

Diagnosis and Subclassification of Primary and Recurrent Lymphoma

The Usefulness and Limitations of Combined Fine-Needle Aspiration Cytomorphology and Flow Cytometry

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Abstract

The primary diagnosis of non-Hodgkin lymphoma/leukemia by fine-needle aspiration (FNA) is still controversial and relatively underused. We evaluated our FNA experience with lymphomas using the revised European-American classification of lymphoid neoplasms to determine the reliability of FNA when combined with flow cytometry in the diagnosis of lymphoma, the types of diagnoses made, and the limitations of this technique. Slides and reports from all lymph node and extranodal FNAs performed during the period January 1, 1993, to December 31, 1998, with a diagnosis of lymphoma or benign lymphoid process were reviewed. There were 290 aspirates from 275 patients. These included 158 cases of lymphoma, of which 86 (54.4%) were primary and 72 (45.6%) were recurrent. There were 44 aspirates suggestive of lymphoma and 81 benign/reactive diagnoses. With diagnoses suggestive of lymphoma considered as positive for lymphoma, levels of diagnostic sensitivity and specificity were 95% and 85%, respectively. Specificity was 100% when only definitive diagnoses of lymphoma were considered. Clearly, FNA and immunophenotyping by flow cytometry are complementary and obviate a more invasive open biopsy for many patients with lymphadenopathy.

The primary diagnosis of non-Hodgkin lymphoma (NHL) by fine-needle aspiration (FNA) is still controversial and relatively underused.¹ Some authorities (clinicians and pathologists) believe this approach should almost never be used, whereas others believe aspiration cytology can diagnose many lymphomas.²⁻⁸ Reasons for this situation include inadequate exposure during pathology residency training to the benefits and limitations of FNA, lack of confidence by oncologists in accepting an FNA diagnosis of a hematopoietic malignant neoplasm, opposition from some hematopathologists about the reliability of such diagnoses, distinguishing between low-grade lymphomas and reactive processes that have cytomorphologic overlap, and, not least of all, the constantly changing lymphoma classification systems that require knowledge of morphologic features and immunophenotyping techniques.^{9,10} Many pathologists are secure diagnosing only recurrences or the most obvious cases of lymphoma by FNA.

Several publications document the important role of FNA in the diagnosis of NHL, particularly when combined with immunophenotyping by flow cytometry (FC).¹¹⁻¹⁵ In this study, we present our FNA experience with lymphomas using the revised European-American classification of lymphoid neoplasms (REAL)⁸ to determine the reliability of FNA in the diagnosis of lymphoma, the types of diagnoses made, and the limitations of this technique. We believe that aspiration cytomorphology and flow cytometry are diagnostically complementary.

Materials and Methods

Records for the period January 1, 1993, to December 31, 1998, were reviewed to find all FNAs with concurrent FC of lymph nodes and extranodal sites that were performed for possible lymphoma, as well as those that revealed an unsuspected lymphoma or lymphoid proliferation. Aspirates of lymph nodes diagnostic of metastatic carcinoma or leukemia were excluded from the study. Slides of cases from 1993 to early 1995 that were diagnosed before the implementation of the REAL classification system were rescreened and recategorized according to this new classification system.

In many instances, pathologists performed the aspiration. In others, cytotechnologists and pathologists assisted the clinicians on site during performance of the FNAs to prepare cytologic smears, determine specimen adequacy, and collect material for FC. Two to 4 needle passes provided adequate material in most cases.

The specimens were obtained by computed tomography guidance or by standard techniques if a palpable lesion was present. A drop of aspirate was expressed onto a glass slide and smeared with another glass slide. Half the slides were air dried and stained using a rapid Romanowsky stain; the other half were alcohol-fixed and stained using a modified rapid Papanicolaou stain. Slides were viewed by a cytopathologist and triaged for special studies depending on the cytomorphologic features. FC was not performed on cases with classic Reed-Sternberg cells seen in an appropriate background; rather, immunocytochemistry was performed on cytocentrifuged specimens or cell blocks.

During the 6-year period of the study, FNA specimens were evaluated and diagnosed by 7 cytopathologists with experience ranging from 1 to 20 years. Consultation among the group was standard practice in problem cases.

A rapid-Romanowsky smear of the flow cytometric material was prepared and reviewed, and the number of cells available for analysis was determined before selecting an appropriate antibody panel. The specimens for FC were analyzed for various antigens using a Cytoron-Absolute flow cytometer (Ortho-Diagnostic Systems, Raritan, NJ) from 1993 until 1997 and a Coulter Epics XL (Coulter Electronics, Hialeah, FL) during 1997 and 1998, using standard techniques and the following commercially available monoclonal antibodies: CD3, CD4, CD8, CD16, CD19, HLA-DR (Ortho-Diagnostic Systems), CD2, CD5, CD10, CD11c, CD19, CD20, CD45, kappa, lambda (Dako, Carpinteria, CA), CD23, CD38 (Coulter Clone, Coulter Immunology, Hialeah, FL), CD7, CD15, CD34, CD45 (Becton Dickinson Immunocytometry Systems, San Jose, CA).

A policy was set that each tube used for monoclonal antibody labeling required at least 100,000 cells per milliliter. A

full study required 7 tubes and at least 700,000 cells to run all the appropriate antibodies. If 700,000 cells per milliliter were not available, tubes were set up in the following order of preference: CD5/20, CD19/kappa/lambda (2 tubes or 1 tube with 3 colors), CD10/CD7, CD11c/CD23, CD4/CD8, and CD2/HLA-DR.

To minimize nonspecific binding, a viability marker, 7-amino-actinomycin D (7-AAD), was used (it was used on most samples after July 1995). One milligram of stock powdered 7-AAD was reconstituted in 500 mL of dimethyl sulfoxide. Ten milliliters of this solution was mixed with 390 mL of standard phosphate-buffered saline to give a final concentration of 25 µg/µL of 7-AAD. This working solution then was added to the cells containing the monoclonal antibodies. The samples were placed on the flow cytometer, and data from the far red (640-nm high-pass filter) spectrum were collected and used for gating purposes. Cells with positive 7-AAD expression and low forward scatter properties were excluded from analysis.

After examination of the forward and side scatter, cells within the lymphocyte gate were examined, excluding the granulocytes and monocytes. However, if the lymphocytes were morphologically mixed, small and large, back gating on the 2 sizes was used and each gate examined separately. Kappa or lambda chain monoclonality was diagnosed when an abnormal lymphocyte population expressed a substantial predominance of either light chain.

Three hematopathologists evaluated and interpreted the FC results during the 6-year period of the study. Two hematopathologists had 20 or more years of morphologic hematopathology experience and had at least 6 years experience in evaluating FC results; the other had recently completed a fellowship in diagnostic FC. Consultation among the 3 was commonplace in problem cases.

Results

Two hundred ninety FNA specimens from nodal and extranodal sites in 275 patients were submitted for flow cytometric immunophenotyping. Of the 290 total aspirates, 158 were diagnosed as lymphoma, 44 as suggestive of lymphoma, and 81 as benign/reactive **Table 1**. None of the extranodal FNAs involved samples from the bone marrow. In 7 cases (2.4%), the aspirated cytomorphologic material was inadequate for evaluation. Failure to obtain cytologically diagnostic material was attributed to necrosis, peripheral blood contamination, and absence of lymphoid tissue.

Of the 158 cases of lymphoma, 155 were non-Hodgkin lymphoma and 3 were Hodgkin lymphoma (HL) **Table 2**. Eighty-six (54.4%) were primary, and 72 (45.6%) were recurrent. Histologic confirmation of the FNA diagnosis was

Table 1
Diagnosis of 290 Fine-Needle Aspiration Specimens by Combined Cytomorphologic and Flow Cytometric Results

	No. of Aspirates	Confirmatory Tissue Biopsy
Lymphoma		
Previous diagnosis of lymphoma		
Nodal	40	8
Extranodal*	32	13
Initial diagnosis of lymphoma		
Nodal	18	8
Extranodal*	68	23
Suggestive of lymphoma		
Nodal	16	12
Extranodal*	28	20
Benign/Reactive		
Nodal	48	15
Extranodal*	33	11
Inadequate		
Nodal	3	—
Extranodal*	4	—
Total	290	110

* Orbital, neck, submandibular, retroperitoneal, lung, mediastinal, lumbar, and hypogastric midline masses.

available for 52 cases, of which 32 represented sampling from the same anatomic site. Twenty biopsy specimens were from different anatomic sites, including 9 cases with bone marrow involvement by documented tissue biopsy. The results of the cytologic findings together with the flow cytometric immunophenotyping on the aspirates diagnosed as B-cell lymphoma are described in the following sections **Table 3**.

Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

Tissue biopsy was performed in 4 of 18 cases of chronic lymphocytic leukemia/small lymphocytic lymphoma. Of the 4 cases, 2 were confirmed as small lymphocytic lymphoma, 1 as chronic lymphocytic leukemia in transformation to prolymphocytic leukemia, and 1 as chronic lymphocytic leukemia in transformation to Richter syndrome.

On aspiration smears, 15 of the cases displayed a uniform population of lymphocytes that were the same size or slightly larger than a normal lymphocyte, with clumped chromatin, a round nucleus, and an occasional nucleolus **Image 1**. Small numbers of admixed large lymphocytes with round nuclei, single distinct nucleoli, and abundant cytoplasm were present in some cases. In 1 case with plasmacytoid features, the predominant cells were small lymphocytes and plasmacytoid lymphocytes with occasional immunoblasts and follicular center cells.

In the case of chronic lymphocytic leukemia in transformation to prolymphocytic leukemia, the predominant cells were small lymphocytes; approximately 15% to 20% of the remaining cells were intermediate-sized with round

nuclei, moderately clumped chromatin, a single prominent nucleolus, and a moderate amount of pale cytoplasm.

The 1 case of chronic lymphocytic leukemia in transformation to Richter syndrome contained a number of large blastic cells with vesicular chromatin, prominent nucleoli, basophilic cytoplasm, or, in a minority of the cells, scant pale cytoplasm.

All 18 cases expressed monotypic staining with kappa (5 cases) or lambda (13 cases) light chains. The B-cell-associated antigens CD19 and CD20 were coexpressed with CD5 in all 18 cases. The B-cell marker CD23 was added to our institution’s antibody panel in 1997; therefore, only the 3 most recent cases of chronic lymphocytic leukemia were evaluated. All 3 cases expressed CD23. CD20 expression was weaker than CD19 expression in all cases except the case of transformation to Richter syndrome.

Follicle Center Lymphoma

There were 66 cases of follicle center lymphoma. Tissue biopsy was performed in 21 cases. Seven were confirmed as follicle center lymphoma, grade I, 5 as follicle center lymphoma, grade II, 5 as follicle center lymphoma, grade III, 1 as bone marrow involvement by follicle center lymphoma, and 2 as bone marrow involvement by NHL (type not specified); and 1 tissue biopsy specimen was signed out as involvement by lymphoma/leukemia (type not specified).

The aspirates in grade I cases were composed predominantly of small cleaved cells **Image 2**. In some cases, the cells were very small and resembled small lymphocytes. Rare large noncleaved cells also were present. When the number of large cells per high-power field approached 20% to 50%, the cases were classified as grade II (mixed small cleaved and large cell) **Image 3**, whereas grade III (large cell) was diagnosed when the large cells constituted more than 50% of the cells per high-power field **Image 4**. The aspirates in grade III were composed of a variable mixture of large noncleaved cells, large cleaved cells, and occasional immunoblasts.

Flow cytometric immunophenotyping was performed in 64 of 66 cases. Two cases were unsatisfactory for interpretation because of peripheral blood contamination or poor viability. Of the 64 cases, 62 showed restricted light-chain expression (33 with kappa and 29 with lambda). In 1 case, surface immunoglobulin was not expressed. CD10 was expressed in 61 cases; 3 were negative. One case was interpreted as no evidence of lymphoma. Grading was performed prospectively on cytology specimens.

Mantle Cell Lymphoma

There were 9 cases of mantle cell lymphoma, of which 3 had histologic confirmation. Two cases were confirmed

Table 2
Diagnosis of 158 Malignant Lymphomas by Combined Cytomorphologic and Flow Cytometric Results

Diagnosis	No. of Lymphomas	Tissue Biopsy Before Treatment
B-cell lymphoma		
Chronic lymphocytic leukemia/small lymphocytic lymphoma	18	4
Follicle center lymphoma		
Grade I (small cleaved)	33	8
Grade II (mixed small cleaved and large cell)	15	7
Grade III (large cell)	18	6
Mantle cell lymphoma	9	3
Marginal zone B-cell lymphoma (mucosa-associated lymphoid tissue)	5	1
Large B-cell lymphoma	35	8
High-grade B-cell, Burkitt-like lymphoma	1	
T-cell lymphoma		
Precursor T-lymphoblastic lymphoma/leukemia	1	1
Peripheral T-cell lymphoma, unspecified	4	2
Anaplastic large cell lymphoma	1	1
Miscellaneous		
Non-Hodgkin lymphoma, unspecified	15	8
Hodgkin lymphoma	3	3
Total	158	52

Table 3
Results of Flow Cytometric Immunophenotyping in Fine-Needle Aspirates of B-Cell Lymphomas*

	CLL/SLL (n = 18)	FCL (n = 66)	MCL (n = 9)	MZL (n = 5)	LBCL (n = 35)	BLL (n = 1)
Restricted light chain expression	18/18 (100)	62/64 (97)	9/9 (100)	5/5 (100)	7/24 (29) [†]	1/1 (100)
Kappa	5/18 (28)	33/62 (53)	6/9 (67)	2/5 (40)	3/7 (43)	1/1 (100)
Lambda	13/18 (72)	29/62 (47)	3/9 (33)	3/5 (60)	4/7 (57)	
CD19/20	18/18 (100)	64/64 (100)	9/9 (100)	5/5 (100)	24/24 (100)	1/1 (100)
CD23	3/3 (100) [‡]	0/64 (0)	0/8 (0)	0/5 (0)		
CD5	18/18 (100)	0/64 (0)	9/9 (100)	1/5 (20)	1/3 (33) [§]	0/1 (0)
CD10	0/18 (0)	61/64 (95)	1/9 (11)	0/5 (0)	2/2 (100)	1/1 (100)
Other						
CD20 bright	1					
Negative surface immunoglobulin		1			1	
CD10+			1			
CD5+	1					
No evidence of lymphoma		1			16	
Technical problems						
Poor viability		1			4	1
Dilute sample	1				4	
Limited study			1	1	9	
Insufficient quantity					3	

BLL, Burkitt-like lymphoma; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; FCL, follicle center lymphoma; LBCL, large B-cell lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma.
* Data are given as number positive/number tested (percentage).
† Because 11 cases were deemed inadequate for interpretation, 24 cases were evaluated by flow cytometry.
‡ CD23 was added to our department's antibody panel in 1996; therefore, only the 3 most recent cases were evaluated for CD23 expression.
§ Only 3 cases had sufficient material to evaluate CD5 expression. One of the 3 cases demonstrated CD5 positivity.
|| Only 2 cases had sufficient material to evaluate CD10 expression.

as mantle cell lymphoma, and the third case was diagnosed as recurrent bone marrow involvement by NHL (type not specified). The aspirates were composed of small to medium lymphoid cells, usually slightly larger than normal lymphocytes, with more dispersed chromatin, scant pale cytoplasm, and inconspicuous nucleoli. The nuclei were irregular in several cells **Image 5**.
Flow cytometric immunophenotyping indicated monotypic staining with kappa (6 cases) or lambda (3 cases). The B-cell-associated antigens CD19 and CD20 were

coexpressed with CD5 in all cases. CD23 was negative in all 9 cases. One case was weakly CD10+.
Marginal Zone Lymphoma: Low-Grade B-Cell Lymphoma of Mucosa-Associated Lymphoid Tissue
There were 5 cases of marginal zone lymphoma. A bone marrow biopsy was performed in 1 case and diagnosed as involvement by NHL (type not specified). On aspirated material, the cells were small with slightly irregular nuclei and relatively abundant pale cytoplasm with an admixture of variable

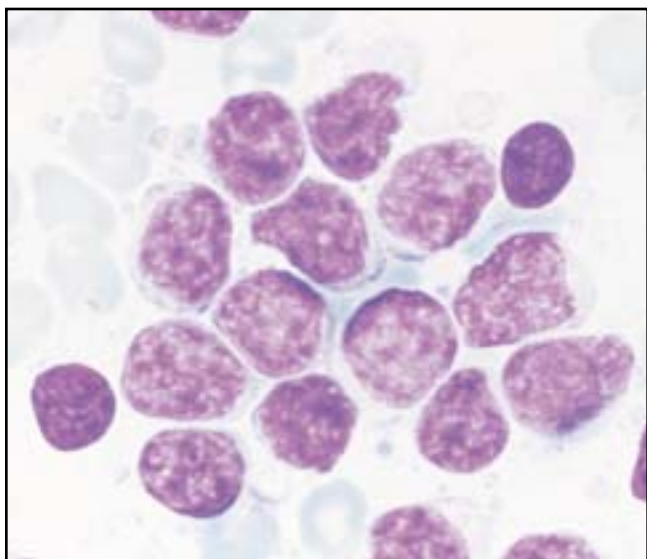


Image 1 Chronic lymphocytic leukemia. A uniform population of lymphocytes slightly larger than a normal lymphocyte, with clumped chromatin, a round nucleus, and an occasional nucleolus (Romanowsky, $\times 1,000$).

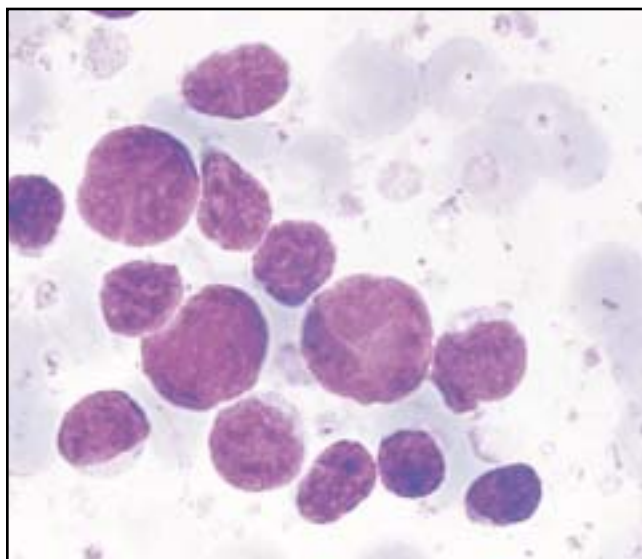


Image 2 Follicle center lymphoma, grade I. A mixed population of small cleaved lymphocytes, some resembling normal-appearing small lymphocytes and rare large noncleaved and cleaved lymphocytes. The number of large lymphocytes constituted less than 20% of the malignant population (Romanowsky, $\times 1,000$).

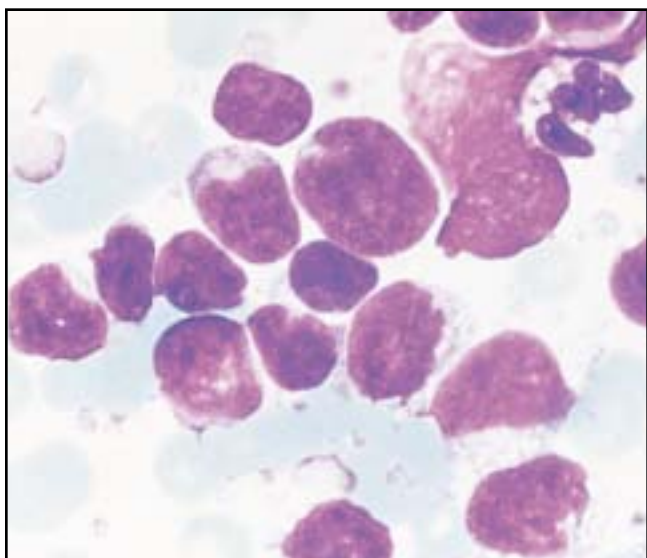


Image 3 Follicle center lymphoma, grade II. A mixed population of small cleaved and large cleaved lymphocytes, each constituting approximately 50% of the malignant population (Romanowsky, $\times 1,000$).

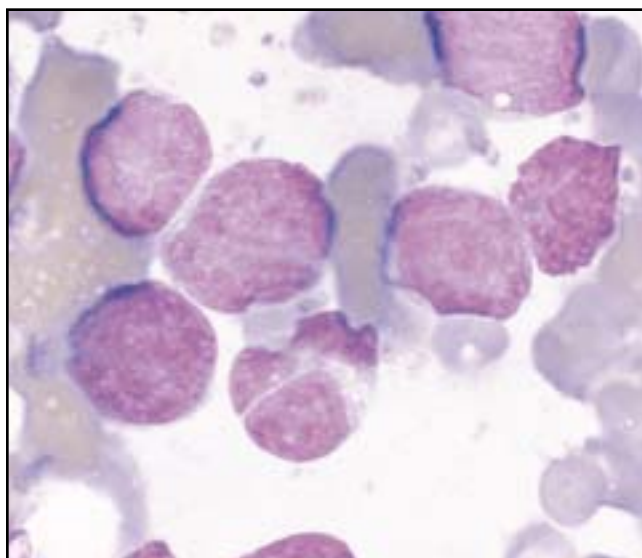


Image 4 Follicle center lymphoma, grade III. A mixed population of large cleaved and noncleaved lymphocytes constituting 100% of the malignant population (Romanowsky, $\times 1,000$).

numbers of monocytoid B cells, small lymphocytes, plasma cells, and an occasional immunoblast **Image 6**.

Flow cytometric immunophenotyping indicated monotypic staining with kappa (2 cases) or lambda (3 cases). The B-cell-associated antigens CD19 and CD20 were not coexpressed with CD23 in all 5 cases. CD5 and CD10 were negative in all but 1 case, which was weakly CD5+.

Large B-Cell Lymphoma

Of 35 cases of large B-cell lymphoma, tissue biopsy was performed in 8 cases. Of the 8 cases, 6 were confirmed as large B-cell lymphoma, 1 as bone marrow examination findings highly suggestive of NHL (type not specified), and 1 as T-cell-rich B-cell lymphoma. On aspiration smears, the majority of the cells were centroblasts with distinct

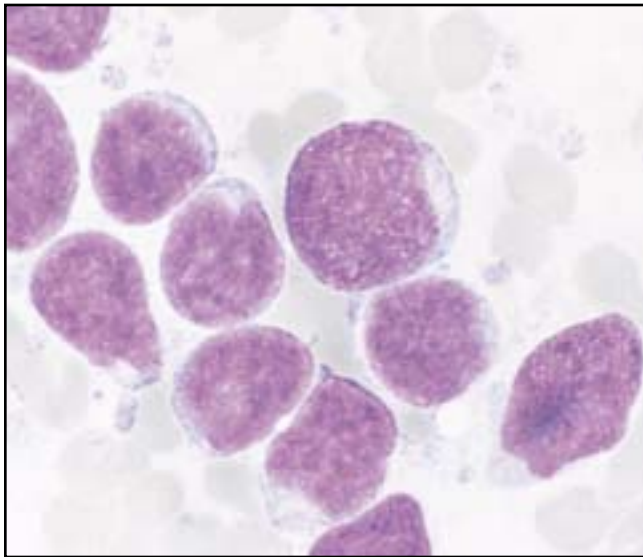


Image 5 Mantle cell lymphoma. A uniform population of medium-sized lymphoid cells, slightly larger than normal lymphocytes, with more dispersed chromatin, scant pale cytoplasm, and inconspicuous nucleoli. The nucleus is irregular in several cells (Romanowsky, $\times 1,000$).

nuclear membranes, vesicular chromatin, prominent nucleoli, and basophilic cytoplasm **Image 7**. Small numbers of centrocytes, immunoblasts, cells with multilobulated nuclei, or a mixture of these cell types also were observed in some specimens.

Flow cytometric immunophenotyping was attempted in all 35 cases, but in 11 cases, the material for FC was inadequate for diagnosis. Failure to obtain diagnostic material was attributed to insufficient quantity (3 cases), poor viability (4 cases), and peripheral blood contamination (4 cases). Of the 24 cases analyzed by FC, 7 expressed monotypic staining with kappa (3 cases) or lambda (4 cases). All 7 with restricted light chain expression, as well as the 1 case with negative surface immunoglobulin expression, retained the B-cell-associated antigens CD19 and CD20. Only 3 cases had sufficient material to evaluate CD5 expression. One of the 3 cases demonstrated CD5 positivity. CD10 positivity was present in the 2 cases that could be evaluated for that marker. In 17 cases, a monotypic population was not demonstrated. A limited study was performed in 9 cases, 6 of which were interpreted as no evidence of lymphoma.

Burkitt-like Lymphoma

One case was diagnosed as high-grade B-cell Burkitt-like lymphoma. On aspirated material, a monomorphic population of medium to large cells with round nuclei containing a coarse granular chromatin pattern, multiple small central nucleoli, and abundant vacuolated basophilic cytoplasm was present. Numerous mitotic figures and macrophages with ingested nuclear debris also were seen **Image 8**. Flow cytometric

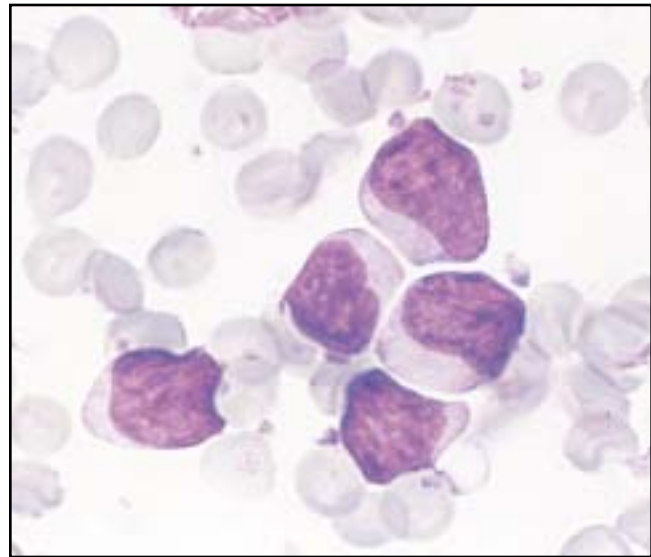


Image 6 Marginal zone lymphoma. A uniform population of medium-sized lymphoid cells, slightly larger than normal lymphocytes, with irregular coffee bean-like nuclei and relatively abundant pale cytoplasm (Romanowsky, $\times 1,000$).

immunophenotyping indicated monotypic staining for kappa and expression of pan-B-cell antigens and CD10.

The results of the cytologic findings together with the flow cytometric immunophenotyping on the aspirates diagnosed as T-cell lymphoma or HL are described in the following sections **Table 4**.

Precursor T-Lymphoblastic Lymphoma/Leukemia

Tissue biopsy confirmed the single case of precursor T-lymphoblastic lymphoma/leukemia. The aspirate was composed of lymphocytes having round to convoluted nuclei, finely dispersed chromatin, inconspicuous nucleoli, scant cytoplasm, and a high mitotic rate. Terminal deoxynucleotidyl transferase staining performed on the cell block was positive.

Flow cytometric immunophenotyping revealed pan-T-cell antigen expression with loss of the CD4 T-helper antigen.

Peripheral T-Cell Lymphoma, Unspecified

Four cases of peripheral T-cell lymphoma were diagnosed. Tissue biopsy was performed in 2 cases, confirming each as a peripheral T-cell lymphoma. Both tissue biopsy specimens consisted of diffuse sheets of predominantly large T cells. On aspirated material, the cells ranged from small to intermediate to large irregular lymphocytes with scattered epithelioid histiocytes **Image 9**. Occasional large hyperchromatic Reed-Sternberg-like cells were present in 1 case with anaplastic features.

Flow cytometric immunophenotyping revealed 1 case with CD4 and CD5 positivity with loss of the CD8 T-suppressor/cytotoxic antigen, 1 case with CD2 positivity and

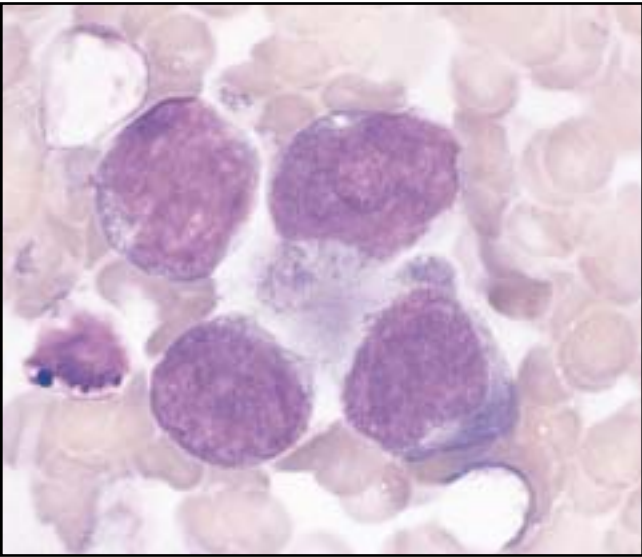


Image 7 Large B-cell lymphoma. A predominant population of large blastic lymphocytes with distinct nuclear membranes, vesicular chromatin, prominent nucleoli, and basophilic cytoplasm. Several lymphoglandular bodies are also present (Romanowsky, ×1,000).

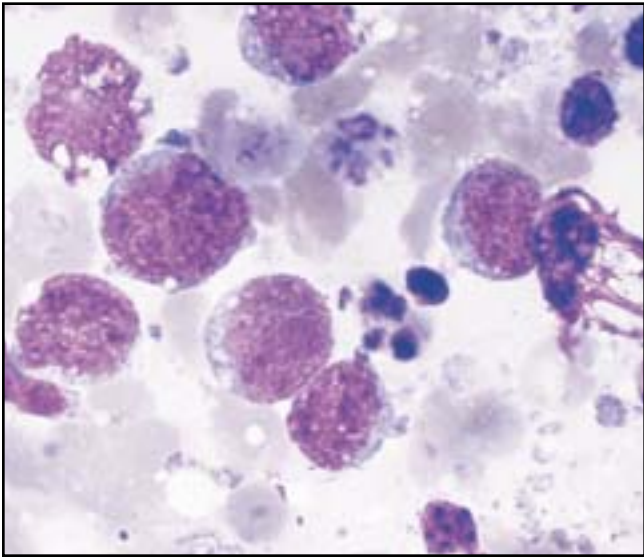


Image 8 Burkitt-like lymphoma. A population of medium-sized cells with smooth nuclear membranes, fine to granular chromatin, conspicuous nuclei, and fine cytoplasmic vacuoles in a dark blue cytoplasm. Numerous mitotic figures, tingible body macrophages, and nuclear debris were seen in other areas (Romanowsky, ×1,000).

Table 4
Results of Flow Cytometric Immunophenotyping in Fine-Needle Aspirates of T-Cell Lymphomas and Hodgkin Lymphoma*

	PTLBL (n = 1)	PTCL (n = 4)	ALCL (n = 1)	HL (n = 3)
CD2	1 (100)	4 (100)	0 (0)	3 (100)
CD3	1 (100)	4 (100)	0 (0)	3 (100)
CD4	0 (0)	3 (75)	1 (100)	3 (100)
CD5	1 (100)	4 (100)	1 (100)	3 (100)
CD7	1 (100)	4 (100)	0 (0)	3 (100)
CD8	1 (100)	2 (50)	0 (0)	3 (100)
CD19/20	0 (0)	2 (50)	0 (0)	3 (100)
Others	—	2†	—	—

ALCL, anaplastic large cell (CD30+) lymphoma; HL, Hodgkin lymphoma; PTCL, peripheral T-cell lymphoma; PTLBL, precursor T-lymphoblastic lymphoma/leukemia.
* Data are given as number (percentage).
† No evidence of lymphoma.

loss of the T-helper and T-suppressor/cytotoxic antigen (CD4–, CD8–), and 2 cases that were interpreted as no evidence of lymphoma.

Anaplastic Large Cell (CD30+) Lymphoma

Tissue biopsy confirmed the single case of anaplastic large cell (CD30+) lymphoma. The aspirate was composed of large bizarre or multinucleated cells with vesicular nuclei, prominent nucleoli, and abundant pale or basophilic cytoplasm **Image 10**. Numerous mitotic figures and macrophages with ingested nuclear debris were present. CD30 (Ki-1) staining performed on the cell block was positive.

Flow cytometric immunophenotyping indicated a CD4+ and CD5+ population with loss of the remaining pan-T-cell antigens and the CD8 T-suppressor/cytotoxic antigen.

Non-Hodgkin Lymphoma, Unspecified

There were 14 cases of NHL, unspecified. Tissue biopsy was performed in 8 cases **Table 5**. The histologic findings included 3 cases of bone marrow involvement by NHL, type not specified, 2 cases of diffuse large B-cell lymphoma, and 1 case each of anaplastic large cell (CD30+) lymphoma, follicle center lymphoma, grade II, and marginal zone lymphoma.

Hodgkin Lymphoma

Three cases were diagnosed as HL. Tissue biopsy confirmed 2 cases as HL, nodular sclerosing type and 1 case as HL, mixed cellularity type. The aspirates contained a variable number of atypical mononuclear Reed-Sternberg variants and classic Reed-Sternberg cells

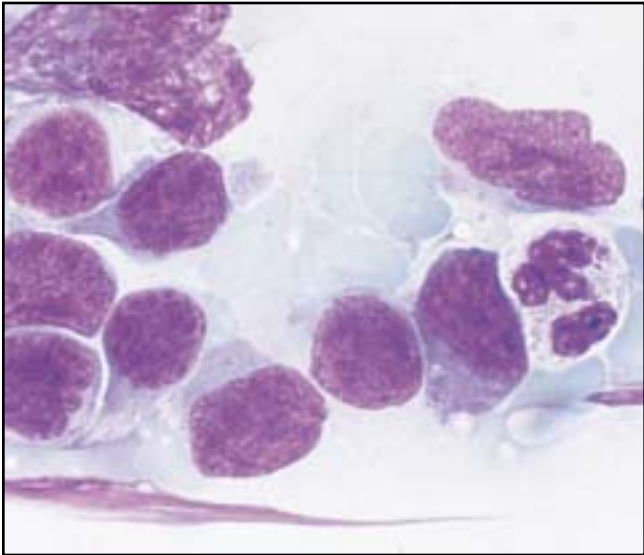


Image 9 Peripheral T-cell lymphoma, unspecified. A mixed population of small to medium-sized lymphoid cells with slightly irregular nuclear contours, coarsely dispersed chromatin, conspicuous nucleoli, and abundant basophilic cytoplasm. Occasional scattered epithelioid histiocytes were also seen (Romanowsky, ×1,000).

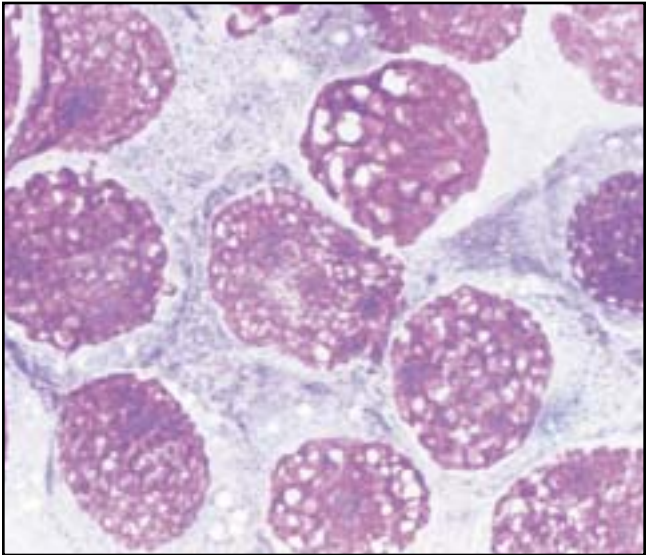


Image 10 Anaplastic large cell (CD30+) lymphoma. A sheet of large bizarre lymphoid cells with vesicular nuclei, prominent nucleoli, and abundant pale or basophilic cytoplasm. Numerous mitotic figures and macrophages with ingested nuclear debris were present in other areas (Romanowsky, ×1,000).

Table 5
Histologic Diagnosis in Non-Hodgkin Lymphoma, Unspecified

Diagnosis	No. of Aspirates
Bone marrow involvement by non-Hodgkin lymphoma	3
Diffuse large B-cell lymphoma	2
Anaplastic large cell (CD30+) lymphoma	1
Follicle center lymphoma, grade II	1
Marginal zone lymphoma	1
Total	8

in a background of small round lymphocytes, plasma cells, and eosinophils.

Flow cytometric immunophenotyping showed a polyclonal B-cell population with a predominance of helper (CD4+) T lymphocytes in all 3 cases.

Aspirates Suggestive of Lymphoma

In 44 cases, the cytomorphology and flow immunophenotyping were discordant or inconclusive, or material for FC was inadequate for a diagnosis of lymphoma **Table 6**. Tissue histology was available for 32 cases.

Benign/Reactive Aspirates

Eighty-one aspirates were interpreted as reactive hyperplasia or lymphadenitis. Tissue confirmation was available for 26 cases **Table 7**.

Discussion

Our experience, which is similar to that of Young et al,¹⁴ indicates that FNA combined with FC is useful in the diagnosis of both primary and recurrent NHL. A definitive diagnosis of NHL was made in 76.7% (158/206) of our lymphoma cases on the basis of combined FNA and FC, including 72.3% (86/119) of the primary lymphomas and 83% (72/87) of the previously diagnosed or recurrent lymphomas. Diagnostic sensitivity, specificity, positive and negative predictive values, and efficiency were calculated on cases with tissue biopsy follow-up (n = 110) and are given in **Table 8**.

A diagnosis of lymphoma based solely on the cytomorphic findings may be quite difficult and sometimes impossible, particularly in the low-grade lymphomas, notably the small cell and mixed cell subgroups. For example, small B-cell lymphocytic lymphoma may show some irregularity of the nuclear membranes, whereas mantle cell lymphoma may consist of round cells that resemble chronic lymphocytic leukemia.⁸ Fortunately, since most low-grade lymphomas are of B-cell lineage, the demonstration of a monotypic B-cell population coupled with appropriate cytomorphic characteristics can be used to diagnose lymphoma.

In our series of diagnoses that were suggestive of lymphoma, many situations occurred in which the cytomorphic findings were worrisome, but FC was not able to meet our criteria for diagnosis. This most commonly occurred when a monotypic surface immunoglobulin could

Table 6
Histologic Diagnosis in Aspirates Suggestive of Lymphoma

Diagnosis	No. of Aspirates	Classification
Diffuse large B-cell lymphoma	11	
Follicle center lymphoma, grade II	7	
Hodgkin lymphoma	3	
Follicle center lymphoma, grade III	2	
Lymphoid follicular hyperplasia	2	False positive
Atypical immune response	1	False positive
Atypical lymphoid hyperplasia	1	False positive
Follicle center lymphoma, grade I	1	
Bone marrow involvement by non-Hodgkin lymphoma	1	
Precursor T-lymphoblastic lymphoma/leukemia	1	
Diffuse large B-cell lymphoma vs high-grade Burkitt-like lymphoma	1	
Suggestive of lymphoma	1	
Total	32	

Table 7
Histologic Diagnosis in Benign/Reactive Aspirates

Diagnosis	No. of Aspirates	Classification
Follicular lymphoid hyperplasia	12	
With progressive transformation of germinal centers	3	
Diffuse large B-cell lymphoma	2	False negative
Atypical follicular lymphoid hyperplasia	1	
Benign lymphoepithelial lesion	1	
Chronic lymphadenitis	1	
Chronic sialadenitis	1	
Fatty lymph node	1	
Follicle center lymphoma, grade I	1	False negative
Necrotizing granulomatous lymphadenitis	1	
Peripheral T-cell lymphoma	1	False negative
Unsatisfactory for diagnosis	1	
Total	26	

Table 8
Sensitivity, Specificity, Positive and Negative Predictive Values, and Efficiency (n = 110)*

Sensitivity	95
Specificity	85/100†
Positive predictive value	95
Negative predictive value	85
Efficiency	93

* Given as percentage.

† Specificity was 100% when only definitive diagnoses of lymphoma were considered.

not be demonstrated (17 cases) or when the specimen for FC had few viable cells, dilute sample, or insufficient material (12 cases). In such situations, a biopsy typically was recommended. Likewise, the clinical staff would be reluctant to treat a patient with a diagnosis that was less than definitive for malignant neoplasm unless the clinical features were compatible with a previously documented malignant lymphoma. Overall, flow cytometry distinguished between a polytypic or monotypic population in 208 cases (71.7%).

Correlating FC results with cytomorphologic findings has been absolutely mandatory in our ability to maximize the use of FNA in the diagnosis of NHL; as a result, close

intradepartmental cooperation between the cytopathologists who view the FNA specimens and the hematopathologists who interpret the flow cytometric results is essential. Often a worrisome FNA specimen will be viewed by the cytopathologist and the hematopathologist; likewise, the hematopathologist always has informed the cytopathologist of the FC results before the cytopathologist provides a final diagnosis. In fact, all interpretations for FNAs of lymphoid lesions with concurrent FC will have a comment section included in the FNA final diagnosis summarizing the results of FC. Based on the combined cytomorphologic characteristics and FC results for the abnormal cells, most categories of the REAL classification system were recognized.

Our experience is similar to others in that FNA with combined FC obviates tissue biopsy for a majority of patients.^{11,14} Of our positive FNA results, 67% of the patients had not undergone a surgical biopsy for tissue confirmation. One may argue that a patient's history of lymphoma or leukemia may influence one's confidence in relying on FC and FNA. However, in our experience, as confidence was gained by the cytopathologists and the hematopathologists in interpreting the FC and FNA, clinicians also began to accept FC and FNA as a reliable means of establishing an initial

diagnosis of NHL. As a result, the number of confirmatory tissue biopsies decreased over time (Figure 1), and clinicians became comfortable treating patients with a diagnosis made by combined FNA and FC. Unfortunately, pitfalls in combined FC and FNA exist. The following sections describe the most frequently encountered problems.

Sampling and Inadequate or Insufficient Material for Flow Cytometry

Sampling error is always a potential problem with FNA and is not limited to the cytodiagnosis of lymphoma. Three or more passes with sampling from several angles in different parts of the lesion or lymph node will substantially reduce sampling error; however, if the cytologic features do not explain the clinical presentation (ie, rapid growth of a lymph node), close follow-up with repeated FNA or surgical biopsy should permit proper diagnosis and timely treatment of the patient.

In our study, inadequate material prevented proper FC studies in 25 cases (8.6%). Failure to obtain adequate material was attributed to tumor necrosis, peripheral blood contamination, or insufficient material. In the latter 2 situations, a repeated FNA may provide the necessary material for an adequate FC panel; however, in cases with extensive tumor necrosis, a repeated FNA is unlikely to be beneficial. Material for FC that is obtained from bone marrow biopsies for staging purposes sometimes can aid in the diagnosis of lymphoma; however, caution is advised when interpreting lymphomatous involvement of the bone marrow. Discordant morphologic findings between lymph node and bone marrow

are not rare, especially in cases of large cell lymphoma. In addition, normal cells within the bone marrow, most notably hematogones, may be mistaken for a lymphoblastic process.

Reactive Lesions

In most cases, a cytomorphologically reactive pattern and polyclonal aspirate suggest a reactive rather than a neoplastic process; however, in our study, 2 cases that were cytomorphologically consistent with a reactive process were revealed by FC to have a clonal excess staining pattern if a kappa/lambda ratio of 3:1 or a lambda/kappa ratio of 2:1 was used. Follow-up biopsy proved both lesions to be follicular hyperplasia. In this small sample, cytomorphologic examination prevailed over immunophenotyping. Samoszuk et al¹⁶ recommend using a kappa/lambda ratio of 5.5:1 and a lambda/kappa ratio of 1.7:1; however, these authors also concluded that when using these ratios, a false-positive rate of 6% and a false-negative rate of 27% occurred.¹⁶ Therefore, specimens with marginal cytologic atypia that show a monotypic staining pattern should be biopsied or closely monitored.

Coexistence of Benign and Neoplastic Lymphoid Cells

A particularly difficult problem is that aspirates of lymphoid neoplasms typically contain a mixed population of benign and neoplastic lymphoid cells. Sometimes partial involvement of a lymph node by malignant lymphoma will result in overshadowing of a small component of neoplastic cells by the predominant polyclonal population. In most instances, the malignant population will originate from the B cells. Back-gating on the B-cell population often confirms a monotypic population. When the benign component is composed of polyclonal B cells, gating specifically on the larger B cells is often helpful. If the T-cell component is abundant (ie, >90%), it often suggests the possibility of a T-cell-rich B-cell lymphoma. If the number of neoplastic cells are too few, one may not be able to prove monoclonality. Our study included 1 case of large B-cell lymphoma and 1 case of follicle center lymphoma, grade III, that by FC were interpreted as polyclonal. Tissue biopsy confirmed the lymph nodes in both cases to be partially involved by lymphoma.

Hodgkin Lymphoma

The primary diagnosis of HL depends on being able to identify cytologically classic Reed-Sternberg cells residing in a background of benign reactive inflammatory cells. The most common differential diagnostic dilemma occurs when trying to distinguish an HL from an NHL composed of immunoblasts, a peripheral T-cell lymphoma, or a reactive paracortical hyperplasia with abundant immunoblasts. Binucleated immunoblasts may be observed in all 3 entities and can mimic Reed-Sternberg variants.

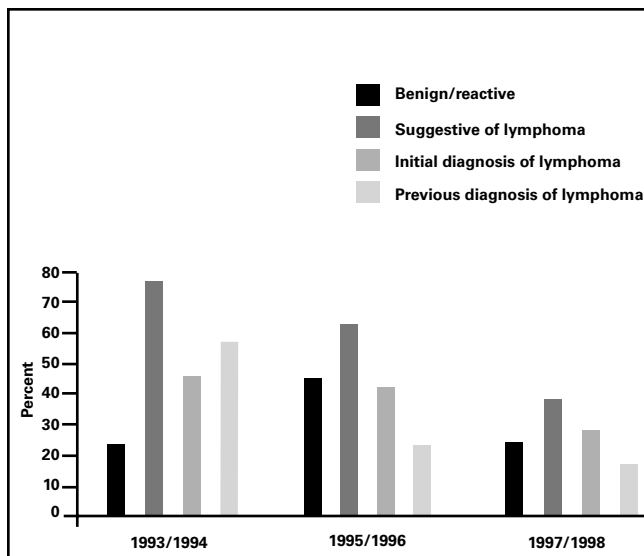


Figure 1 Frequency in which tissue biopsies were obtained during the period 1993-1998, before initiating patient treatment. Separated into categories based on combined cytomorphology and flow cytometric results (Table 1).

Classically, in benign immunoblastic proliferations and T-cell lymphomas, there should be a continuous spectrum between the atypical Reed-Sternberg-like variants and the background lymphoid cells, whereas HL has a sharp morphologic contrast between the relatively benign background and the malignant cells. T-cell lymphomas generally show more marked anisonucleosis, nuclear membrane irregularity, hyperchromatism, less conspicuous nuclei, and cytoplasmic basophilia.¹⁷

Our results of flow cytometric immunophenotyping on 6 cases of HL indicated a small population of polyclonal B lymphocytes and a preponderance of T-helper lymphocytes. In our opinion and that of others,¹⁴ HL should not be diagnosed by FC. In cases of suspected HL, it is better to perform immunocytochemistry on cytocentrifuged specimens for the appropriate markers rather than rely on FC; thus, our policy has been to perform immunocytochemistry on cytocentrifuged specimens or cell blocks. This explains why our series contains only 6 cases of HL. Three cases of HL were diagnosed definitively on the cytomorphic features, and immunocytochemistry was performed on the cell block. These cases contained several classical Reed-Sternberg cells. However, the 3 cases diagnosed as suggestive of lymphoma contained only rare Reed-Sternberg variants in a reactive-appearing background. In cases such as these, a conservative diagnosis of "suggestive of lymphoma" with a recommendation for surgical biopsy is indicated. In most settings, tissue biopsy confirmation is recommended for a primary diagnosis of HL.

T-Cell Lymphomas

Most peripheral T-cell lymphomas arise from the T-helper population, although some may arise from the T-suppressor population and occasionally from cells that are CD4+/CD8+. Although no immunophenotypic marker analogous to immunoglobulin light chain restriction is presently available, the diagnosis can be suspected by the identification of an abnormal T-cell phenotype (eg, expression of exclusively helper or cytotoxic/suppressor phenotype) and/or the loss of 1 or more of the pan-T-cell antigens. Unfortunately, a benign, reactive, cellular infiltrate also is present characteristically. In fact, only a fraction of the cells present may actually represent the malignant population. Thus, flow cytometric immunophenotyping will not distinguish between reactive and neoplastic processes but can help confirm the T-cell nature of a known lymphoma. Potentially helpful clues are that T-cell lymphomas generally show more marked anisonucleosis, nuclear membrane irregularity, hyperchromatism, less conspicuous nuclei, and cytoplasmic basophilia.¹⁷ In addition, many cases often have a reactive component consisting of eosinophils, plasma cells, or epithelioid histiocytes.

Lymphoglandular bodies often are less numerous than those seen in B-cell lymphomas.¹⁷

In our series, 3 cases of peripheral T-cell lymphoma were interpreted by FC to contain a benign polymorphous population of T cells. Fortunately, 2 of 3 cases had been diagnosed previously, and the cytomorphic features were similar to the original diagnostic material. Unfortunately, 1 case was interpreted incorrectly as benign by cytology and FC. In 4 of our cases, morphologic findings with concurrent FC documenting an atypical T-cell population with loss of 1 or more pan-T-cell antigens enabled us to make an initial diagnosis of T-cell lymphoma.

If a T-cell lymphoma is suspected because of clinical history or preliminary morphologic findings, a panel of T-cell antibodies can be used according to the number of cells available. Tissue biopsy confirmation is recommended when a primary diagnosis of T-cell lymphoma is suspected. Molecular diagnostic testing also may help or be necessary.

Large Cell Lymphomas

In our experience and that of others,¹³ the cells of large cell lymphoma are particularly fragile compared with the background lymphocytes and, thus, frequently are underrepresented in flow cytometric immunophenotyping. Often a false interpretation of a polyclonal population occurs. Misleading information often results from limited samples. Therefore, when there is a limited sample, reviewing the cells before labeling is important so that appropriate antibodies are chosen and the specimen is not wasted.

In our study, inadequate material prevented proper FC studies in 13 cases. Of the 13 cases, 11 were large cell lymphomas. In addition, of the 24 cases of large cell lymphoma interpreted by FC, 16 cases were interpreted as benign polyclonal populations. Of these 16 cases, a limited panel was performed in 9.

Another source of confusion stems from the fact that large B-cell lymphomas occasionally may be negative for surface immunoglobulin.¹³ B-lineage markers, such as CD19 and CD20, are more reliable markers, and the inability to demonstrate light chain clonality does not necessarily exclude malignant neoplasm. In our series, 1 case of large B-cell lymphoma did not express surface immunoglobulin. The large cells were specifically gated and were proved to be B cells. Absence of immunoglobulin on these cells was abnormal and strongly suggestive of malignant neoplasm, and a diagnosis of large B-cell lymphoma was made with confidence without a confirmatory tissue biopsy. A case like this demonstrates how important it is to correlate FC results with the cytologic features. The collaboration between the cytopathologist and the hematopathologist who interprets the FC has accounted for much of our success with the cytologic diagnosis of lymphomas.

FNA cytology in conjunction with flow cytometric immunophenotyping is a reliable and accurate method for the diagnosis and REAL classification of the majority of cases of NHL. FC often allows immunologic subtyping of lymphoma without open biopsy and enables a confirmation of the diagnosis of lymphoma. Polyclonality in clinically and cytologically "suspicious" cases does not exclude malignant neoplasm, but instead suggests HL, T-cell lymphoma, or, occasionally, partial tissue involvement by B-cell lymphoma. In these cases, obtaining an excisional biopsy specimen is strongly recommended. Clearly, FNA and immunophenotyping by flow cytometry are complementary and eliminate the need to perform open biopsy for many patients with lymphadenopathy.

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