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A Comparative Study of Frozen-Section Immunoperoxidase and Flow Cytometry for Immunophenotypic Analysis of Lymph Node Biopsies

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Immunophenotyping by flow cytometry and frozen-section immunoperoxidase was compared on 21 consecutive lymph node biopsy specimens, of which a diagnosis of lymphoma was made for 11 specimens. Samples for flow cytometry were obtained by a fine-needle aspiration technique. Concordance between frozen-section immunoperoxidase and flow cytometry for all routine markers on all specimens ranged from 76 to 100%. In general, B-cell markers showed poorer concordance than T-cell markers, with κ and λ light chains having the poorest concordance, at 76% each. Flow cytometry was significantly more sensitive (90 versus 30%; $P < 0.006$) and had a significantly higher negative predictive value (100 versus 63%; $P < 0.006$) than frozen-section immunoperoxidase for demonstrating light-chain restriction. There was no significant difference in the specificities (100 versus 91%) or positive predictive values (100% each) between the two methods. Both methods demonstrated characteristic immunophenotypes for intermediate cell lymphomas, small lymphocytic lymphomas, and T-cell lymphoblastic lymphomas. Frozen-section immunoperoxidase and flow cytometry appear to be significantly concordant methods for immunophenotypic analysis of lymph node biopsies. Light-chain restriction is more readily demonstrated by flow cytometry than frozen-section immunoperoxidase. We believe that ex vivo fine-needle aspiration is a simple and reliable method of obtaining cell suspensions of lymph nodes for flow cytometry.

The advent of immunophenotyping of samples from patients with lymphoproliferative disorders has added much to the classification and understanding of these processes. It is now recognized that the majority of normal lymphoid subsets and developmental stages are also represented in clonal lymphoproliferative disorders (8, 10, 33). Immunophenotyping also has practical diagnostic utility in supporting the diagnosis of malignancy by demonstrating clonality (15, 18, 28, 32). Clonality for B-cell processes is demonstrated by evidence of monotypic light-chain expression or light-chain restriction (11, 21, 22). Since committed B cells produce a single immunoglobulin light chain, monoclonal populations display a single immunoglobulin light chain, κ or λ, on the surfaces of all of their cells. Polyclonal populations are made up of a mixture of cells that produce either light chain. Therefore, they do not demonstrate light-chain restriction. For T-cell processes, monoclonality is supported by demonstration of an aberrant immunophenotype for the majority of cells. For an immunophenotyping method to be useful as an aid in the classification and diagnosis of lymphomas and lymphoid leukemia, it should be able to demonstrate light-chain restriction in monoclonal B-cell processes and its absence in polyclonal B-cell processes, demonstrate aberrant immunophenotypes in T-cell processes, and demonstrate characteristic immunophenotypes.

Several methods exist for immunophenotyping lymphomas in lymph node biopsies, including immunoperoxidase on fixed paraffin-embedded tissue (18–20), immunoperoxidase on fresh frozen tissue (frozen-section immunoperoxidase [FSIP]) (3, 30, 31), immunofluorescence on fresh frozen tissue (34), immunoperoxidase or immunofluorescence on cytospin preparations (26), or flow cytometry (FCM) on cell suspensions (2, 5, 6, 12, 16). Each of these methods has unique advantages and disadvantages. Immunoperoxidase on formalin-fixed, paraffin-embedded tissue provides the best morphology for correlation with staining. However, this is limited by poor antigen preservation and difficulties in demonstrating antigens restricted to cell surfaces, which include the majority of lymphoid markers. Sufficient antibodies have been developed for the separation of B- and T-cell processes on formalin-fixed, paraffin-embedded tissue, but immunoglobulin light chains are not reliably demonstrated on cell surfaces by this method (18–20, 25). FSIP allows demonstration of most surface markers and some morphology for correlation with staining, but FSIP may also have difficulty reliably demonstrating immunoglobulin light chains on cell surfaces (24, 25, 28, 29, 31, 34). This common limitation of these immunophenotyping techniques likely results from the high level of background staining of ubiquitous immunoglobulins in extracellular fluids and connective tissues and variable immunoglobulin expression by lymphomas. This might be compounded by the reduced cell surface area in tissue sections. Immunofluorescence on frozen tissue has sufficiently strong positive staining with a sufficiently low background for demonstration of immunoglobulin light chains on cell surfaces, but it provides only general architectural morphologic correlation, requires a fluorescence microscope, and does not provide permanent material for future review (25, 34). FCM has proven to be extremely useful for immunophenotyping leukemia, providing statistical power, sensitivity, and automation (3, 6, 9). Because of these benefits, FCM is now more widely used for immunophenotyping lymphomas as well. The major disadvantages of FCM for immunophenotyping node-based lymphomas are limited morphologic correlation,

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potential sampling error, and the need to prepare viable cell suspensions (13, 16).

We undertook a comparative immunophenotyping study of two of these methods, FSIP and FCM, on consecutive lymph node biopsies to determine the concordance of FSIP and FCM for routine markers, assess the diagnostic performance of each method for demonstrating light-chain restriction, assess the ability of each method to demonstrate characteristic immunophenotypes, and determine the utility of ex vivo fine-needle aspiration (FNA) for obtaining cell suspensions for FCM.

MATERIALS AND METHODS

Sample collection. Samples for both FCM and FSIP consisted of all lymph nodes submitted to the University of North Carolina Hospitals Surgical Pathology Laboratory for lymphoma workup from November 1991 to March 1992. Samples for FCM were collected by an ex vivo FNA technique by using a 10-ml syringe holder (gun) and a 22-gauge needle (1, 7). Multiple passes of each lymph node were made, sampling all areas. The aspirated material was flushed into RPMI tissue culture medium (Lineberger Comprehensive Cancer Center Tissue Culture Facility, University of North Carolina-Chapel Hill) to obtain disaggregated cell suspensions. Simultaneous air-dried, Diff-Quik (Baxter Scientific Products, Charlotte, N.C.)-stained smears were prepared to verify the collection of representative samples and to aid in diagnosis. The viabilities of the cell suspensions were assessed with trypan blue, yielding sample viabilities of 80% ± 14%. A representative portion of each lymph node specimen was soaked in phosphate-buffered saline (PBS; pH 7.2 to 7.4) for 20 min, covered with Tissue-Tek OCT tissue embedding medium (Baxter), and snap-frozen in liquid nitrogen for FSIP. Human palatine tonsils for lymphoid tissue controls were processed in the same manner.

Monoclonal antibodies. Samples from all lymph nodes were labeled for FCM and FSIP analysis with antibodies directed to a core set of lymphoid antigens (CD3, CD4, CD8, CD20, CD45, κ light chain, and λ light chain). All samples for FCM were also labeled with antibodies to CD2, CD5, CD7, CD10, CD19, CD25, CD38, and HLA-DR. Samples from selected lymph nodes were labeled for FSIP with antibodies to CD2, CD5, CD7, CD19, and CD22. Commercial antibodies for FSIP were obtained from Dako Corporation, Carpintina, Calif., and ICN, Irvine, Calif. Commercial fluorochrome (fluorescein isothiocyanate and phycoerythrin)-conjugated monoclonal antibodies were obtained from Becton Dickinson Immunocytometry Systems, San Jose, Calif., and GenTrak, Inc., Plymouth Meeting, Pa.

FCM. Aliquots of the cell suspensions were added to staining tubes containing the appropriate fluorochrome-conjugated monoclonal antibodies. Following incubation at 4°C for 15 min, the samples were centrifuged, washed with PBS containing bovine serum albumin and sodium azide, and resuspended in 0.5 ml of 1% paraformaldehyde in PBS. The labeled specimens were then run on a FACScan flow cytometer with an argon ion laser emitting at 498 nm (Becton Dickinson Immunocytometry Systems). Two-color analysis was performed by using Consort 30, SIMULSET, and LYSYS analysis software (Becton Dickinson Immunocytometry Systems), yielding a percentage of total cells positive for each antigen. Gates were set by light-scatter characteristics and were verified by staining for CD45 and CD14. Light-chain results were considered indeterminate when >75% of the CD19-positive cells stained for both light chains.

FSIP. Frozen sections of the lymph nodes and control tonsils of 4 μm were cut onto silanized slides (ProbeOn Plus; Fisher Scientific, Pittsburgh, Pa.), labeled with the appropriate antibodies, and developed by the ABC method (Vectastain Elite kit; Vector Laboratories, Inc., Burlingame, Calif.) with DAB chromogen (Biomeda Corp., Foster City, Calif.) by using a capillary gap apparatus (Microprobe; Fisher Scientific). Specific details of the staining procedure were described previously (4). Sections of lymph nodes were paired against sections of control tissue. The staining was graded for each antigen by light microscopy into five categories, each reflecting a range of percentage of total cells that were positive, as follows: negative, <10%; 1+, 10 to 25%; 2+, 26 to 50%; 3+, 51 to 75%; and 4+, >75%. Results for light chains were considered indeterminate when staining was 4+ with both markers.

Statistical analysis. The concordance of immunostaining between the two methods, defined as FSIP within ±1 corresponding grade of FCM, was determined for each marker in each lymph node specimen. A concordance percentage [(number concordant/total) × 100] was then determined for each marker for all lymph nodes and the subgroups of samples representing B-cell lymphomas, benign lymphadenopathies, and Hodgkin's disease. The 95% confidence intervals for concordance percentages were obtained from a binomial distribution chart (23), and the observed values were compared with 0.5 (50%) by using binomial probability tables (17). Demonstration of light-chain restriction (LCR) was determined for each method according to the following definitions: LCR ≡ $\kappa/\lambda < 0.25$ or $\kappa/\lambda > 7.5$. This definition was translated into categories of grading with ratios of corresponding percentages that met or exceeded the definition, as follows: for FSIP, LCR ≡ κ = 4+, λ = negative ($\kappa/\lambda > 7.5$); λ ≥ 2+, κ = negative; or λ = 4+, κ = 1+ ($\kappa/\lambda < 0.25$). The sensitivity, specificity, positive predictive value, and negative predictive value for demonstrating LCR were then calculated for each method. For the purposes of these calculations, the "gold standard" LCR was defined as present in B-cell lymphomas and absent from all other samples. B-cell lymphomas were defined by the combination of a light microscopy diagnosis of lymphoma and the presence of surface immunoglobulin on tumor cells. This approach is based on current understanding of B-cell lymphomas, specifically, that those tumors that express light chains do so in a monotypic or restricted fashion and other lymphoid processes do not (11, 21, 22). The 95% confidence intervals for these diagnostic performance statistics were obtained as discussed above for concordance percentages, and those of each method were compared by using a normal approximation to the distribution of the difference of two binomial proportions (17).

RESULTS

Concordance of FCM and FSIP. Twenty-one lymph node biopsy specimens were analyzed by FSIP and FCM (Table 1). The final diagnoses included the following: 10 B-cell lymphomas, 1 T-cell lymphoma, 3 cases of Hodgkin's disease, and 7 benign lymphadenopathies.

The concordance of the two methods was determined for each marker. Concordance for all markers in all cases ranged from 76 to 100% (Table 2). These proportions were significantly greater than 50% for all commonly determined markers (all markers, excluding CD2, CD5, and CD7, determined by both methods; $P < 0.0001$ to 0.0207). In general, the T-cell markers showed greater concordance than the B-cell markers, with κ and λ light chains having the lowest concordance (76%). Similar concordances were found in the subgroups of B-cell lymphomas, Hodgkin's disease, and benign lymphadenopathies.

TABLE 1. Immunophenotyping results by sample[a]

Sample	Diagnosis	CD3		CD4		CD5		CD7		CD8		CD10, FCM	CD19		CD20		κ		λ		κ/λ, FCM
		FCM[b]	FSIP[c]	FCM	FSIP	FCM	FSIP	FCM	FSIP	FCM	FSIP		FCM	FSIP	FCM	FSIP	FCM	FSIP	FCM	FSIP	
1	ICL	18	2+	9	Neg	98	4+	16	ND	7	Neg	3	81	ND	59	3+	4	Neg	84	Neg	0.03
2	ICL	23	1+	18	1+	31	1+	8	ND	3	Neg	0	83	ND	87	3+	82	2+	1	1+	82
3	ICL	36	1+	8	1+	37	1+	8	ND	5	Neg	1	55	4+	84	3+	58	4+	4	1+	27
4	ICL	23	1+	10	1+	93	4+	17	ND	12	1+	0	75	4+	80	Neg	2	Neg	77	4+	0.01
5	SLL	5	1+	3	Neg	92	4+	2	ND	1	Neg	0	90	ND	4	Neg	86	1+	1	Neg	86
6	SNCNB	2	1+	3	Neg	0	2+	1	ND	3	1+	1	47	ND	93	3+	1	Neg	88	Neg	0.01
7	SNCNB	5	1+	2	Neg	5	ND	3	ND	5	1+	37	39	ND	54	4+	1	Neg	34	3+	0.03
8	FML	35	2+	36	2+	42	2+	24	ND	8	Neg	59	58	ND	83	4+	3	4+	47	4+	0.06
9	FML	35	2+	29	1+	30	ND	18	ND	4	Neg	57	68	ND	81	3+	66	2+	67	Neg	0.99
10	FSCL	50	2+	43	2+	50	ND	37	ND	4	Neg	3	37	ND	74	1+	3	Neg	50	3+	0.06
11	TLBL	16	4+	88	4+	93	4+	7	Neg	3	Neg	1	3	ND	32	1+	2	Neg	3	Neg	0.67
12	MC HD	31	3+	14	2+	30	ND	28	ND	20	2+	0	48	2+	58	1+	37	1+	30	1+	1.2
13	NS HD	45	2+	26	1+	47	ND	43	ND	21	1+	0	50	1+	57	1+	26	1+	25	Neg	1
14	LD HD	72	3+	54	3+	70	ND	61	ND	17	1+	1	16	1+	30	1+	23	Neg	15	Neg	1.5
15	Granulomas	77	4+	49	2+	66	ND	50	ND	13	2+	0	14	1+	16	1+	8	Neg	5	Neg	1.6
16	Sarcoid	32	3+	21	3+	28	ND	25	ND	7	1+	3	62	2+	63	2+	38	1+	29	1+	1.3
17	DLA	83	3+	52	2+	84	ND	76	ND	29	1+	0	15	2+	18	2+	8	1+	5	1+	1.6
18	Histiocytosis	77	4+	61	4+	71	ND	70	ND	12	1+	0	19	1+	20	1+	64	Neg	23	Neg	2.8
19	BLH	69	3+	46	2+	73	ND	64	ND	24	1+	1	27	ND	27	1+	14	Neg	13	Neg	1
20	BLH	45	3+	38	3+	46	ND	36	ND	6	1+	1	43	2+	45	2+	22	Ind	17	Ind	1.3
21	BLH	49	4+	31	3+	54	ND	41	ND	18	3+	4	46	1+	47	1+	34	1+	16	1+	2.1

[a] ICL, intermediate cell lymphoma; SLL, small lymphocytic lymphoma; SNCNB, small noncleaved non-Burkitt's lymphoma; FML, follicular mixed lymphoma; FSCL, follicular small cleaved lymphoma; TLBL, T-cell lymphoblastic lymphoma; MC HD, mixed cellularity Hodgkin's disease; NS HD, nodular sclerosis Hodgkin's disease; LD HD, lymphocyte depleted Hodgkin's disease; DLA, dermatopathic lymphadenopathy; BLH, benign lymphoid hyperplasia; FCM, flow cytometry; FSIP, immunoperoxidase; ND, not determined.

[b] Values are percent positive cells.

[c] Grade corresponding to percent positive cells (neg, negative = <10%; 1+, 10 to 25%; 2+, 26 to 50%; 3+, 51 to 75%; 4+, >75%; Ind, indeterminate).

Demonstration of LCR. FCM was significantly more sensitive (90 versus 30%; $P = 0.006$) and had a significantly higher negative predictive value (100 versus 63%; $P < 0.006$) than FSIP for demonstrating LCR. There was no significant difference in the specificities (100 versus 91%; $P > 0.3$) or positive predictive values (100% each; $P > 0.9$) between the two methods (Table 3). FCM yielded indeterminate results in one follicular mixed lymphoma (sample 9; Table 1). FSIP yielded indeterminate results in one follicular mixed lymphoma (sample 8; Table 1) and one benign sample (sample 20; Table 1).

Demonstration of characteristic immunophenotypes. The T-cell lymphoblastic lymphoma (sample 11; Table 1) had an aberrant immunophenotype (strong CD2 and CD5 expression but low CD7 expression) by both FCM and FSIP, although the methods were discordant with respect to CD3 (16% by FCM; 4+ surface staining by FSIP). The four intermediate cell lymphomas and one small lymphocytic lymphoma (samples 1 to 5; Table 1) showed expression of CD19 or CD20 and CD5 by both FCM and FSIP. Coexpression of these markers by individual cells was demonstrated by dual staining with FCM. The two follicular mixed lymphomas (samples 8 and 9; Table 1) showed CD10 expression by FCM.

DISCUSSION

Although FCM and FSIP are both widely used for immunophenotyping of lymphomas, both have been evaluated on large numbers of samples, and both have shown advantages over other methods, little has been published regarding the comparative performance of each method. The present study was performed to directly compare immunophenotyping by FCM and FSIP. By the frequency of utilization of each method, one

TABLE 2. Concordance for markers by FCM and FSIP

Marker	All samples		B-cell lymphomas		Benign samples		Hodgkin's disease	
	% Concordance[a]	No.	% Concordance	No.	% Concordance	No.	% Concordance	No.
CD2	100 (I[b])	2	100 (I)	1	ND[c] (ND)	0	ND	0
CD3	95 (76–100)	21	100 (69–100)	10	86 (41–100)	7	100	3
CD4	95 (76–100)	21	100 (69–100)	10	86 (41–100)	7	100	3
CD5	88 (48–100)	8	86 (41–100)	7	ND (ND)	0	ND	0
CD7	100 (I)	1	ND (ND)	0	ND (ND)	0	ND	0
CD8	95 (76–100)	21	100 (69–100)	10	86 (41–100)	7	100	3
CD19	100 (71–100)	11	ND (ND)	0	100 (57–100)	7	33	3
CD20	81 (58–94)	21	80 (44–98)	10	100 (57–100)	7	100	3
CD45	100 (84–100)	21	100 (69–100)	10	100 (57–100)	7	100	3
κ	76 (55–92)	21	70 (35–95)	10	71 (27–96)	7	100	3
λ	76 (55–92)	21	60 (26–90)	10	86 (41–100)	7	100	3

[a] Values are percent observed concordance (95% confidence interval).

[b] I, insufficient sample size.

[c] ND, not determined.

TABLE 3. Demonstration of LCR by FCM and FSIP[a]

Method	% Sensitivity[a]	% Specificity	% PV+[b]	% PV−[c]
FCM	90 (56–100)	100 (71–100)	100 (66–100)	100 (71–100)
FSIP	30 (7–65)	91 (58–100)	100	63 (35–87)

[a] Observed value (95% confidence interval).
[b] PV+, positive predictive value.
[c] PV−, negative predictive value.

would expect each to perform reasonably well with most markers, and therefore that the two methods would give similar results for the same markers on the same samples (high concordance). Reasonable concordances were found in the present study. As a result of the potential problems with demonstration of light chains discussed above, one might expect antibodies to light chains to demonstrate the poorest concordance. This again was the case in the study, although the sample size ($n = 21$) does not allow adequate confidence in the magnitude of the difference between the two methods.

Even though the two methods were compared by using antibodies directed against the same antigens, antibodies from the same source were not used. Some difference in staining between the two methods might therefore relate to differences in the antibody specificities since these are monoclonal antibodies. It is unlikely that this difference would be great enough to prevent the observation of concordance as defined in the present study. Because our goal was to compare FCM and FSIP as they are routinely performed in skilled laboratories, which carefully select and titrate antibodies specifically for their respective methodologies, changes in the antibodies used by each laboratory were not instituted for the present study.

FCM and FSIP were both able to assign a B- or a T-cell lineage to the lymphomas. This does, however, raise a point regarding the arbitrary assignment of markers to lymphocyte subsets. In the present study, there were some apparent discrepancies in "pan B-cell markers" within individual samples by FCM (Table 1). This may relate as much to the heterogeneity of both tumors and benign lymphocyte subsets as to the antibodies that were used and the markers that they detected. In particular, CD20 as obtained in our laboratory is not restricted to B cells but is present on a subset of T cells which comprise on average 11% of the peripheral lymphocytes in our healthy controls. Although most of the lymphoma samples we presented had higher CD20 than CD19 values, the differences often were small and could be attributed to CD20-positive T cells.

Of foremost diagnostic importance in the immunophenotyping of lymph nodes is the demonstration of LCR in lymphomas and the absence of LCR in benign lymph nodes. The first obstacle in assessing LCR and comparing methods is one of definition. LCR has been loosely described as a preponderance of one light chain type, but the ability to quantify light-chain expression by such methods as FCM has led investigators to use various numerical definitions. These range from the most liberal of a κ/λ ratio of ≥ 3.0 or ≤ 0.5 (27) to the most conservative of a κ/λ ratio of ≥ 10 or ≤ 0.1 (21). Because benign reactive lymphoid hyperplasias generally have κ/λ ratios in the ranges of 0.3 to 1.0 and 3.0 to 7.0 (11, 14), we chose the criteria for LCR = $\kappa/\lambda > 7.5$ or $\kappa/\lambda < 0.25$ in order to provide an optimum separation of benign and malignant disorders while maintaining a realistic uniform criterion for application to FSIP as well as FCM. Because routine assessment of FSIP is subjective, the definition of a "preponderance of one light chain" is more widely used; however, we adopted a semiquantitative method of grading FSIP results to allow comparison with the numeric data generated by FCM.

The second obstacle in comparing each method's ability to demonstrate LCR is to establish a reference. For this purpose, we arbitrarily assigned LCR to the B-cell lymphomas as defined by morphology and surface immunoglobulin expression and its absence to all other samples. An alternate approach for comparing the methods would be to use molecular studies for light-chain gene rearrangement as the reference. Gene rearrangement analysis should be the most specific test for demonstration of LCR and an ultimate gold standard for comparing immunophenotyping methods. We did not perform light-chain or T-cell receptor gene rearrangement analysis on any of the study samples because this was beyond the scope of our study and is not required for diagnosis. One would expect molecular studies to confirm the theoretical assignment of LCR to the B-cell lymphomas with surface immunoglobulins and the lack thereof to the remaining samples. The only predictable discrepancies would be those B-cell lymphomas which fail to express surface immunoglobulins. There were no such cases in the present study, although they reportedly make up 12 to 15% of B-cell lymphomas and potentially hinder the ability to demonstrate LCR by any method (11, 16).

Having thus compared the methods, the better sensitivity and negative predictive value of FCM in the present study likely relate to the problems in demonstrating surface immunoglobulins in tissue as described above, including the presence of polyclonal immunoglobulins in extracellular fluid and connective tissue, variable immunoglobulin expression by lymphomas, and the difficulty in quantifying FSIP. In addition, the small sample size precludes confidence in the magnitude of the differences in diagnostic statistics between methods. Use of image analysis to improve the quantification of FSIP might improve the performance of FSIP.

Sampling error is another concern in studying lymph nodes. FSIP requires the use of some portion of the total lymph node, allowing the potential for sampling error in focal processes. No sampling error in FSIP was encountered in the present study on the basis of comparison of frozen sections with paraffin-embedded sections. FCM requires preparation of a cell suspension. Traditional methods include mechanical disaggregation of a portion of the total lymph node (35), which is subject to the same potential for sampling error as FSIP. In addition, the technique might introduce a selection bias if some cells are more readily disaggregated than others. The ex vivo FNA technique used in the present study had the potential benefit of allowing sampling from all portions of the lymph node by multiple redirected passes prior to sectioning. This likely would decrease the potential for inadequate sampling of focal processes; however, the possibility of selection bias would still exist. On the basis of a comparison of smears of the aspirated material, the FSIP and FCM results, and the paraffin-embedded sections, appreciable sampling error was not experienced with the FNA method in the present study.

In summary, FCM and FSIP are concordant methods for immunophenotypic analysis of lymph node biopsies. FCM is significantly more sensitive and has a significantly higher negative predictive value for demonstration of LCR in lymphoid processes involving lymph nodes than FSIP. FCM and FSIP can both identify characteristic immunophenotypes for some T- and B-cell lymphomas. An ex vivo FNA technique is a simple and reliable method for the collection of cell suspension samples of lymph node biopsies for FCM.

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REFERENCES

1. **Bach, B. A., W. A. Knape, M. G. Edinger, and R. R. Tubbs.** 1991. Improved sensitivity and resolution in the flow cytometric DNA analysis of human solid tumor specimens. Use of in vitro fine-needle aspiration and uniform staining reagents. Am. J. Clin. Pathol. **96:**615–627.
2. **Braylan, R. C., and N. A. Benson.** 1989. Flow cytometric analysis of lymphomas. Arch. Pathol. Lab. Med. **113:**627–633.
3. **Carey, J. L.** 1989. Flow cytometric immunoanalysis of leukemia and lymphoma. J. Clin. Immunoassay **12:**21–29.
4. **Cowan, D. W., G. A. Dent, and K. B. Pryzwansky.** 1991. Application of automated immunocytochemistry for assessing lymphocyte surface markers in frozen sections. J. Histotechnol. **14:**231–235.
5. **De Martini, R. M., R. R. Turner, D. C. Boone, R. J. Lukes, and J. W. Parker.** 1988. Lymphocyte immunophenotyping of B-cell lymphomas: a flow cytometric analysis of neoplastic and nonneoplastic cells in 271 cases. Clin. Immunol. Immunopathol. **49:**365–379.
6. **Diamond, L. W., B. N. Nathwani, and H. Rappaport.** 1982. Flow cytometry in the diagnosis and classification of malignant lymphoma and leukemia. Cancer **50:**1122–1135.
7. **Eliasen, C. A., L. M. Opitz, E. C. Vamvakas, E. C. Espiritu, E. R. Marsh, D. F. Roses, M. N. Harris, and H. D. Feiner.** 1991. Flow cytometric analysis of DNA ploidy and S-phase fraction in breast cancer using cells obtained by ex vivo fine-needle aspiration: an optimal method for sample collection. Mod. Pathol. **4:**196–200.
8. **Foon, K. A., and R. F. Todd.** 1986. Immunologic classification of leukemia and lymphoma. Blood **68:**1–31.
9. **Foucar, K., I. M. Chen, and S. Crago.** 1989. Organization and operation of a flow cytometric immunophenotyping laboratory. Semin. Diagn. Pathol. **6:**13–36.
10. **Kadin, M. E., and J. W. Said.** 1990. Pathology of malignant lymphomas. Curr. Opin. Oncol. **2:**822–831.
11. **Knowles, D. M., A. Chadburn, and G. Inghirami.** 1992. Immunophenotypic markers useful in the diagnosis and classification of hematopoietic neoplasms, p. 73–167. *In* D. M. Knowles (ed.), Neoplastic hematopathology. The Williams & Wilkins Co., Baltimore.
12. **La Via, M. F., and S. E. Self.** 1990. Immunophenotypic analysis of non-Hodgkin's lymphomas. Recenti Prog. Med. **81:**629–634.
13. **Leith, C., and K. Foucar.** 1992. Flow cytometry in the diagnosis of non-Hodgkin's lymphoma. J. Histotechnol. **15:**219–227.
14. **Levy, N., J. Nelson, P. Meyer, R. J. Lukes, and J. W. Parker.** 1983. Reactive lymphoid hyperplasia with single class (monoclonal) surface immunoglobulin. Am. J. Clin. Pathol. **80:**300–308.
15. **Levy, R., R. Warnke, R. F. Dorfman, and J. Haimovich.** 1977. The monoclonality of human B cell lymphomas. J. Exp. Med. **145:** 1014–1028.
16. **Little, J. V., K. Foucar, A. Horvath, and S. Crago.** 1989. Flow cytometric analysis of lymphoma and lymphoma-like disorders. Semin. Diagn. Pathol. **6:**37–54.
17. **Milton, J. S.** 1992. Statistical methods in the biological and health sciences, 2nd ed. McGraw-Hill Book Co., New York.
18. **Nemes, Z., V. Thomazy, and G. Szeifert.** 1983. Demonstration of light chain monotypia in B cell non-Hodgkin's lymphomas using unfixed freeze-dried and formalin-fixed trypsinised paraffin sections. J. Clin. Pathol. **36:**883–893.
19. **Norton, A. J., and P. G. Isaacson.** 1989. Lymphoma phenotyping in formalin-fixed and paraffin wax-embedded tissues. I. Range of antibodies and staining patterns. Histopathology **14:**437–446.
20. **Norton, A. J., and P. G. Isaacson.** 1989. Lymphoma phenotyping in formalin-fixed and paraffin wax-embedded tissues. II. Profiles of reactivity in the various tumor types. Histopathology **14:**557–579.
21. **Picker, L. J., L. M. Weiss, J. L. Medeiros, G. S. Wood, and R. A. Warnke.** 1987. Immunophenotypic criteria for the diagnosis of non-Hodgkin's lymphoma. Am. J. Pathol. **128:**181–201.
22. **Ratech, H., and S. Litwin.** 1989. Surface immunoglobulin light chain restriction in B-cell non-Hodgkin's malignant lymphomas. Am. J. Clin. Pathol. **91:**583–586.
23. **Remington, R. D., and M. A. Schork.** 1985. Statistics with applications to the biological and health sciences. Prentice-Hall, Inc., Englewood Cliffs, N.J.
24. **Sheibani, K., B. N. Nathwani, W. G. Swartz, J. Ben-ezra, M. D. Brownell, J. S. Burke, J. L. Kennedy, C. H. Koo, and C. D. Winberg.** 1988. Variability in interpretation of immunohistologic findings in lymphoproliferative disorders by hematopathologists. A comprehensive statistical analysis of interobserver performance. Cancer **62:**657–664.
25. **Sheibani, K., and R. R. Tubbs.** 1984. Enzyme immunohistochemistry: technical aspects. Semin. Diagn. Pathol. **1:**235–250.
26. **Sneige, N., R. Dekmezian, A. el Naggar, and J. Manning.** 1991. Cytomorphologic, immunocytochemical, and nucleic acid flow cytometric study of 50 lymph nodes by fine needle aspiration. Comparison with results obtained by subsequent excisional biopsy. Cancer **67:**1003–1007.
27. **Taylor, C. R.** 1979. Results of multiparameter studies of B cell lymphomas. Am. J. Clin. Pathol. **72:**687–697.
28. **Tetu, B., J. T. Manning, and N. G. Ordonez.** 1986. Comparison of monoclonal and polyclonal antibodies directed against immunoglobulin light and heavy chains in non-Hodgkin's lymphoma. Am. J. Clin. Pathol. **85:**25–31.
29. **Tubbs, R. R., and K. Sheibani.** 1984. Immunohistology of lymphoproliferative disorders. Semin. Diagn. Pathol. **1:**272–284.
30. **Tubbs, R. R., K. Sheibani, and B. A. Sebek.** 1980. Immunohistochemistry versus immunofluorescence for non-Hodgkin's lymphomas. Am. J. Clin. Pathol. **73:**144–145.
31. **Tubbs, R. R., K. Sheibani, R. A. Weiss, and B. A. Sebek.** 1981. Immunohistochemistry of fresh-frozen lymphoid tissue with the direct immunoperoxidase technic. Am. J. Clin. Pathol. **75:**172–174.
32. **Tubbs, R. R., K. Sheibani, R. A. Weiss, B. A. Sebek, and S. D. Deodhar.** 1981. Tissue immunomicroscopic evaluation of monoclonality of B-cell lymphomas. Comparison with cell suspension studies. Am. J. Clin. Pathol. **76:**24–28.
33. **Vaickus, L., E. D. Ball, and K. A. Foon.** 1991. Immune markers in hematologic malignancies. Crit. Rev. Oncol. Hematol. **11:**267–297.
34. **Warnke, R., N. Pederson, C. Williams, and R. Levy.** 1978. The study of lymphoproliferative diseases comparing immunofluorescence with immunohistochemistry. Am. J. Clin. Pathol. **70:**867–875.
35. **Warzynski, M. J., A. E. Podgurski, D. M. Boldt, and R. N. Otto.** 1990. An automated method to prepare cell suspensions from human biopsy samples for immunophenotyping by flow cytometry. Am. J. Clin. Pathol. **93:**104–108.