Abstract
We determined the prevalence and significance of finding B cells without surface immunoglobulin (SIg) light chain expression. The flow cytometry database at Johns Hopkins Medical Institutions was searched for cases in which immunoglobulin light chain staining was performed to rule out a B-cell malignant neoplasm between January 1994 and February 2000. We excluded plasma cell dyscrasias, precursor B-cell acute lymphoblastic leukemia/lymphomas, and hematogones. Cases with more than 25% of B cells lacking SIg light chain expression were retrieved. Polymerase chain reaction assays for immunoglobulin heavy chain gene rearrangements were performed in SIg-negative cases with available tissue blocks. We identified 36 cases; all represented lymphoma. Their diagnoses included diffuse large B-cell lymphoma (20), HIV-related lymphoma (5), follicular lymphoma (5), Burkitt lymphoma (2), monomorphic posttransplant lymphoproliferative disorder (1), chronic lymphocytic leukemia/small lymphocytic lymphoma (1), marginal zone B-cell lymphoma (1), and low grade B-cell lymphoma (1). Of the 17 SIg-negative cases with amplifiable DNAs, 12 (71%) showed a clonal immunoglobulin heavy chain gene rearrangement. SIg-negative B-cell lymphomas are rare. Complete absence of SIg light chain expression in a mature B cell proliferation can be used as a surrogate marker to help diagnose peripheral B-cell lymphoma.

Malignant B-cell lymphomas derived from mature B cells encompass a spectrum of monoclonal proliferation of B cells at different stages of differentiation and comprise the majority of non-Hodgkin lymphomas in the Western populations. Except for plasma cells, mature B lymphocytes express either kappa or lambda immunoglobulin light chain proteins on their surface. The demonstration of surface immunoglobulin (SIg) light chain restriction by immunohistochemical or flow cytometric immunophenotyping (FCI) indicates monoclonality of the proliferating mature B cells, a feature that usually is used to support the diagnosis of malignant B-cell non-Hodgkin lymphomas.

Previous studies have demonstrated that the neoplastic cells in up to one third of B-cell non-Hodgkin lymphomas did not express either kappa or lambda SIg light chain proteins. These early studies, however, were done using the relatively insensitive immunohistochemical method. Data obtained with the more sensitive FCI technique indicated that SIg-negative malignant B-cell non-Hodgkin lymphomas were rare. The frequency ranged from 3.4% to 12.2%, depending on the criteria used in the studies. Since the number of cases reported so far is limited, the true prevalence of SIg-negative non-Hodgkin lymphomas is still unknown. Furthermore, whether the absence of SIg light chain expression can be used as an indicator of peripheral B-cell lymphomas in the absence of demonstration of clonality remains to be determined.

We retrospectively studied, by using FCI in a large referral laboratory, the frequency and significance of finding lymphoid proliferations in which B cells lacked SIg light chain expression. By correlating these findings with clinicopathologic and molecular genetic features of cases, we found...
that complete lack of SIg light chain expression indicated an abnormal B-cell proliferation and can be used to support the diagnosis of malignant lymphoma.

**Material and Methods**

**Case Selection**

The flow cytometric database in the Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD, was searched for cases in which immunoglobulin light chain staining was precluded owing to the unavailability of specimens. Intracellular staining on the remaining SIg-negative cases was performed in a subset of cases at the time of original diagnosis. The FCI results were compared with the stated diagnosis of malignant lymphoma. Abnormal B-cell proliferation and can be used to support the histologic diagnosis of malignant lymphoma.

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**Flow Cytometric Immunophenotyping**

FCI was performed on fresh biopsy tissues collected in RPMI 1640 culture medium, fine-needle aspirates, or body fluid samples. Specimens were processed routinely, and single cell suspensions were stained with 3 or 4 fluorochrome-conjugated antibody combinations (fluorescein isothiocyanate, phycoerythrin, peridinin chlorophyll protein, and allophycocyanin) using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA). Monoclonal antibodies against kappa, lambda, CD3, CD19, and CD45 were used in all cases (Becton Dickinson, San Diego, CA). In most cases, antibodies against CD5, CD10, CD20, CD22, CD23, FMC7, and HLA-DR were included in the panel. Antibodies against CD13, CD14, CD33, CD34, CD71, CD38, and terminal deoxynucleotidyl transferase were used in some cases. Polyclonal antibodies against kappa and lambda immunoglobulin light chain proteins were added to the panel if the B cells failed to stain with the monoclonal antibodies. Intracellular staining for immunoglobulin light chains with monoclonal kappa and lambda antibodies was performed in a subset of cases at the time of original diagnosis. Intracellular staining on the remaining SIg-negative cases was precluded owing to the unavailability of specimens.

**Results**

Between January 1994 and February 2000, more than 5,000 specimens were sent to the flow cytometry laboratory at the Johns Hopkins Medical Institutions for immunophenotyping to rule out malignant B-cell non-Hodgkin lymphomas. Among those, 1,561 showed clonality with either kappa (971 cases) or lambda (590 cases) light chain restriction, supporting the histologic diagnosis of malignant B-cell lymphoma. After excluding plasma cell dyscrasia, precursor B-cell acute lymphoblastic leukemia/lymphoma, and cases with hematogones, 36 cases were identified in which more than 25% of the B cells negative for SIg light chain proteins according to the aforementioned criteria were selected for this study. Monoclonal light chain expression was recognized by a distinct shift in the cluster of CD19+ B cells with respect to control samples or by a significant difference in either the light scatter or the CD19 intensity distribution of kappa- and lambda-positive events; these cases were considered SIg-positive. Cases with more than 25% of the B cells negative for SIg light chain proteins according to the aforementioned criteria were selected for this study.

**PCR Assay**

Approximately 10,000 events were acquired and analyzed using the Paint-A-Gate computer software program (Becton Dickinson, San Diego). SIg negativity was defined as complete lack of B-cell surface staining for kappa and lambda light chain proteins with both monoclonal and polyclonal antibodies in a pattern similar to that of the isotype control samples. Cases with high nonspecific staining for both light chains that precluded identification of a clear-cut staining pattern were considered indeterminate and not included in this study. Monoclonal light chain expression was recognized by a distinct shift in the cluster of CD19+ B cells with respect to control samples or by a significant difference in either the light scatter or the CD19 intensity distribution of kappa- and lambda-positive events; these cases were considered SIg-positive. Cases with more than 25% of the B cells negative for SIg light chain proteins according to the aforementioned criteria were selected for this study.

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diagnosis of lymphoma and comprised about 2.3% of all peripheral B-cell non-Hodgkin lymphomas during the period studied.

As summarized in Table 1, the age of these SIg-negative lymphoma patients ranged from 1 to 93 years, with a median age of 53 years. The male/female ratio was 1.4:1. There was no preferential involvement of extranodal anatomic sites. The majority of cases were aggressive lymphomas. Twenty of the 36 cases were typical diffuse large B-cell lymphoma. The diagnoses of the remaining cases included HIV-related lymphoma (5), follicular lymphoma (5), Burkitt lymphoma (2), monomorphic post-transplant lymphoproliferative disorder (1), chronic lymphocytic leukemia/small lymphocytic lymphoma (1), marginal zone B-cell lymphoma, (1) and low grade B-cell lymphoma, unclassifiable (1).

The percentage of SIg-negative B cells varied from 29% to more than 99% of all B cells, with a median of about 95%. In all these cases, the SIg-negative B-cell population was distinctly separated from the normal polytypic B lymphocytes present (Image 1 and Image 2). In almost 90% of the cases (32/36), forward angle light scatter indicated that the SIg-negative B cells were larger than the background reactive T cells or the polytypic B cells (Image 3). Except for 1 case in which B-cell lineage derivation of the neoplastic cells was determined by immunohistochemical stain for CD79a (case 22), at least 1 of the B-cell markers (CD19, CD20, CD22) was positive by FCI analysis (Table 2). The activation marker CD71 was expressed in most cases examined, and CD10 was expressed in about one third of the cases tested. CD5 was present in 1 case of chronic lymphocytic leukemia and 1 diffuse large B-cell lymphoma. Intracellular staining for bcl-2 was positive in cases 5 and 22, further supporting the diagnosis of follicular lymphoma in both cases (data not shown). CD45 was positive in all cases.

### Table 1

<table>
<thead>
<tr>
<th>Case No./Sex/ Age (y)</th>
<th>Site/Specimen Type</th>
<th>Diagnosis</th>
<th>IgH PCR*</th>
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<td>1/F/66</td>
<td>LN, left side of neck/biopsy</td>
<td>MZL</td>
<td>+</td>
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<tr>
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<td>4/M/1</td>
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<td>5/M/41</td>
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<td>FL</td>
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</tr>
<tr>
<td>6/M/54</td>
<td>Left cervical LN/FNA and biopsy</td>
<td>DLBCL</td>
<td>+</td>
</tr>
<tr>
<td>7/M/62</td>
<td>Kidney/FNA</td>
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<tr>
<td>8/F/74</td>
<td>Bone marrow/biopsy</td>
<td>CLL/SLL</td>
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<td>9/M/83</td>
<td>Left axillary LN/FNA and biopsy</td>
<td>DLBCL</td>
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<td>10/M/29</td>
<td>Left cervical LN/FNA and biopsy</td>
<td>HIV-related lymphoma</td>
<td>No blocks</td>
</tr>
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<td>12/F/37</td>
<td>Cervical LN/biopsy</td>
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<td>15/F/42</td>
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<td>16/F/53</td>
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<td>18/F/23</td>
<td>Pelvic mass/FNA</td>
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<td>19/F/26</td>
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<td>DLBCL</td>
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<td>20/M/78</td>
<td>Peritoneal fluid/abdominal paracentesis</td>
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<td>23/M/70</td>
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<td>Thoracic mass/biopsy</td>
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<td>28/F/67</td>
<td>Retroperitoneal mass/biopsy</td>
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<td>+</td>
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<td>29/F/56</td>
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<td>No blocks</td>
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<td>30/M/20</td>
<td>CSF and bone marrow/biopsy</td>
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<td>DLBCL</td>
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<td>32/F/93</td>
<td>Epidermal mass/biopsy</td>
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<td>Right cervical LN/biopsy</td>
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<td>HIV-related lymphoma</td>
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<tr>
<td>36/F/47</td>
<td>Right cervical LN/FNA and biopsy</td>
<td>Lymphoma</td>
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</tr>
</tbody>
</table>

| IGH PCR* | No blocks indicates that no blocks were available; no DNA, no amplifiable DNA was obtained. |

CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; CSF, cerebrospinal fluid; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; FNA, fine-needle aspirate; IgH, immunoglobulin heavy chain gene; LN, lymph node; MZL, marginal zone B-cell lymphoma; PCR, polymerase chain reaction; PTLD, posttransplant lymphoproliferative disorder; +, positive; –, negative.
Isolation of genomic DNAs was attempted in 20 cases with tissue blocks. Among the 17 cases with amplifiable genomic DNAs, 12 had a clonal immunoglobulin heavy chain gene rearrangement (Table 1), a positive rate similar to that reported for peripheral B-cell lymphomas with either SIg kappa or lambda light chain restriction.

To estimate the specificity of finding SIg-negative B cells, the flow cytometric database was reviewed to identify cases of reactive lymphoid hyperplasia in tissues other than bone marrow. Among 490 such cases in which the percentage of total B cells and kappa- and lambda-positive B cells were recorded, the highest percentage of SIg-negative B cells noted was 17%.

Discussion

SIg light chain expression is the hallmark of a mature B cell. Normally, either kappa or lambda light chains are expressed, and the great majority of B cell lymphomas show skewing of the normal kappa/lambda ratio or an otherwise obvious light chain–restricted population.1-8

The absence of SIg light chain expression has been observed in about one third of B-cell non-Hodgkin lymphomas using immunohistochemical stain on frozen tissues; about 30% to 40% of these SIg-negative cases were diffuse large B-cell lymphoma and follicular lymphoma.1 Subsequent studies using the relatively more sensitive FCI technique demonstrated that lack of SIg light chain expression in B-cell non-Hodgkin lymphoma was a rare phenomenon.2-8 In the earliest study using FCI, Liendo et al2 reported that 4 of 47 cases of B-cell non-Hodgkin lymphoma lacked SIg light chains, but the criteria for SIg negativity was not described. In a subsequent study by de Martini et al,3 the absence of SIg was defined as B cells expressing less than 15% kappa and less than 10% lambda immunoglobulin light chains. By using this definition, they identified 33 cases of SIg-negative B-cell lymphomas among the 271 cases analyzed, a prevalence of 12.2%. Most recently, Kaleem et al8 described 10 SIg-negative B-cell lymphomas among 298 consecutive cases. In their study,
SIg negativity was defined when light chain expression was present in fewer than 5% of all gated B lymphocytes. Although they speculated that the 5% expression may have represented background autofluorescence, low-level expression of SIg is not uncommon in typical B-cell non-Hodgkin lymphomas. In our series, therefore, we defined SIg negativity as complete absence of staining for kappa and lambda antibodies in a pattern similar to that of the isotype control samples, avoiding the calculation of the percentage of kappa or lambda light chain expression among the B lymphocytes of interest. With this strict criterion, it was found that approximately 2.25% (36/1,597) of all peripheral B-cell non-Hodgkin lymphomas failed to express SIg light chain proteins in this large series.

Little is known about the status of immunoglobulin gene rearrangements in these SIg-negative B-cell non-Hodgkin lymphomas because they are relatively uncommon. In the only study reported by Kaleem et al,8 they demonstrated clonal immunoglobulin heavy chain gene rearrangements in 3 cases studied. Of these 3 cases, 2 also were found to undergo clonal immunoglobulin kappa light chain gene rearrangement.® They postulated that lack of SIg light chain expression was probably due to a defect(s) at the postranscriptional level such as protein translation, posttranslational protein modification, or intracellular protein trafficking. We also showed that approximately 71% (12/17) of the SIg-negative B-cell non-Hodgkin lymphomas harbored a clonally rearranged immunoglobulin heavy chain gene as revealed by PCR assays, a detection frequency similar to that reported in SIg-positive B-cell non-Hodgkin lymphomas, further supporting the speculation of Kaleem et al.® We did not determine the status of kappa or lambda immunoglobulin light chain genes.

From a practical standpoint, finding B cells without SIg light chain expression raises the possibility of several different diagnoses. First, SIg-negative mature B-cell non-Hodgkin lymphomas need to be distinguished from precursor B-cell lymphoblastic lymphoma/leukemia. In most situations, this is not a problem because the neoplastic precursor B cells have a distinct, very different phenotype, with low density or absent CD45 expression, frequent expression of CD34 and terminal deoxynucleotidyl transferase, and frequent partial or complete loss of CD20.9 SIg-negative B-cell lymphomas also should be differentiated from plasma cell dyscrasias, as these sometimes express CD19 and may reveal a significant population of CD19+, SIg-negative cells. However, plasma cells show very strong expression of CD38 and are generally negative for CD45; in difficult cases expression of CD138 (Syndecan) may be a useful marker.10 Third, when bone marrow samples are studied, SIg-negative normal B-cell precursors need to be excluded, but, again, these have a characteristic phenotype.9,11

![Image 4](image_url) A representative polyacrylamide gel showing the clonally rearranged immunoglobulin heavy chain genes. M, 50-base-pair DNA molecular weight marker; +, positive control; –, negative control; lanes 1-7, patient samples. IgH, immunoglobulin heavy chain gene; beta-GB, human beta-globin gene.

<table>
<thead>
<tr>
<th>Table 2A</th>
<th>Flow Cytometric Immunophenotyping Findings</th>
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ND, not done; +, positive; –, negative.
* Light scatter of surface immunoglobulin–negative B cells compared with normal lymphocytes: L, larger; S, same or smaller.
Of greater interest is whether the finding of SIg-negative B cells outside the situations noted excludes the possibility of a reactive B-cell proliferation. In some cases of florid follicular hyperplasia, a subset of germinal center B cells in a polyclonal background seems to lack SIg. However, in a review of almost 500 reactive lymphoid populations, we found no case with more than 17% of SIg-negative B cells. Thus, while finding a small number of SIg-negative B cells cannot be used as a criterion for monoclonality, the presence of a significant population of such cells in our series was invariably associated with malignant B-cell lymphoma, indicating the clonal nature of the SIg-negative B cells. Even though we found no overlap in the percentage of B cells between benign and malignant cases, it may be useful in borderline cases to perform intracellular staining for bcl-2, as its presence would exclude the interpretation of activated SIg-negative follicle center cells and thus support the diagnosis of lymphoma.12 Cytogenetic and/or molecular studies also might be helpful. In any case, correlation with morphologic features, appropriately supplemented by immunohistochemical analysis, is essential for an accurate diagnosis of malignant lymphoma.

SIg-negative B-cell non-Hodgkin lymphomas, defined by our strict criteria, are rare, accounting for less than 5% of all peripheral B-cell lymphomas. Because we defined our criteria for SIg negativity without validating it on an independent data set, additional prospective confirmatory studies would be useful, especially to define a more accurate threshold for discriminating between SIg-negative lymphomas and normal cases. Nevertheless, our results strongly suggest that finding a significant population of SIg-negative B cells is a surrogate marker that can be used to diagnose peripheral B-cell lymphomas.

References

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