

Cytologic Diagnosis of Burkitt Lymphoma

Role of Ancillary Studies

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BACKGROUND. The diagnosis and classification of lymphoma require correlation of morphologic, immunophenotypic, and molecular-cytogenetic studies. Fine-needle aspiration biopsy (FNAB) is a valuable diagnostic technique that allows material to be collected for these ancillary studies, and for morphologic evaluation.

METHODS. The authors report a series of seven cases clinically or morphologically suspicious for Burkitt lymphoma. Fluorescence in situ hybridization studies (FISH) for *c-myc* were performed on FNAB material and correlated with cytologic and immunophenotypic data.

RESULTS. Six of seven specimens were positive for *c-myc* rearrangement by FISH. However, only three of these cases represented Burkitt lymphoma, with one additional case of atypical Burkitt lymphoma. The other cases included diffuse large B-cell lymphoma, monomorphic posttransplant B-cell lymphoma, and an aggressive B-cell lymphoma, with the latter case negative for *c-myc* rearrangement by FISH. Of 2 non-Burkitt lymphoma specimens tested, 1 was positive for the immunoglobulin H/*bcl-2* rearrangement, in addition to the *c-myc* rearrangement, suggesting transformation from a lower grade lymphoma.

CONCLUSIONS. These cases illustrated the value of FNAB in the diagnosis of Burkitt lymphoma, as well as the importance of obtaining material for, and integrating results of, ancillary studies for the final diagnosis. *Cancer (Cancer Cytopathol)* 2005;105:310–8. © 2005 American Cancer Society.

KEYWORDS: Burkitt lymphoma, *c-myc*, fluorescence in situ hybridization, fine-needle aspiration biopsy, t (14;18).

The distinction between Burkitt lymphoma and other B-cell lymphomas, including diffuse large B-cell lymphoma (DLBCL), precursor B-acute lymphoblastic lymphoma/leukemia (B-ALL), and so-called atypical Burkitt/Burkitt-like lymphoma, often proves difficult, even on tissue biopsy specimens. Diagnostic criteria for Burkitt and atypical Burkitt lymphoma have been refined in the recent World Health Organization (WHO) lymphoma classification system.¹ A number of ancillary diagnostic techniques are currently employed in the diagnosis of Burkitt lymphoma and other hematolymphoid neoplasms, including immunohistochemistry, flow cytometry, and molecular-cytogenetic studies. The typical immunophenotype of Burkitt lymphoma includes expression of the B-cell antigens CD19 and CD20, expression of CD10, lack of *bcl-2* or terminal deoxynucleotid transferase (TdT), and a very high growth fraction. Rearrangement of *c-myc* is also essentially required for the diagnosis. The t(8;14) translocation involving the immunoglobulin (Ig) heavy chain locus is the most common, whereas other rearrangements include variants t(2;8) or t(8;22) involving the Ig light chain loci.¹

Reliable fluorescence in situ hybridization (FISH) and polymerase

TABLE 1
Patient Data

Patient no.	Age	Gender	FNAB site	Bone marrow biopsy	Excision
1	70	F	Submental LN	Positive	Yes
2	26	M	Neck LN	Negative	No
3	64	M	Submandibular LN	Negative	No
4	36	M	Retroperitoneum	Negative	No
5	49	M	Liver	Positive	Yes
6	77	M	Retroperitoneum, Thoracentesis	Minimal (< 1%)	No
7	22	F	Parotid	Negative	No

F: female; M: male; FNAB: fine-needle aspiration biopsy; LN: lymph nodes.

chain reaction (PCR) methods have made feasible the testing of small samples, including those obtained by fine-needle aspiration biopsy (FNAB), with probes readily available for the detection of the rearrangement of the *c-myc* gene.²⁻⁴ However, *c-myc* rearrangements are not specific for Burkitt lymphoma, as they also may be seen in other aggressive lymphomas, especially those transformed from low-grade lymphomas.⁵⁻¹⁶

We report a series of seven FNAB specimens with concurrent *c-myc* analysis by FISH. These cases illustrate the importance of correlating data from several modalities, including flow cytometry, immunohistochemistry, and molecular diagnostic techniques, in addition to standard morphologic assessment.

MATERIALS AND METHODS

Case Selection

Case files of the Department of Pathology, Stanford University Medical Center (Stanford, CA) were searched for FNAB samples with a diagnosis of B-cell lymphoma that had concurrent *c-myc* analysis by FISH. Seven cases were identified (Table 1). Two of these FNAB samples were requested by clinicians for the primary purpose of obtaining additional material for *c-myc* rearrangement analysis. Cytologic specimens were obtained using standard techniques. Smears were alcohol fixed for Papanicolaou (Pap) staining, and air dried for Diff-Quik (Richard-Allen Scientific; Kalamazoo, MI) staining (Fig. 1). Cell block material was prepared by concentrating material transported in RPMI-1640 by centrifugation at 2200 rpm for 10 minutes (with a second centrifugation step with added formalin fixative as necessary), and transferring pelleted material to lens paper. The folded lens paper was then transferred to a cassette, fixed in formalin, and processed routinely. Aspirate material was submitted for FISH analysis and/or flow cytometry.

Cytology

Cytologic preparations were reviewed, along with any formalin-fixed, paraffin-embedded cell block or excisional biopsy material. For a diagnosis of Burkitt lymphoma, we required morphologic features as defined in the 2001 WHO leukemia/lymphoma classification: medium-sized cells with round nuclei (approximate size of histiocyte nucleus), finely granular (blast-like) chromatin, relatively clear parachromatin, multiple basophilic centrally situated nucleoli, deeply basophilic cytoplasm, usually containing lipid vacuoles, and a high mitotic rate.^{1,17} The WHO classification system cites the expected immunophenotype as positive for immunoglobulin M (IgM), CD19 or CD20, CD10, and *Bcl-6*, and typically negative for CD5, CD23, TdT, and *bcl-2*, with few infiltrating T cells (of these, we assayed CD20, CD10, *bcl-2*, and Ki-67).^{1,15,17-19} Additional previously characterized features of Burkitt lymphoma as seen on Pap-stained smears include coarse nuclear chromatin and multiple nucleoli.^{20,21} Atypical Burkitt/Burkitt-like lymphoma, according to the WHO classification, is defined as showing some of the above features, but with greater pleomorphism in nuclear size and shape, and fewer, more prominent nucleoli.¹ For both typical and atypical Burkitt lymphoma, at least presumptive evidence of *c-myc* translocation is required for a diagnosis.^{1,17}

Flow Cytometry

Material was sent fresh in RPMI-1640 media or saline for flow cytometry. Flow cytometry was performed at Stanford Clinical Laboratories on the primary aspirate specimen in 4 of 7 cases, and on a concurrent thoracentesis specimen for Case 6, using standard whole blood lysis. Case 1 had flow cytometry performed at an outside institution on the concurrent excisional biopsy specimen, with results confirmed by immunohistochemical staining on tissue sections. Case 7 had no flow immunophenotypic data available. A FACScan

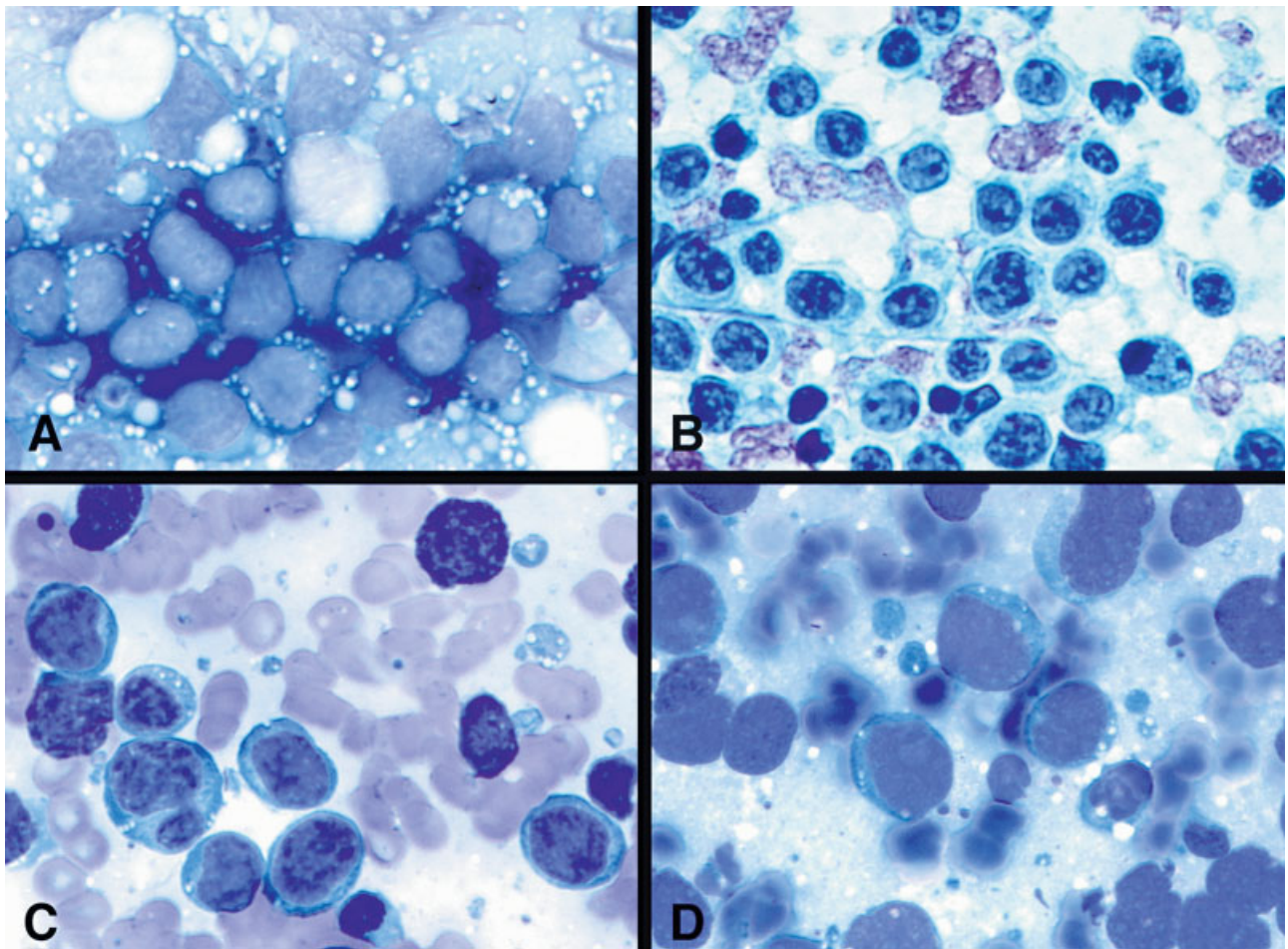


FIGURE 1. Cytologic features of study cases. (A) Burkitt lymphoma, Diff-Quik stain, Case 6. (B) Burkitt lymphoma, Papanicolaou stain, Case 5. (C) Monomorphic posttransplant lymphoproliferative disorder, favor diffuse large B-cell lymphoma, Diff-Quik stain, Case 3. (D) Atypical Burkitt lymphoma, Diff Quik stain, Case 2 (original magnification $\times 600$).

(Becton Dickinson, San Jose, CA) direct dual-parameter flow cytometer was used, with commercially available fluorescein isothiocyanate or phycoerythrin-conjugated monoclonal antibodies (MoAb) (Table 2). Cytoplasmic antigens were detected after standard fixation and permeabilization procedures. An analysis gate was selected on the forward/side scatter plot to include the population of atypical lymphocytes (Fig. 2). The number of events reactive with each MoAb was expressed as a percentage (positive $> 20\%$), with thresholds determined based on isotypic controls.

Immunohistochemistry

Immunohistochemical staining for Ki-67 and *bcl-2* was performed at Stanford on material from 5 cases, and also was performed on previous outside surgical or FNAB specimens for Case 1 and 2, respectively. Additional immunohistochemical markers, includ-

ing CD10 and CD20, were performed on selected specimens. Four-micron sections were prepared, deparaffinized, and rehydrated using standard methods. Sections were treated to unmask epitopes before incubation with primary antibodies. Details of pretreatment, antibodies, and antibody concentrations are presented in Table 3. The entire procedure, including pretreatment, primary and secondary antibody incubation, washing, chromogen development (biotin/horseradish peroxidase/diaminobenzidine), and counterstaining, was performed with a Ventana Benchmark (Tucson, AZ) automated stainer for CD20 and CD10. For *bcl-2* and Ki-67, pretreatment in a pressure cooker (30 minutes, 115°C , citrate buffer, pH 6.0) was performed by hand, and slides were transferred to a DakoCytomation autostainer (Carpinteria, CA) for subsequent steps, using the Envision Plus™ system for signal detection.

TABLE 2
Antibody Reagents Used for Flow Cytometry

Antibody	Clone	Source
CD3	SK7	Becton Dickinson ^a
CD4	SK3	Becton Dickinson
CD5	L17F12	Becton Dickinson
CD7	4H9	Becton Dickinson
CD8	SK1	Becton Dickinson
CD10	W8E7	Becton Dickinson
CD13	L138	Becton Dickinson
CD14	MOP9	Becton Dickinson
CD16	3G8	Coulter ^b
CD19	J4.119	Coulter
CD20	L27	Becton Dickinson
CD23	EBUCS-5	Becton Dickinson
CD33	P67.6	Becton Dickinson
CD34	HPCA-2	Becton Dickinson
CD56	MY31	Becton Dickinson
CD61	Y2-51	DakoCytomation ^c
CD64	FCGR-1	Coulter
CD79a	Mb-1	Coulter
HLA DR	L243	Becton Dickinson
FMC 7	FMC7	Coulter
KAPPA	Kappa	Becton Dickinson
LAMBDA	Lambda	Becton Dickinson
CD45	2d1	Becton Dickinson
MPO	MPO7	DakoCytomation

^a Becton Dickinson (San Jose, CA).

^b Coulter (Hiialeah, FL).

^c DakoCytomation (Carpinteria, CA).

Fluorescence In Situ Hybridization Analysis

Six of 7 cases had FISH performed on the FNAB material, whereas 1 had FISH performed on a concurrent positive ascites specimen (Case 4). A cell suspension was submitted for FISH analysis in 4 cases, whereas 3 cases had the study performed on paraffin sections of formalin-fixed tissue specimens (Table 4). Interphase FISH analysis was performed with the *c-myc* dual-color breakapart probe (Vysis, Danners Grove, IL), or with the LSI[®] IgH/*bcl-2* dual-color, dual-fusion translocation probe (Vysis). Submitted cell suspensions were processed by standard cytogenetic methodologies of hypotonic shock with 0.075 M KCl, fixation with a 3:1 ratio of methanol to acetic acid and air-dried slide preparation. Slides were subsequently pretreated using a VP2000 processor (Vysis) programmed to include a 2 ×X sodium chloride sodium citrate (SSC) incubation (73 °C, 2 minutes), deproteinization (Protease I solution [Vysis], 37 °C, 5 minutes), fixation in 1% buffered formaldehyde/20 mM MgCl₂ (20 °C, 5 minutes), and a 70%/85%/95% ethanol dehydration series. Paraffin sections were pretreated with the following parameters: deparaffinization with Hemo-De (Fisher Scientific, Fair Lawn, NJ), pretreatment (Pre-

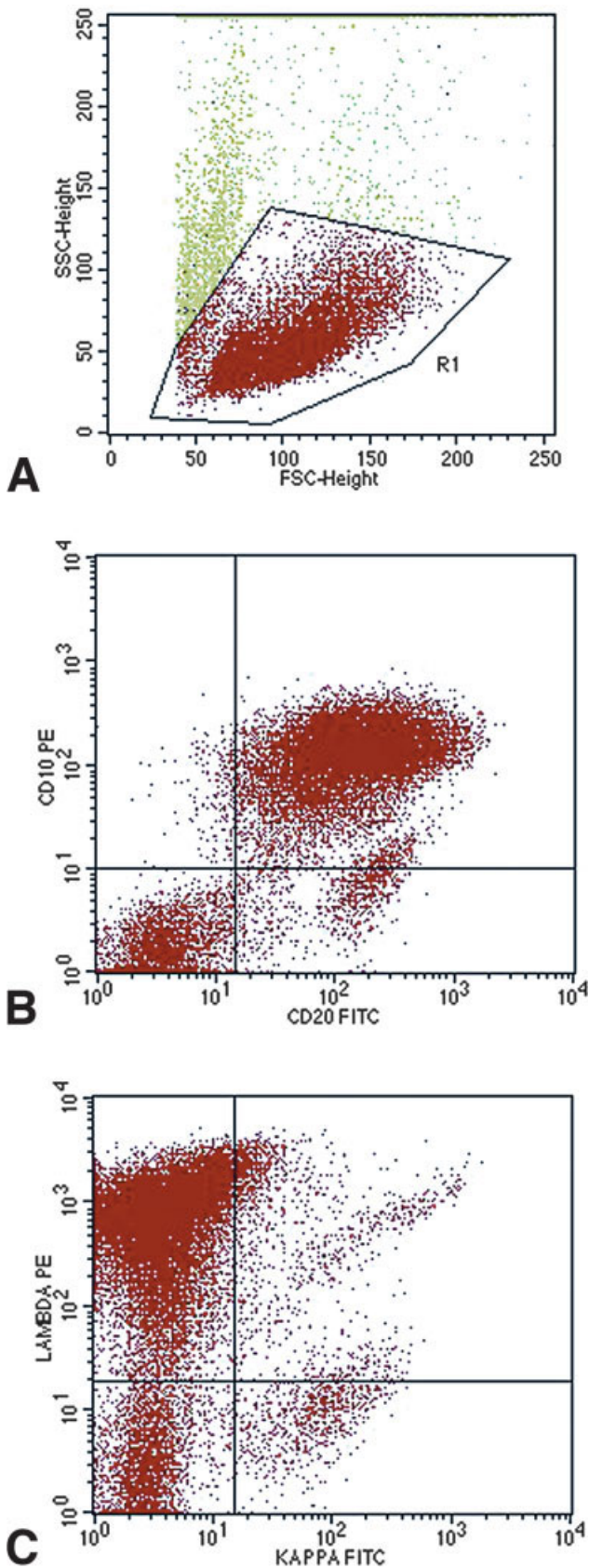
treatment Reagent [Vysis], 80 °C, 30 minutes), deproteinization (Protease I solution [Vysis], 37 °C, 45 minutes), 10% buffered formalin fixation (20 °C, 10 minutes), and ethanol dehydration. FISH probes were prepared and applied per vendor instruction and hybridized using a HyBrite hybridization instrument (Vysis) with the following parameters: denaturation (75 °C, 1 minute)/hybridization (37 °C, 16 hours) for cell suspension specimens or denaturation (73 °C, 6 minutes)/hybridization (37 °C, 16 hours) for paraffin sections. A posthybridization wash was performed in 0.4 × SSC, 73 °C/2 minutes followed by 2 × SSC, 20 °C/1 minute. Slides were coverslipped with 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI) II solution (Vysis) and visualized using a CytoVysion (Applied Imaging, San Jose, CA) capture system-equipped Olympus BH51 fluorescent microscope with DAPI, and with spectrum orange and spectrum green single-pass filter sets (Chroma Technology Corp, Rockingham, NC) and a spectrum orange/spectrum green dual-pass filter set (Chroma Technology Corp). For each probe/specimen, 200 nuclei were screened for a signal pattern consistent with *c-myc* or IgH/*bcl-2* gene rearrangement.

RESULTS

Seven cases were identified from the files of Stanford University Medical Center. *c-myc* analysis was performed on cytologic specimens by FISH. These cases were either clinically or histopathologically suspicious for a high-grade lymphoma, particularly Burkitt lymphoma. Immunophenotyping by either flow cytometry, or immunohistochemical studies on formalin-fixed tissue specimens, or both was available on all FNAB specimens. Patients' demographic data are presented in Table 1.

Cytologic Features

We applied the 2001 WHO leukemia/lymphoma classification system criteria for the diagnosis of Burkitt or atypical Burkitt lymphoma. We required a monomorphic population of medium sized cells, with rounded nuclear contours, and deep blue cytoplasm containing small clear vacuoles with crisp borders.¹ However, 3 of our cases did not demonstrate these features (Cases 1, 3, and 7). Cases 4, 5, and 6 demonstrated cytologic features compatible with a diagnosis of Burkitt lymphoma, as illustrated in Figure 1. Case 2 had the characteristic cytoplasmic features, but had variably sized nuclei, including many large nuclei, some with cleaved nuclear contours. This case was interpreted as atypical Burkitt lymphoma. In contrast, smears for Case 1 were paucicellular, but clearly demonstrated medium to large lymphocytes with pale cytoplasm,



irregular nuclear contours, small nucleoli, but only rare cells with small cytoplasmic vacuoles. Case 1 had a concurrent excisional biopsy which showed effacement of the involved lymph node by a proliferation of atypical cells. Focally, the so-called “starry-sky” pattern was seen at low power. High-power examination revealed sheets of malignant cells of variable size, mostly large, and some with abundant cytoplasm. Nuclei were pleomorphic, with multiple nucleoli, and coarse chromatin. The large cell size, pleomorphic nuclei, and chromatin features seen on the tissue biopsy sample also precluded a diagnosis of Burkitt lymphoma. FNAB material from Case 3 revealed a heterogenous atypical lymphoid population consisting of medium to large cells, a few with cleaved nuclei. Only occasional cells had cytoplasmic vacuoles, and some cells had more abundant pale cytoplasm, typical of plasmacytoid lymphocytes. Case 7 consisted of scant medium to large lymphoid cells with pale grey-blue cytoplasm, rare vacuoles, and irregular nuclear contours. Thus, Cases 1, 3, and 7 did not demonstrate cytologic features of Burkitt or atypical Burkitt lymphoma.

Immunophenotypic Analysis

Immunophenotyping by flow cytometry was performed on the FNAB samples from Patients 2, 3, 4, 5, and on a concurrent biopsy or thoracentesis specimen (Patients 1, 6, respectively). Flow cytometry was not performed for the specimen from Patient 7. Gating on medium to large cells demonstrated that the atypical population was CD19+/CD20+/CD10+/CD5- for all specimens, with the exception of Case 4, which had no CD10 detectable by flow cytometry. Cases 1, 3, 5, 6 were tested for CD23 expression by flow cytometry, and all were negative. Five of 6 cases demonstrated light chain restriction, Cases 1 and 5 were kappa monotypic, whereas Cases 3, 4, and 6 were lambda monotypic (Fig. 2, Table 4). Case 2 demonstrated a kappa-to-lambda ratio within normal limits, both by surface and cytoplasmic light chain analysis (Table 4).

Immunohistochemical studies were performed on cell block material from 6 of 7 FNAB specimens, and on the concurrent excisional biopsy material for Case 1. Immunoperoxidase stains for CD10 and/or B-cell markers confirmed the flow cytometry results, with 1 discrepancy. In Case 4, no CD10 staining was demon-

FIGURE 2. Selected flow cytometry data from Case 6. (A) Forward versus side scatter plot with atypical lymphoid gate delineated. (B) CD20 versus CD10 demonstrating B-cell phenotype with CD10 coexpression. (C) Kappa versus lambda light chains showing lambda monotypia.

TABLE 3
Antibody Reagents and Conditions Used for Immunohistochemistry

Antibody	Clone	Source ^a	Dilution	Treatment
CD20	L26	Dako	1:2000	Ventana-standard
CD10	56C6	Novocastra	1:20	Ventana-standard
<i>bcl-2</i>	124	Dako	1:50	Dako-pressure, citrate, pH6
Ki-67	IVAK-2	Dako	1:200	Dako-pressure, citrate, pH6

^a DakoCytomation (Carpinteria, CA); Novocastra (Hingham, MA); Ventana (Tucson, AZ).

strated by flow cytometry, but CD10 was positive by immunohistochemistry. All 7 cases had staining of > 90–100% of nuclei by the proliferation marker Ki-67. *bcl-2* was negative in 4 of 7 cases, whereas strong staining was seen in Case 1. *bcl-2* staining was equivocal in Cases 6 and 7, with positive staining in a subset of mostly small cells.

Fluorescence In Situ Hybridization Analysis

FISH studies were performed on the FNAB material from all 7 specimens using the Vysis LSI[®] MYC dual-color breakapart rearrangement probe, and in 2 cases with the Vysis LSI[®] IgH/*bcl-2* dual-color, dual-fusion translocation probe. In four cases, FISH was performed on a cell suspension from the FNAB specimen processed by standard cytogenetic methodologies (hypotonic shock and methanol-acetic acid fixation). In three cases, FISH was performed on sections cut from formalin-fixed, paraffin-embedded material. Although equivalent results were obtained with both methods, scoring of signals from individual cells was more straightforward in the specimens prepared from cell suspensions. Scoring of FISH signals on paraffin-embedded material can be complicated by overlapping of nuclei present on the tissue sections, hybridization inefficiency caused by the more complex prehybridization methodology, and loss of signal caused by sectioning through nuclei. Six of 7 cases demonstrated rearrangement of *c-myc* (Fig. 3, Table 4). Case 7, which was morphologically inconsistent with Burkitt lymphoma, was negative for the *c-myc* rearrangement. Two cases were assayed for IgH/*bcl-2* fusion (Cases 1, 3), and Case 1 was positive for the fusion product (Fig. 3).

Diagnostic Summary

Although 6 of our 7 cases were positive for *c-myc* rearrangement, only 3 cases (Cases 4, 5, and 6) had morphologic, immunophenotypic and cytogenetic features diagnostic of Burkitt lymphoma. One case (Case 2) had atypical morphologic features, compatible with atypical Burkitt lymphoma; immunophenotypic and cytogenetic findings supported this diagno-

sis, though the lack of light chain monotypia is not characteristic. Three cases (Cases 1, 3, 7) did not meet criteria for a diagnosis of Burkitt lymphoma. Case 1 was comprised of large cells morphologically incompatible with Burkitt lymphoma, as described above, and demonstrated *bcl-2* expression, as well as IgH/*bcl-2* fusion indicative of t(14;18), suggesting origin from follicular lymphoma. Both the FNAB and biopsy specimens were diagnosed as DLBCL. Case 3 also demonstrated cytologic features precluding a diagnosis of Burkitt lymphoma, with plasmacytoid large cells. In the setting of a patient status post kidney transplantation, a diagnosis of monomorphic posttransplant lymphoproliferative disorder (PTLD), favor DLBCL was made. Case 7 was negative for *c-myc* rearrangement and also lacked morphologic features of Burkitt lymphoma. This case was diagnosed as an aggressive B-cell lymphoma, but further classification was not possible given the paucicellular nature of the specimen.

DISCUSSION

The diagnostic categories of Burkitt lymphoma and atypical Burkitt/Burkitt-like lymphoma have been defined more precisely with the WHO 2001 leukemia/lymphoma classification system.¹ Nevertheless, a definitive diagnosis of Burkitt lymphoma may be difficult, even on tissue sections. The application of ancillary diagnostic techniques such as immunophenotyping and genotypic analysis has become central to the evaluation of hematolymphoid neoplasms, including Burkitt lymphoma.¹ Refinement of these techniques has allowed application to small samples, including those obtained by FNAB.^{17,22–29} Of our series of seven FNAB samples, six had *c-myc* rearrangement demonstrated by FISH analysis. However, only three of these were ultimately diagnosed as Burkitt lymphoma, with one additional case of atypical Burkitt lymphoma. The other 2 *c-myc*-positive cases were aggressive mature B-cell neoplasms: DLBCL (Case 1) and monomorphic PTLD, favor DLBCL (Case 3). The *c-myc*-negative case represented an aggressive B-cell lymphoma (Case 7). These cases reemphasize the importance of integrating data from morphology and ancillary techniques in the FNAB diagnosis of high-grade B-cell lymphomas.

To facilitate these important studies, the FNAB material must be carefully allocated at the time of aspiration. In addition to air-dried and alcohol-fixed smears, we recommend collecting fresh material in RPMI-1640 or saline for ancillary studies. Placing material into transport media rather than fixative allows the greatest flexibility in the studies that can be performed. Fresh material is required for flow cytometry,

TABLE 4
Immunophenotypic and Molecular Characterization of Cases

Case	<i>c-myc</i> FISH	CD19/20	CD10	<i>bcl-2</i>	Light chain	Ki-67	Final diagnosis
1	Pos	Pos	Pos	Pos ^a	kappa	Variable ^a	Large B-cell lymphoma
2	Pos	Pos	Pos	Neg ^{a,b}	none	> 95% ^a	Atypical Burkitt lymphoma
3	Pos	Pos	Pos	Neg ^a	Lambda	> 95% ^a	Monomorphic PTLD
4	Pos	Pos	Pos ^a	Neg ^a	Lambda	100% ^a	Burkitt lymphoma
5	Pos ^a	Pos	Pos	Neg ^a	kappa	100% ^a	Burkitt lymphoma
6	Pos ^a	Pos	Pos	Eq ^a	Lambda	100% ^a	Burkitt lymphoma
7	Neg ^a	Pos ^a	ND	Eq ^a	ND	> 90% ^a	Aggressive B-cell lymphoma

ND: not done; Pos: positive; Neg: negative; Eq: equivocal; FISH: fluorescence in situ hybridization; PTLD: posttransplant lymphoproliferative disorder.

^a Formalin-fixed, paraffin-embedded tissue section used for immunohistochemistry or FISH.

^b By outside report.

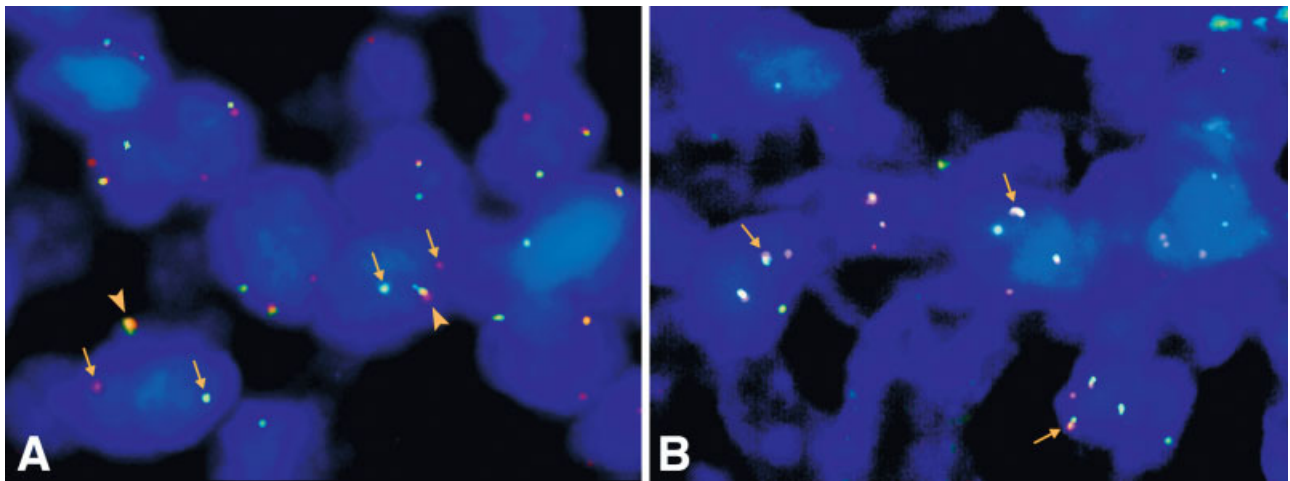


FIGURE 3. Fluorescence in situ hybridization studies. (A) *c-myc* breakapart probe (Case 6) demonstrates cells with spatial separation of red 5'-*myc* and green 3'-*myc* signals consistent with rearrangement of one *myc* allele (arrows). The normal allele appears as a fused yellow (as overlapping of green and red, arrowhead) signal. (B) Immunoglobulin H (IgH)/*bcl-2* translocation fusion probe (Case 1) demonstrates cells with dual IgH-*bcl-2*/*bcl-2*-IgH red-green signal fusions (generating yellow, arrows), consistent with t(14;18)-mediated IgH-*bcl-2* gene rearrangement.

which is essential when lymph node aspirates are obtained for the evaluation of lymphoproliferative disorders. Fresh material can also be sent for molecular-cytogenetic and FISH analysis, as well as used to prepare a paraffin-embedded cell block or unstained cytopsin slides. Cell block material and cytopsin slides can be used for the determination of growth fraction by Ki-67 immunohistochemical staining, and *bcl-2* immunophenotyping, as well as for more extensive immunohistochemical studies that may be needed to confirm or extend immunophenotypic results.²²⁻²⁹(and references therein) In addition, sections of formalin-fixed, paraffin-embedded tissue can now be used for FISH studies (e.g. *c-myc* and *bcl-2* rearrangements), as demonstrated in the current study, and can also be sent for PCR studies, for example, t(14;18) and T-cell receptor gene rearrangements.²²⁻²⁹ Although FISH analysis and immu-

noperoxidase stains also can be performed on cytopsin slides or on air-dried unstained direct smears, immunoperoxidase protocols in our laboratory, including epitope retrieval, are optimized for paraffin-embedded tissue specimens, and we prefer to prepare cell blocks for these studies. In preparing cell blocks, alcohol-based fixatives should be avoided because they can interfere with immunohistochemical staining. In addition, eosin should not be used to facilitate embedding, because it can lead to autofluorescence in FISH analysis.

Although evidence of the *c-myc* rearrangement is essentially required for a diagnosis of Burkitt or atypical Burkitt/Burkitt-like lymphoma, the presence of the *c-myc* rearrangement is not specific for Burkitt or Burkitt-like lymphoma. *c-myc* rearrangements, or translocations involving 8q24, have been reported in a number of other types of B-cell lymphomas, including

B-ALL, follicular lymphoma, DLBCL, and multiple myeloma, as well as cases in which the *c-myc* rearrangement was documented only after transformation from a low-grade to a high-grade lymphoma.⁵⁻¹⁶ Published prevalence rates of the *c-myc* alteration by lymphoma subtype are variable. In general, molecular and/or cytogenetic studies show that < 10% of adult DLBCL cases harbor the *c-myc* alteration,^{5,7-12,15} and approximately 8-10% of high-grade or transformed follicular lymphomas have the *c-myc* alteration,^{5,6,13,16} as detected by molecular methods. Yano et al.⁶ studied 38 cases of transformed follicular lymphomas, 3 (8%) of which showed the *c-myc* rearrangement by Southern blot analysis (1 small, noncleaved and 2 DLBCL). In these three cases, patient survival was < 16 months posttransformation. Two of the three cases with the *c-myc* rearrangement had material from the antecedent low-grade lymphomas available for analysis, and the *c-myc* rearrangement was not present in either.⁶ Likewise, Lee et al.³⁰ reported transformation of a Grade I follicular lymphoma with t(14;18) to a high-grade, small, noncleaved lymphoma with the identical t(14;18) breakpoints, and acquisition of the *c-myc* rearrangement. Karsan et al.⁸ report 4 novel cases with t(14;18) and Burkitt-type rearrangement, all with extremely aggressive clinical courses. In addition, they reviewed 22 cases previously reported in the literature with the *c-myc* rearrangement, some with documented antecedent lymphoma of lower grade. In the reviewed cases, morphologic diagnoses included many cases of ALL (ALL-L2 or Burkitt type), several cases of high-grade non-Hodgkin lymphoma (large cell or not otherwise specified), and individual cases of chronic lymphocytic leukemia/lymphoma and prolymphocytic leukemia/lymphoma.⁸ Our Case 1 may represent an example of follicular lymphoma transformed to DLBCL with acquisition of the *c-myc* rearrangement. Given that the *c-myc* alteration appears to confer a poor prognosis on patients with lymphoma,^{5,10-13,16,31,32} investigation of *c-myc* on such FNAB samples can provide useful clinical information even if the diagnosis of Burkitt or atypical Burkitt lymphoma is not made.

The same caution must be applied to overinterpretation of immunophenotypic data, especially given an FNAB sample, where the architectural context is absent. CD10 is commonly known as a marker of germinal center derivation, and germinal center-derived lymphomas include follicular lymphoma, Burkitt lymphoma, and some cases of DLBCL.^{33,34} Normal or abnormal early lymphoid progenitors are also CD10 positive, including those in the thymus, pediatric bone marrow specimens, and ALL (where CD10 derived its original name common acute lymphoblastic leukemia

antigen [CALLA]).³³ *bcl-2* is overexpressed in a number of different types of lymphoma, including 30-50% of DLBCL.¹ *bcl-2* is most commonly negative in cases of Burkitt lymphoma. However, cases of *bcl-2* positivity have been reported in 12-15% of Burkitt or atypical Burkitt lymphomas in several small series.^{18,19,35} As mature B-cell neoplasms, Burkitt lymphoma should be derived from cells with rearranged Ig, most often seen as monotypic light chain expression.^{21,36} Although the CD10+/*bcl-2*-/TdT-/light chain monotypic immunophenotype can be helpful in distinguishing Burkitt-type lymphoma from other distinct entities with blastic morphology, such as ALL and blastic mantle cell lymphoma, it is also not specific. In addition, these entities may also harbor *c-myc* rearrangements.^{8,37} Morphology remains essential for distinguishing Burkitt lymphoma from other lymphomas with a similar immunophenotype.

The cases reported in the current study confirm that a diagnosis of Burkitt and atypical Burkitt lymphoma can be made on cytologic specimens, as previously reported by Stastny et al.²¹ and Das et al.²⁰ Further, they illustrate that FNAB allows material to be obtained for important ancillary studies. However, these cases also demonstrate that careful correlation of cytologic, immunophenotypic, and molecular diagnostic data is essential when considering the diagnosis of Burkitt lymphoma.

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