22 expression pattern and clinical response to anti-CD22 therapy.

As a diagnostic marker, CD22 was expressed aberrantly in more than two thirds of B-CLPDs, including cases that showed normal expression of CD19 and CD20. Therefore, we recommend that whenever κ+ and λ+ populations are present, special attention be given to any B-cell subset with aberrant CD22 expression. Examining the light chain distribution pattern in the different CD22-expressing populations might reveal a clonal B-cell population. Because neoplastic B cells do not always show a CD22 abnormality, the light chain distribution pattern should be considered along with other immunologic features to completely rule out the presence of monoclonal B cells. Neoplastic B cells often can be differentiated from normal B cells based on their size or differences in antigen intensity using surface markers such as CD5, CD10, CD19, CD20, CD25, and CD103 or immunoglobulin light chains. Also, on occasion, mature neoplastic B cells might not express immunoglobulin light

\[\text{Image 5I} \text{ Underexpression of CD22 in a bone marrow specimen in a case of lymphoplasmacytic lymphoma. Three B-cell subsets are identified: CD19+CD5+ (A, R1, green), CD19+CD10– (B, R2), and CD19+CD10+ (B, R3, blue). These 3 B-cell subsets with different antigen intensities of CD22 are shown (C). The light chain expression of each B-cell subset is shown: hematogones without light chain expression (D), polyclonal B cells (E), predominantly monoclonal B cells (F).}\]
chains, whereas residual normal B cells express polyclonal light chains, allowing astute pathologists to further investigate the aberrant, light chain–negative, mature B-cell immunologic profile. Whenever different B-cell subsets are identified immunologically, one should evaluate light chain restriction separately in each B-cell subset.

Our study shows the usefulness of CD22 in flow cytometry for detection of a small number of neoplastic B cells in a background of polyclonal B cells. It is especially applicable in the early stages of disease and in cases of partial involvement and for determining residual disease status.

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References