Fine-Needle Aspiration in Non-Hodgkin Lymphoma

Evaluation of Cell Size by Cytomorphology and Flow Cytometry

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Key Words: Fine-needle aspiration; FNA; Non-Hodgkin lymphoma; NHL; Large cell lymphoma; Large cell transformation; Cytomorphology; Flow cytometry

Abstract

We studied 48 non-Hodgkin lymphoma (NHL) fine-needle aspiration (FNA) specimens with initial cytomorphology (CM) and flow cytometry (FC) and subsequent surgical biopsy of the same lesion to determine whether a reliable diagnosis of large cell lymphoma or large cell transformation could be made. CM was evaluated by examining 200 lymphocytes in each specimen. FC was performed by analyzing monoclonal or abnormal B-cell populations. Percentages of large cells were evaluated by CM and FC and results correlated with the histologic diagnosis. All small cell NHLs showed fewer than 40% large cells by CM and FC; 100% (9/9; FC) and 67% (6/9; CM) of diffuse large B-cell lymphomas demonstrated greater than 40% large cells. Variable numbers of large cells were detected in grade III follicular lymphoma, low-grade lymphoma with partial large cell transformation, and large B-cell lymphoma containing fewer than 10% neoplastic cells. By using combined CM and FC, large cell lymphoma and large cell transformation can be diagnosed reliably by FNA if greater than 40% large cells are present. Surgical biopsy is necessary when there is necrosis, fewer than 10% neoplastic cells by FC, or fewer than 40% large cells with clinical signs of transformation.

Diagnosis and classification of non-Hodgkin lymphoma (NHL) are traditionally made by surgical biopsy and histologic examination. During the past 10 years, with the application of flow cytometric (FC) techniques to fine-needle aspiration (FNA) specimens, increasing numbers of initial lymphoma diagnoses have been made by FNA.1-4 Many studies have concluded that by using a combination of cytomorphologic (CM) examination and FC analysis, a substantial portion of lymphomas could be diagnosed based on FNA alone without further tissue biopsy. The sensitivity of FNA diagnosis in NHL ranges from 66% to 100%.5-13 Furthermore, a substantial number of these lymphomas could be subclassified based on the FNA material.7,13-15 However, these studies also indicated that FNA could not completely replace tissue biopsy, and there were still cases of NHL that required surgical biopsy for confirmation of the diagnosis. One of the main limitations of the cytologic preparation is the lack of architectural pattern. In the current lymphoma classification systems, architectural morphologic features remain an important parameter, although lymphoma diagnosis is now increasingly based on combined information from immunophenotype, genotype, and clinical manifestations, in addition to histomorphologic features.16,17 Nevertheless, in certain clinical conditions, FNA is accepted as the primary tool in the diagnosis of NHL. FNA provides faster turnaround, less morbidity and mortality, and decreased cost compared with surgical biopsy. The documentation of recurrent lymphoma is a widely accepted use of FNA.

Using FNA in the diagnosis of large cell lymphoma and large cell transformation has been approached by several studies.13,18-22 The recognition of large cell lymphoma and
large cell transformation is important in FNA diagnosis because of the implications for therapy. In the majority of NHLs, the percentage of large cells is associated directly with the clinical aggressiveness of the NHL. Follicular lymphoma grades I and II, small lymphocytic lymphoma, marginal zone lymphoma, and lymphoplasmacytoid lymphoma consist predominantly of small cells and exhibit indolent clinical behavior. These tumors usually are managed with more conservative treatment regimens. NHLs with increased numbers of large cells include follicular lymphoma grade III, diffuse large B-cell lymphoma (DLBCL), and any transformed low-grade NHL. These lymphomas often have a more aggressive clinical course and are treated by more aggressive combination chemotherapy. For clinical reasons, it is particularly important to document DLBCL or large cell transformation in patients with a history of low-grade lymphoma.

We analyzed the percentage of large cells in various subtypes of NHL using combined FNA CM examination and FNA FC analysis. For all cases, CM and FC data and subsequent surgical biopsy specimens were available from the same lesions. Our goal was to correlate the CM and FC findings with the histologic diagnosis to evaluate the accuracy of FNA in the diagnosis of large cell lymphoma and large cell transformation.

Materials and Methods

Samples

The cases were obtained by searching the Duke University Medical Center (Durham, NC) pathology database for hematologic-related FNA samples obtained within a 5-year period from July 1996 to June 2001. Cases of reactive lymphoid hyperplasia and Hodgkin lymphoma were excluded from the study. Samples were further selected according to the following criteria: initial FNA with both cytologic preparation and FC studies and subsequent surgical biopsy at the same site with adequate histologic diagnosis before therapy. A total of 48 samples were found to fulfill these criteria.

Cytologic Examination

The FNA procedures were performed using standard methods as previously described. These included both superficial, palpable lesions and those requiring image (ultrasound or computed tomography) guidance. Multiple passes were made depending on the amount of material obtained from each pass. Several cytologic smears were prepared for both Diff-Quik (rapid Romanowsky) and Papanicolaou stains (DAKO, Miami, FL). The remaining material was rinsed in saline for immunophenotypic analysis. Rapid Romanowsky–stained smears were used to quantitate the percentage of large cells. Papanicolaou-stained smears were reviewed only if rapid Romanowsky–stained slides were not adequate for evaluation. Cell counts were performed independently by two of us (J.Z.G. and C.J.), and an average was calculated for each case. A large cell was defined as equal to or greater than 2 times the diameter of a small lymphocyte, with open chromatin and 1 to several nucleoli. An intermediate cell was defined as at least 1.5 but less than 2 times the diameter of a small lymphocyte. The entire slide was reviewed initially under low magnification to determine the best representative areas. A total of 200 cells were counted in 4 or more separate fields. The percentages of the large cells were recorded

FC Analysis

FC analysis was performed as previously described. Three-color staining methods were used for all specimens. Antibodies preconjugated with fluorescein isothiocyanate, phycoerythrin, or peridinin chlorophyll protein were used. The target antigens and monoclonal antibody clones (in parentheses) included CD19 (HIB19), CD20 (L27), CD10 (W8E7), CD23 (EBVCS-5), FMC7, kappa (TB28-2), lambda (1-155-2), CD3 (SK7), and CD5 (L17F12). All antibodies were obtained from BD Biosciences, San Jose, CA. The stained cells were acquired on a bench-top flow cytometer (FACScan or FACScalibur, BD Biosciences) and analyzed by using CellQuest software (BD Biosciences). The antibody combinations and priority to be used in the analysis were as follows: CD19/kappa/lambda, CD19/CD3/CD5, CD19/CD10/CD20, CD19/FMC7/CD23. The neoplastic cells were selected by combination gating strategy with the identification of the CD19 gate and the monoclonal immunoglobulin light-chain gate (either kappa or lambda) or by recognition of a kappa and lambda double-negative population when the neoplastic cells lacked expression of surface immunoglobulin light chain. The percentages of large cells were determined by comparison with the reactive T-cell population and were calculated by using CellQuest software statistics. A small cell population was defined as complete overlap of the neoplastic population with the T-cell population on forward scatter histogram. A large cell population was defined as a complete shift of the neoplastic population from the T-cell population without overlap. If the neoplastic population exhibited a significant shift but substantial overlap with the T-cell population, the cells were defined as intermediate. In addition to the determination of the percentage of large or intermediate cells, the percentages of neoplastic cells in total nucleated cells also were calculated using CellQuest software statistics.
Histologic and Immunohistochemical Analysis

We stained 4- to 5-µm sections of formalin-fixed, paraffin-embedded tissues by H&E for morphologic examination. Immunoperoxidase stains were performed as previously described.23 Briefly, a panel of antibodies that included CD20 (L26, DAKO, Carpinteria, CA), CD79a (DAKO), CD3 (DAKO), CD5 (NCL/Vector, Burlingame, CA), CD23 (NCL/Vector), cyclin D1 (Zymed, San Francisco, CA), and bcl-2 (Zymed) was used. Formalin-fixed, paraffin-embedded tissue sections were air dried and deparaffinized in xylene and alcohol. Primary mouse monoclonal antibodies with appropriate dilutions were applied followed by biotinylated horse antimouse IgG (Vector Laboratories, Burlingame, CA), ABC Elite tertiary complex (Vector), and 3,3’ diaminobenzidine (Sigma Chemical, St Louis, MO). Antigen retrieval was achieved with either a steamer cooker (CD20, CD79a, CD3) or a pressure cooker (CD5, CD23, cyclin D1, and bcl-2). Appropriate negative and positive controls were used in each case.

Results

A total of 48 NHLs were studied, including 41 cases in which a primary diagnosis of lymphoma was made and 7 cases of recurrent lymphoma with an established previous...
Flow cytometry results in representative cases. A, Follicular lymphoma grade II. B, Mantle cell lymphoma. C, Burkitt lymphoma. D and E, Diffuse large B-cell lymphoma. Neoplastic B cells (red events) are cells within both CD19 gate (R1) and monoclonal kappa or lambda gate (R2). Reactive T cells (green events) are gated based on CD19–population (R3). The histograms represent comparison of neoplastic B cells (red line) and reactive T cells (green line). The percentages of large cells are calculated by histogram statistics (M2). Only small numbers of large cells are present in follicular lymphoma grade II and mantle cell lymphoma. Burkitt lymphoma cells are intermediate in size. Diffuse large B-cell lymphomas have greater than 40% large cells, and the lymphoma cells may show different patterns in CD19–side scatter plots (D and E).
The biopsy sites included 33 superficial locations (cervical, supraclavicular, axillary, and inguinal) and 15 deep locations (retroperitoneal and mediastinal). The histologic diagnoses were made on the surgical biopsy specimens based on the Revised European-American classification of lymphoid neoplasms and the new World Health Organization classification and were categorized by cell size.

Table 1
Fine-Needle Aspiration Cytologic and Flow Cytometric Diagnosis in Non-Hodgkin Lymphoma (NHL)*

<table>
<thead>
<tr>
<th>Histologic Diagnosis</th>
<th>Flow Cytometry</th>
<th>Cytomorphology</th>
<th>Neoplastic Cells in Total Analyzed Events</th>
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<tr>
<td>Small cell NHL</td>
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<tr>
<td>Follicular lymphoma grade I (n = 5)</td>
<td>7.7 (6.3-9.1)</td>
<td>13.0 (7.0-20.0)</td>
<td>55.5 (36.0-73.2)</td>
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<tr>
<td>Follicular lymphoma grade II (n = 7)</td>
<td>11.0 (2.6-19.9)</td>
<td>16.6 (4.0-33.0)</td>
<td>44.7 (15.9-68.6)</td>
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<tr>
<td>Other small cell lymphoma (n = 4)</td>
<td>7.8 (4.2-11.9)</td>
<td>5.1 (3.5-7.5)</td>
<td>38.9 (23.4-77.6)</td>
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<tr>
<td>Intermediate cell NHL</td>
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<tr>
<td>Burkitt lymphoma (n = 4)</td>
<td>95.3 (91.1-97.6)</td>
<td>93.5 (90.0-96.0)</td>
<td>64.3 (40.9-84.7)</td>
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<td>NHL with variable numbers of large cells</td>
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<tr>
<td>Follicular lymphoma grade III (n = 3)</td>
<td>38.2 (11.2-52.7)</td>
<td>56.7 (42.5-71.0)</td>
<td>40.4 (25.2-62.6)</td>
</tr>
<tr>
<td>Low-grade NHL with partial large cell transformation (n = 4)</td>
<td>46.4 (17.9-79.5)</td>
<td>34.1 (15.5-60.0)</td>
<td>30.5 (11.5-81.5)</td>
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<tr>
<td>Large cell NHL</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Diffuse large B-cell lymphoma (n = 9)</td>
<td>66.6 (41.9-96.4)</td>
<td>55.4 (16.5-84.5)</td>
<td>32.3 (11.1-64.9)</td>
</tr>
<tr>
<td>NHL with noncontributory fine-needle aspiration results</td>
<td></td>
<td></td>
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<tr>
<td>NHL with necrosis (n = 2)</td>
<td>26.5 (6.7-46.2)</td>
<td>30.0 (10.0-50.0)</td>
<td>0.3 (0.3-0.3)</td>
</tr>
<tr>
<td>Diffuse large B-cell lymphoma with &lt;10% neoplastic cells (n = 9)</td>
<td>37.0 (10.3-73.0)</td>
<td>37.6 (70.82.0)</td>
<td>5.5 (3.1-8.6)</td>
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<tr>
<td>NHL with no detectable monoclonal population (n = 1)</td>
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* Data are given as mean percentage (range).
† Histologic diagnosis was based on subsequent surgical biopsy.
‡ Percentage of intermediate cells in Burkitt lymphoma.
§ Marginal zone lymphoma, 1 case; lymphoplasmacytoid lymphoma, 1 case; mantle cell lymphoma, 1 case; small cell lymphoma, unclassified, 1 case.
|| Follicular lymphoma grade I, 2 cases; follicular lymphoma grade II, 1 case; mucosa-associated lymphoid tissue lymphoma, 1 case.

Table 2
Summary of Fine-Needle Aspiration Cytologic and Flow Cytometric Findings in the Diagnosis of Non-Hodgkin Lymphoma (NHL)*

<table>
<thead>
<tr>
<th>Lymphoma Category</th>
<th>Flow Cytometry</th>
<th>Cytomorphology</th>
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<tbody>
<tr>
<td></td>
<td>&lt;20%</td>
<td>20%-40%</td>
</tr>
<tr>
<td>Small cell NHL (n = 16)</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Intermediate cell NHL (n = 4)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NHL with variable numbers of large cells (n = 7)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Diffuse large B-cell lymphoma (n = 9)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Data are given as number of cases.
† Percentage of intermediate cells in Burkitt lymphoma.
‡ Follicular lymphoma grade I and grade II, small lymphocytic lymphoma, marginal zone lymphoma, mantle cell lymphoma, lymphoplasmacytoid lymphoma.
§ Burkitt lymphoma.
∥ Follicular lymphoma grade III, low-grade lymphoma with partial large cell transformation.

The grading of follicular lymphoma was based on the criteria defined by Mann and Berard. The category of low-grade lymphoma with partial large cell transformation included 4 small cell low-grade lymphomas (2 grade I follicular lymphomas, 1 grade II follicular lymphoma, and 1 mucosa-associated lymphoid tissue [MALT] lymphoma) with large cell transformation identified only in focal areas of the lymphomas.

The FC and CM results are summarized in Table 1 and Table 2. Based on flow cytometric analysis of the large cells, substantial differences were observed between small cell NHLs and DLBCLs. All small cell NHLs showed fewer than 20% large cells, ranging from 2.6% to 19.9% with an average of 9.2%. All cases with a histologic diagnosis of DLBCL contained more than 40% large cells, ranging from 41.9% to 96.4%, with an average of 66.6%. Grade III follicular lymphoma and low-grade lymphoma with partial large cell transformation showed wider ranges, containing variable numbers of large cells (11.2% to 52.7%, and 17.9% to 79.5%, respectively). Using a threshold value of greater than 40% large cells for DLBCL and fewer than 20% large cells for small cell NHL, the sensitivity of diagnosing large cell lymphoma and small cell NHL reached 100% by FC analysis.

The FNA CM examination showed an average of 12.6% large cells in reviewed cases of small cell NHL, with a range from 3.5% to 33.0%. All small cell NHLs had fewer than
40% large cells, with 3 cases (19%) containing a percentage of large cells between 20% and 40%. In DLBCL, 6 (67%) of 9 cases showed more than 40% large cells, and 8 (89%) showed 20% or more large cells. With greater than 40% large cells as the threshold value, the sensitivity of diagnosing large cell lymphoma by CM examination reached 100%. However, with fewer than 20% large cells as the threshold value in the diagnosis of small cell NHL, the sensitivity was 81%.

Based on the cell size analysis by CM and FC analyses, a small number of lymphoma (4 [8%] of 48 cases) showed a high-percentage, monomorphic population of intermediate-sized cells. All 4 cases were confirmed as Burkitt lymphoma by subsequent surgical biopsies. Both FC and CM analyses were highly sensitive in delineating cell size in Burkitt lymphoma, in addition to its characteristic cytologic features observed in CM examination. We found an average of 95.3% intermediate-sized cells (range, 91.1%-97.6%) by FC analysis and an average of 93.5% intermediate-sized cells (range, 90.0%-96.0%) by CM examination.

Grade III follicular lymphoma and low-grade lymphoma with partial large cell transformation were analyzed in a separate category. These entities have relatively wider ranges in the percentages of large cells. Based on the Mann and Berard criteria, grade III follicular lymphomas may have 15 large cells per high-power field up to 100% large cells in the neoplastic follicles. Low-grade lymphoma with partial large cell transformation may have variable proportions of the tumor showing large cell transformation. By CM and FC analyses, our results showed wide ranges of large cells in both entities. In grade III follicular lymphoma, the percentages of large cells were 11.2% to 52.7% by FC analysis and 42.5% to 71.0% by CM examination. In low-grade lymphoma with partial large cell transformation, 17.9% to 79.5% large cells and 15.5% to 60.0% large cells were detected by FC analysis and CM examination, respectively.

Several categories showed noncontributory results, which included NHL with necrosis, large cell lymphoma with fewer than 10% neoplastic cells, and NHL with no detectable monoclonal or abnormal populations. Two cases of NHL with necrosis had very few neoplastic cells detected by FC analysis (<1%), in addition to necrotic debris seen in CM examination. Of the DLBCLs, 50% (9 cases) had fewer than 10% of neoplastic cells detected by FC analysis. In this group, highly variable numbers of large cells were observed by both CM and FC analyses. In addition, some of these cases showed either undetectable monoclonal populations or were FNA specimens containing insufficient material. A single case of MALT lymphoma also showed an undetectable monoclonal population.

**Discussion**

Cell size is one of the most important criteria in the diagnosis and classification of NHL. Several types of cell nuclei, including histiocytes, endothelial cells, reactive small lymphocytes, and RBCs, are commonly used as standards in the assessment of lymphoma cell size. In the evaluation of hematologic disorders by FNA, it is customary to use small reactive lymphocyte nuclei as a standard, mainly because small lymphocytes are relatively consistent in their nuclear size and are almost universally present in the FNA specimens. DLBCLs have predominantly large neoplastic cells, which are defined as 2 times or more the diameter of a small lymphocyte and often contain nucleoli, whereas small cell–type NHLs have predominantly small cells with nuclei similar to or slightly larger than a small reactive lymphocyte. Burkitt lymphoma cells are intermediate in size, and their nuclei average 1.5 times the diameter of a small lymphocyte.

Evaluation of lymphoma cell size by FNA has been attempted by several authors. In these studies, cell size was analyzed mainly by CM examination. Chernoff et al initially used the transformed lymphocyte count to determine the number of large cells. Young et al initially used the transformed lymphocyte count to determine the number of large cells. In their series of 58 FNA specimens, they found that low-grade or indolent lymphoma contained 10% or fewer transformed cells, while aggressive lymphoma had 20% or more transformed cells. They further expanded their study using p53 and proliferating cell nuclear antigen expression in FNA materials by immunoperoxidase staining methods. They found the transformed lymphocyte count was still the best parameter for differentiating large cell lymphoma from indolent small B-cell lymphoma. Dong et al attempted to use FC analysis to determine cell size in 34 cases of DLBCL, in addition to CM examination. They found an increase of sensitivity from initial clinical diagnosis of 65% to 95% after review. However, not all of their cases were confirmed by histologic examination, and the criteria for diagnosis by FC analysis were not discussed in detail.

Evaluation of large cells based on CM features alone has several disadvantages. First, large cells are more fragile than small cells. Cytologic smear preparation may crush the large cells, especially in air-dried preparations, leaving
many naked nuclei that hamper appropriate morphologic examination. Second, in addition to neoplastic cells, the sample may contain both small and large reactive lymphocytes. Merely counting the number of large cells may underestimate or overestimate the number of large cells in the neoplastic population. Third, many of the transformed lymphomas may have variable numbers of cells in the process of transformation. These cells are often intermediate in size. Evaluation of these cells by CM examination can be problematic. These disadvantages can be largely overcome by the concomitant use of FC analysis. In FC analysis, cells are processed in suspension and are less influenced by mechanical factors. The coexisting nonneoplastic lymphocytes can be eliminated by gating and analyzing the mononuclear or abnormal populations. The cell size is more accurately determined by using a forward scatter histogram. Last, FC analysis has the ability to analyze a large number of cells, facilitating more accurate interpretation. However, it also is recognized that large cells often are underrepresented in FC analysis owing to low viability, and a substantial proportion of large B-cell lymphomas may have false-negative or nondiagnostic FC results.22,25

We attempted to use a combined CM and FC method to evaluate large cells in NHL. All cases in the present study had cytologic preparations, adequate flow cytometric analysis of the same specimen, and subsequent surgical biopsy at the same site. Our data showed that only DLBCLs had more than 40% large cells by both CM and FC methods and that none of the small cell NHLs showed more than 40% large cells. Hence, we suggest using a minimum large cell percentage of 40% in the diagnosis of DLBCL. In the assessment of small cell NHLs, FC analysis seemed to be more sensitive than CM examination. All small cell NHLs had 20% or fewer large cells by FC analysis, whereas by CM examination, 13 (81%) of 16 small cell NHLs showed fewer than 20% large cells. Based on these results, we believe that fewer than 20% large cells are required to exclude DLBCL using both FNA CM and FC analyses. Compared with the study by Young et al,20 our study showed a slightly higher percentage of large cells in the diagnosis of both large cell lymphoma and small cell NHL. This is perhaps because of the different methods and classification systems used in the studies. Nevertheless, while none of the small cell NHLs in our study showed between 20% and 40% large cells by FC analysis, 3 cases (19%) of the small cell NHLs had large cell counts between 20% and 40% by CM examination. We believe interpretation should be cautious in this range, and further study may be necessary to confirm the diagnosis.

Burkitt lymphoma is a rapidly proliferating, highly aggressive malignant lymphoma and requires early therapeutic intervention. We found cell size determination by CM and FC analyses was useful in the diagnosis of Burkitt lymphoma. In addition to the characteristic morphologic features, both CM and FC analyses disclosed more than 90% intermediate-sized cells in all cases studied. However, owing to the small number of cases (4 cases) included in this series, evaluation of larger numbers of cases is necessary to confirm these findings.

FNA evaluation of grade III follicular lymphoma and low-grade lymphoma with partial large cell transformation is less reliable. In these 2 categories, there were variable numbers of large cells (ranging from 11.2% to 79.5% by FC analysis and 15.5% to 71.0% by CM examination). Approximately one third of the cases showed fewer than 40% large cells. These results correlated with the histologic findings in which wide ranges of large cells were observed. Similar issues were addressed by Dong and colleagues,13 who found it difficult to distinguish some of the DLBCL cases from grade III follicular lymphomas by FNA. The presence of these categories complicated the issue of FNA evaluation of large cells. Since follicular large cell lymphoma and low-grade lymphoma with partial large cell transformation often are treated in a manner similar to DLBCL, the main clinical issue is underdiagnosis when smaller numbers of large cells are detected by FNA. In our series, 57% (4/7) of these cases showed clinical signs of transformation, which included persistent and progressive lymphadenopathy, rapid enlargement of lymph nodes, and/or widely spread disease with clinical symptoms. In these situations, an increased percentage of large cells is highly suspected and surgical biopsy is necessary for more accurate assessment.

The presence of necrosis or a small number (<10%) of neoplastic cells is an indication for surgical biopsy. In our series, 2 cases of lymphoma containing necrosis showed very few neoplastic cells (<1%) by FC analysis and required surgical biopsy for final diagnosis. This finding is in agreement with the findings of Duphly and Ramos26 in a study of 73 FNA cases. They found that necrosis or increased numbers of polymorphonuclear leukocytes were indications for tissue biopsy. Similarly, finding an insufficient number of neoplastic cells is an indication for additional study. Half of the DLBCL cases in our series showed fewer than 10% neoplastic cells by FC analysis and contained variable numbers of large cells (10.3%-73.0% by FC analysis and 7.0%-82% by CM examination). Three cases had undetectable monoclonal or abnormal populations by FC analysis, and 2 cases had inadequate cytologic smears owing to scant cellularity. An increase of inadequate samples in DLBCL also was observed by other authors. Meda and colleagues11 found that most of their unsuccessful cases were due to inadequate material or lack of monoclonal populations for FC study. Zander et al25 found low viability in the aggressive B-cell lymphoma analyzed by FC analysis.
Bertram et al\textsuperscript{22} reported an increase of “complex” and falsely negative flow cytometric results in 63 DLBCLs, many being FNA samples. The morphologic features of necrosis, apoptosis, or sclerosis did not correlate with the FC patterns.\textsuperscript{22} In our series, we also found low viability, lack of monoclonal populations or abnormal populations, and/or scant cellularity in cytocologic preparations in some of our inconclusive cases of DLBCL. The remaining inconclusive cases contained variable numbers of large cells. By using flow cytometry, we found that low numbers of neoplastic cells (<10\%) often were present in these cases. Of note is that in our series, the high percentage of inadequate DLBCL specimens may be overrepresented, as only the FNA cases with subsequent surgical biopsy specimens were selected, and this group might represent an increased number of inadequate FNA specimens.

Although we conclude that a combined CM and FC approach is the preferred method in the evaluation of large cells by FNA, either CM examination or FC analysis may stand on its own in the diagnosis of large cell lymphoma. More investigation is necessary to determine the percentage of large cells needed to optimize the sensitivity of the diagnosis. Currently, using CM examination alone, the criteria in the diagnosis of large cell lymphoma were either poorly defined or inconsistent.\textsuperscript{13,18-21} Likewise, additional study is necessary to more precisely define the criteria for using FC analysis as the independent method in the diagnosis of large cell lymphoma. In the present study, we found that CM and FC analyses are complementary, and a combined approach is particularly important since both methods have their advantages and shortcomings. FC analysis is particularly useful for identifying FNA specimens containing low numbers of neoplastic cells, when counting large cells by CM examination can be misleading in these specimens. Likewise, CM examination has the advantage of correlation of cytocologic morphologic features with the cell count. We are currently examining the addition of other modalities, such as core needle biopsy, to further improve the accuracy of lymphoma diagnosis by FNA.

We found that FNA FC analysis in combination with FNA CM examination is a useful method in the assessment of large cells in NHL for initial diagnosis and for follow-up of recurrent lymphoma. If the percentage of large cells is 40\% or greater by both FC analysis and CM examination, then DLBCL or large cell transformation is likely, and surgical biopsy may not be necessary in these cases. However, a surgical biopsy is warranted when fewer than 10\% neoplastic cells are detected by FC analysis or when a large amount of necrotic debris is present. In these cases, we believe FNA alone is not adequate for diagnosis. Likewise, surgical biopsy is recommended when a predominance of small cells is detected by CM examination or FC analysis while clinical signs of transformation are present, such as in grade III follicular lymphoma or low-grade lymphoma with partial large cell transformation.

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\textbf{References}


