

Fine needle aspiration cytology diagnosis of malignant lymphoma and reactive lymphoid hyperplasia

C J R Stewart, J A Duncan, M Farquharson, J Richmond

Abstract

Aims—To assess the diagnostic accuracy of lymph node fine needle aspiration (FNA) cytology to distinguish reactive lymphoid hyperplasia from malignant lymphoma, and to evaluate the contribution of ancillary techniques applied to cytological material.

Methods—Two hundred and seventy seven consecutive lymph node FNA specimens reported to be consistent with reactive lymphoid hyperplasia (n = 213) or suggestive/diagnostic of malignant lymphoma (n = 64) were reviewed. Follow up data were obtained by case record review or by histological correlation. The value of immunocytochemistry, in situ hybridisation for immunoglobulin light chain mRNA, and polymerase chain reaction (PCR) towards the final clinicopathological diagnosis was assessed in 92, 61, and 45 cases, respectively.

Results—Sixty one of 67 lymphomas and 207 of 209 reactive lymph nodes were accurately diagnosed by FNA cytology. There were six false negative aspirates including three cases of follicular lymphoma, two cases of Hodgkin's disease, and one chronic lymphocytic leukaemia. Two FNA specimens considered suspicious of lymphoma proved reactive on histology or clinical follow up. One metastatic small cell carcinoma was wrongly diagnosed as lymphoma. Ancillary studies contributed to the correct diagnosis in most cases although occasional misleading results were obtained, particularly with PCR.

Conclusions—FNA cytology accurately distinguished reactive lymphoid hyperplasia from malignant lymphoma in 97% of cases. However, occasional wrong diagnoses occurred owing to sampling error or misinterpretation. Ancillary studies can be applied to cytological samples and contribute to the diagnosis in most cases.

(*J Clin Pathol* 1998;51:197-203)

Keywords: fine needle aspiration; cytology; lymphoma; lymph node

Fine needle aspiration (FNA) cytology is being used increasingly in the assessment of patients with lymph node enlargement. The technique is safe and simple and can be used to sample multiple sites. The use of rapid staining techniques often allows a provisional diagnosis to be made at the patient's initial presentation

and, therefore, helps to guide appropriate specialist referral and further investigation.¹

FNA cytology is highly reliable in the identification of metastatic carcinoma and melanoma in lymph nodes, limiting the requirement for diagnostic excision biopsy in many patients. However, the role of aspiration cytology in the assessment of primary lymphoproliferative disorders has been less certain.² Both clinicians and pathologists have doubted whether an accurate cytological diagnosis of lymphoma is possible, and early reports suggested that FNA produced high false negative rates in patients with Hodgkin's disease and low grade non-Hodgkin's lymphoma. In addition, the complexity of lymphoma classifications and the prognostic importance of architectural assessment in some lymphoid tumours limits the extent of cytological correlation with histology. However, recent studies have indicated that an accurate diagnosis of lymphoma can be achieved by FNA in 85-90% of cases, particularly when morphological assessment is complemented by the use of immunocytochemical techniques.²⁻⁴ Furthermore, genotypic analysis including Southern blot and polymerase chain reaction (PCR) to detect immunoglobulin and T cell receptor gene rearrangements, and in situ hybridisation (ISH) to detect immunoglobulin light chain mRNA expression may be applied successfully to FNA specimens.⁵⁻⁹ Skoog and Tani have suggested that lymph node aspiration cytology supplemented by appropriate ancillary investigations offers similar diagnostic accuracy to excision biopsy.¹⁰

Previously, we described the use of immunocytochemistry, ISH, and PCR in the assessment of small series of lymphoid aspirates derived from both lymph nodes and extranodal sites.^{8,9} In this report, we studied the value of these techniques in routine practice, and examined the diagnostic accuracy of FNA in distinguishing malignant lymphoma from reactive lymphoid hyperplasia in a series of 277 consecutive lymph node aspirates performed in Glasgow Royal Infirmary.

Methods

DIAGNOSTIC CASES

All fine needle aspiration performed on patients with palpable lymph node enlargement in Glasgow Royal Infirmary between January 1993 and May 1997 were reviewed. The aspirates were performed by cytology staff using 23 or 25 G needles. In most cases, needles were attached to 10 ml syringes with a syringe holder (Cameco, London, UK), but a few

Department of Pathology, The Royal Infirmary, Castle Street, Glasgow G4 0SF, UK

C J R Stewart
M Farquharson
J Richmond

Department of Medical Oncology, The Royal Infirmary
J A Duncan

Correspondence to:
Dr Stewart.

Accepted for publication
9 December 1997

Table 1 Correlation of FNA diagnosis and final clinicopathological diagnosis in 277 lymphoid aspirates

FNA diagnosis	Final clinicopathological diagnosis			
	Reactive	NHL	HD	Met CA
Reactive lymphoid hyperplasia (n = 213)	207	4	2	–
Diagnostic of NHL (n = 40)	–	39	–	1
Suggestive of NHL (n = 8)	1	7	–	–
Diagnostic of HD (n = 13)	–	–	13	–
Suggestive of HD (n = 3)	1	–	2	–

NHL, non-Hodgkin's lymphoma; HD, Hodgkin's disease; Met CA, metastatic carcinoma.

specimens were obtained using the non-aspiration technique. Routinely, two aspirates were performed and direct smears were prepared for staining with Diff-Quick (Dade, Abingdon, UK), May-Grunwald-Geimsa, and/or Papanicolaou methods. Thereafter, the needles were rinsed in 10 ml normal saline. In selected cases, cytospin preparations from the saline washes were used for special techniques, and the washes were submitted for culture in those cases where infection was suspected.

In total, 549 lymph node aspirates were performed during the study period of which 235 (42.8%) showed metastatic carcinoma or melanoma. Thirty seven (6.7%) FNA specimens were inadequate for diagnosis. The remaining 277 aspirates showed a lymphoid pattern that was considered reactive in 213 cases (38.8%) and diagnostic or suggestive of malignant lymphoma in 64 cases (11.7%); these lymphoid aspirates form the basis of this study.

The diagnostic accuracy of FNA cytology was assessed by histological correlation or by clinical follow up. Clinical data were obtained by review of case records or by correspondence with referring physicians and general practitioners. The follow up period for those specimens diagnosed as reactive lymphoid hyperplasia averaged 13 months.

SPECIAL TECHNIQUES

Additional studies were performed on selected aspirates to aid distinction between reactive and neoplastic lymphoid proliferations and, in a few cases, to distinguish lymphoid from non-lymphoid neoplasms.

Immunocytochemistry, ISH to detect immunoglobulin light chain mRNA, and PCR for immunoglobulin heavy chain gene rearrangement were used, as described previously.^{8,9} Some specimens were not subject to ancillary studies owing to insufficient material in the needle rinse sample. FNA sampling was not usually repeated if the cytomorphology on routine preparations in conjunction with the clinical findings were sufficient to warrant excision biopsy of the node. Immunocytochemistry was used throughout the period of the study. In most cases, a panel of antisera to CD45, CD20, CD79a, CD43 (all Dako, High Wycombe, UK), CD3 (SAPU, Carlisle, UK), and immunoglobulin light chains was used for routine diagnostic assessment. Antisera to CD15 (SAPU) and CD30 (Dako) were included if Hodgkin's disease was suspected. Immunostaining for cytokeratin (Dako), S100 protein (Biomen, Finchampstead, UK), and HMB45

(Dako) was performed in those cases in which metastatic carcinoma or melanoma were included in the differential diagnosis. In situ hybridisation and PCR were used in cases of suspected B cell lymphoma, mainly in the latter half of the study period.

In each case, an attempt was made to categorise the value of the special techniques in reaching the final clinicopathological diagnosis. The technique was considered helpful if it supported or confirmed the correct diagnosis, unhelpful if it was essentially non-contributory, and misleading if it suggested either a false negative (reactive pattern in proven lymphoma) or a false positive (monoclonal pattern in proven reactive lymph node) diagnosis.

Results

CLINICOPATHOLOGICAL CORRELATION

The 277 lymphoid aspirates were obtained from 260 patients. Fifteen patients underwent FNA on two occasions and one patient had three aspirates.

The correlation between the cytological diagnoses and the final clinicopathological assessment is summarised in table 1.

Sixty four aspirates were considered diagnostic (n = 53) or suspicious (n = 11) of lymphoma and, of these, 47 represented the primary diagnosis of a lymphoproliferative disorder while 17 represented recurrent disease after treatment. Forty eight specimens were considered to be derived from non-Hodgkin's lymphoma, of which 46 were reported as B cell lymphomas on the basis of cytomorphology and ancillary investigations; two non-Hodgkin's lymphomas were not otherwise specified cytologically. Sixteen cases were reported as suggestive or diagnostic of Hodgkin's disease. In general, no attempt was made to subclassify lymphomas further on FNA cytology.

Of those cases considered diagnostic of lymphoma cytologically, clinicopathological correlation confirmed the FNA diagnosis in 39 of 40 non-Hodgkin's lymphomas and in all 13 cases of Hodgkin's disease. However, one aspirate considered diagnostic of non-Hodgkin's lymphoma was shown to be metastatic small cell carcinoma on excision biopsy. Lymph node biopsy was confirmatory in eight of 11 aspirates reported as suspicious of lymphoma, while one further case (not biopsied) was clinically consistent with a Burkitt-type lymphoma. Histology in one case and clinical follow up in another indicated reactive lymphoid changes in the remaining two suspicious cases.

Histological correlation was available in 44 non-Hodgkin's lymphomas from 40 patients. The biopsy diagnoses were follicular lymphoma (n = 16), diffuse large B cell lymphoma (n = 12), lymphocytic lymphoma/chronic lymphocytic leukaemia (n = 5), monocytoid B cell lymphoma (n = 3), lymphoplasmacytic lymphoma/myeloma (n = 2), low grade B cell lymphoma not otherwise specified (n = 3), centrocytic lymphoma, B lymphoblastic lymphoma (n = 1), and post-transplant lymphoproliferative disorder (n = 1). Lymph node biopsies were performed in 15 cases of Hodgkin's disease from 13 patients. These

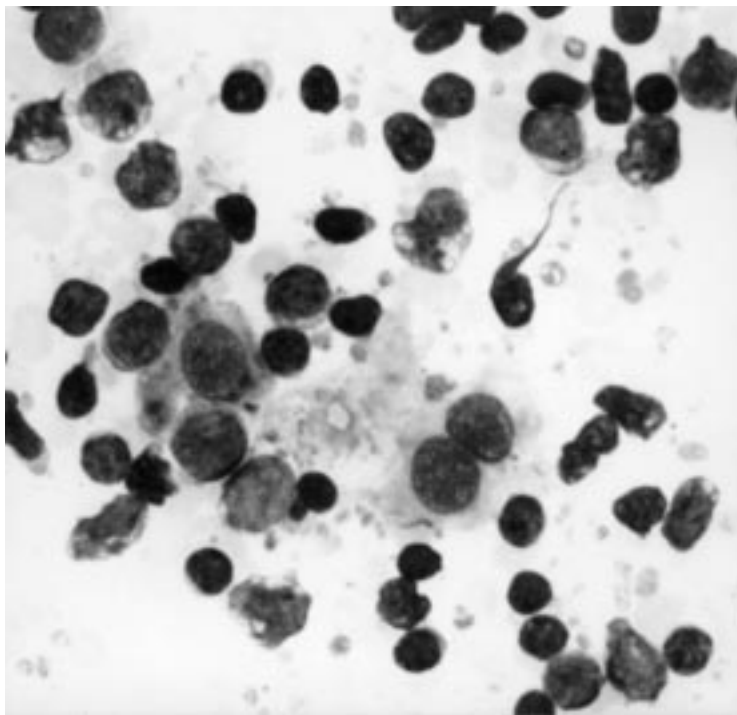


Figure 1 Lymph node fine needle aspirate showing histiocytes (centre), small lymphocytes and occasional intermediate sized cells of probable follicle centre cell origin (May-Grunwald-Giemsa stain). Biopsy revealed follicular lymphoma.

were classified histologically as nodular sclerosis ($n = 9$), mixed cellularity ($n = 3$), lymphocyte predominance nodular ($n = 1$), and follicular variant ($n = 1$) subtypes; one case was not further categorised histologically because only a core biopsy was received.

Although histological assessment was usually advocated to confirm a primary cytological diagnosis of lymphoma, eight patients (six non-Hodgkin's lymphoma, two Hodgkin's disease) were considered medically unfit for lymph node biopsy. The FNA diagnoses were consistent with the clinical and radiological findings in these patients. In general, biopsy was not performed in cases of recurrent lymphoma.

Clinical follow up data alone was consistent with the cytological diagnosis of reactive lymphoid hyperplasia in 181 of 213 cases. Thirty two patients with clinically suspicious or persistent lymphadenopathy underwent lymph node biopsy. Histological assessment confirmed reactive changes in 26 cases, three of which showed a granulomatous lymphadenitis due to tuberculosis. Biopsy in six cases revealed malignant lymphoma; these false negative aspirates are detailed below.

Case 1—A 21 year old, otherwise asymptomatic woman presented with a 1–2 cm right supraclavicular lymph node. The FNA specimen was of low cellularity but showed a mixed lymphoid population with occasional histiocytes consistent with a reactive node. Special techniques were not performed. The patient was discharged and presented again four months later with systemic symptoms and a 5 cm supraclavicular mass which, on repeat FNA, was suspicious of Hodgkin's disease, this being confirmed on subsequent excision bi-

opsy. Review of the initial aspirate showed no evidence of Hodgkin's disease.

Case 2—A 52 year old man presented with a four week history of painless right submandibular swelling. FNA showed a polymorphous lymphoid population with scattered histiocytes consistent with a reactive lymph node (fig 1). Immunocytochemistry revealed an admixed T and B cell population with apparent polytypic immunoglobulin light chain expression. ISH was technically unsatisfactory. PCR showed a polytypic pattern of immunoglobulin heavy chain rearrangement. The mass was clinically suspicious and therefore biopsied three weeks later, revealing a follicular lymphoma, centrocytic/centroblastic. On review, the cytomorphology was still felt to favour a reactive process.

Case 3—A 43 year old man presented with bilateral groin lymph node enlargement. The cytological appearances and special techniques were similar to case 2, except that ISH was not performed. Biopsy, four weeks after FNA, revealed partial lymph node involvement by a follicular lymphoma, centrocytic/centroblastic.

Case 4—A 67 year old man with a history of follicular lymphoma involving the scalp presented with a small cervical lymph node. The cytological appearances were similar to case 2. ISH showed no light chain expression. Biopsy one week later revealed recurrent follicular lymphoma, centrocytic/centroblastic.

Case 5—A 75 year old man with a history of oral squamous carcinoma presented with small right cervical lymph nodes. FNA was interpreted as reactive lymphoid hyperplasia with no evidence of metastatic carcinoma. Special techniques were not performed. Peripheral blood and bone marrow examinations performed shortly thereafter revealed typical features of chronic lymphocytic leukaemia. Review of the aspirate showed a relatively monotonous population of small lymphocytes consistent with chronic lymphocytic leukaemia.

Case 6—A 74 year old man presented with weight loss. Radiological investigations revealed intra-abdominal and intrathoracic lymphadenopathy. Small palpable axillary nodes were aspirated, providing a sample of low cellularity but including non-caseating epithelioid granulomas (fig 2A). A differential diagnosis including tuberculosis, sarcoidosis, and neoplasia was offered. The needle washes were submitted for culture and further studies were not performed. Lymph node biopsy one week after FNA revealed mixed cellularity Hodgkin's disease with a marked granulomatous reaction (fig 2B). No Reed-Sternberg cells were identified on review of the aspirate material.

While most reactive aspirates showed a non-specific admixture of lymphoid cells and histiocytes, 11 cases showed a granulomatous pattern. Seven of these patients had a subsequent clinical diagnosis of tuberculosis, four of whom showed positive mycobacterial culture on the needle aspirate sample. One patient had clinical features of sarcoidosis and another, who presented with bilateral groin lymphaden-

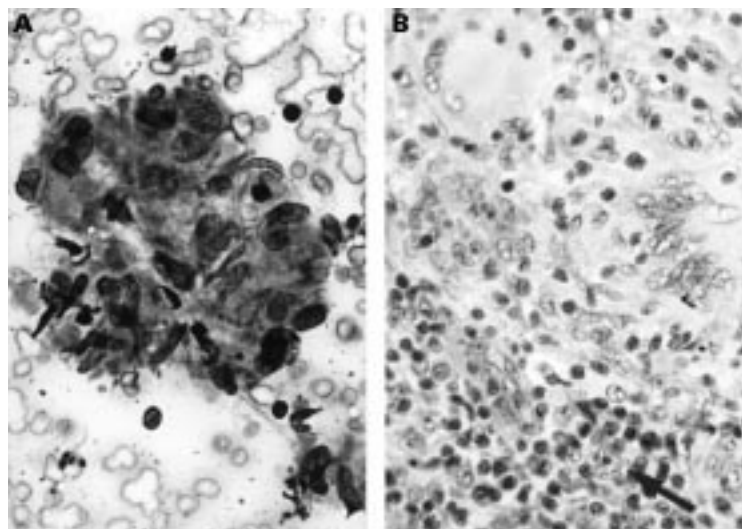


Figure 2 Lymph node FNA showing a cluster of epithelioid histiocytes (A). Sparse small lymphocytes are present at the margin of the granuloma. Excision biopsy showing mixed cellularity Hodgkin's disease with a marked granulomatous reaction (B). Scant Reed-Sternberg cells (arrow) are present (haematoxylin and eosin stain).

opathy, later had serological evidence of chlamydial infection. Excision biopsy in a further case confirmed the FNA diagnosis of a granulomatous reaction to dust pigment in a patient with silicosis. The remaining granulomatous aspirate proved to be from a case of mixed cellularity Hodgkin's disease (false negative case 6).

In total, there were 61 true positive, 207 true negative (reactive), three false positive, and six false negative diagnoses of lymphoma in this series. Therefore, the sensitivity and specificity for a diagnosis of lymphoma were 91% and 95%, respectively. Overall accuracy within the series of lymphoid aspirates was 97%.

USE OF SPECIAL TECHNIQUES

Special techniques were performed in 100 (36%) of the 277 FNA samples and, of these, 49 had a final diagnosis of lymphoma and 51 had a final diagnosis of reactive lymphoid hyperplasia. Immunocytochemistry was performed in 92 cases, ISH in 61, and PCR in 45.

The value of the special techniques towards the final clinicopathological diagnosis is summarised in table 2. Immunocytochemistry was considered helpful in 80 of 92 cases. Typically, reactive lymph nodes exhibited a mixed T and B cell population, and most cases showed polytypic expression of immunoglobulin light chain. In B cell lymphomas, immunocytochemistry usually defined the B cell phenotype of the dominant or abnormal lymphoid element, although T lymphocytes were also present in variable number. Light chain restriction was demonstrated in approximately one third of B cell lymphomas tested by immunocytochemistry while, in other

Table 2 Use and value of special techniques in lymphoid aspirates

	Value of technique		
	Helpful	Unhelpful	Misleading
Immunocytochemistry (n = 92)	80	10	2
In situ hybridisation (n = 61)	47	13	1
Polymerase chain reaction (n = 45)	28	10	7

cases, equivocal immunoreactivity with both kappa and lambda antisera made definite assessment of clonality impossible. Two aspirates showed a small cell malignant population in which immunocytochemistry was required to differentiate lymphoma from small cell carcinoma. The Reed-Sternberg cell associated antigens, CD15 and CD30, were demonstrated in most cases of Hodgkin's disease.

Immunocytochemistry was unhelpful in 10 aspirates, either as a result of insufficient material or technically unsatisfactory staining, and was misleading in two cases, both of which were shown to be follicular lymphoma on biopsy (false negative cases 2 and 3). These cases showed an admixed B and T cell population in the cytospin preparations and immunoglobulin light chain expression appeared polytypic.

ISH was considered helpful in 47 of 61 cases. Immunoglobulin light chain restriction was seen in 16 of 24 B cell lymphomas and polytypic light chain expression was seen in 31 of 36 reactive lymph nodes. The technique was unhelpful in 13 cases because no light chain expression was identified. ISH was potentially misleading in only one case, a Burkitt-type lymphoma in which polytypic light chain expression was identified.

PCR was considered to be helpful in 28 of 45 cases. A clonal gene rearrangement was seen in nine of 18 B cell lymphomas and a polyclonal B cell pattern in 19 of 26 reactive lymph nodes. The technique was non-contributory in 10 specimens, because no rearrangement bands were observed. PCR was misleading in seven cases; an apparent monoclonal band was seen in two proven reactive lymph nodes, while a polyclonal pattern was seen in five B cell lymphomas.

Discussion

The distinction between reactive and malignant lymphoid proliferations is the most problematical area in lymph node FNA cytology. This is not surprising, given that excised lymph nodes commonly cause diagnostic difficulty despite the advantage of architectural preservation in biopsy specimens. Aspirate specimens from cases of high grade lymphoma and Hodgkin's disease may show an obvious cytological abnormality, but the diagnosis of low grade lymphomas in cytological preparations is most often based on the presence of a relatively monomorphic lymphoid population, contrasting with the typically polymorphous cell pattern seen in reactive proliferations.^{11 12} Therefore, potential cytological misdiagnoses may occur, either in lymphomas that present an apparently admixed cell pattern (false negative cases), or in reactive proliferations in which atypical cells are identified (false positive cases). For these reasons, excision biopsy is advocated by most authors to confirm a primary cytological diagnosis of lymphoma. However, FNA is being used increasingly to document recurrence of lymphoma or tumour transformation, to allow sampling of multiple sites for staging, to exclude other unrelated causes of lymphadenopathy, and to obtain

samples from surgically inaccessible sites or medially unfit patients.² In our series, eight of 67 cases (12%) of lymphoma did not undergo confirmatory biopsy because of poor general medical condition. However, precise classification of lymphoma was not required in these patients because most were fit only for general supportive therapy. In addition, the cytological diagnosis of reactive lymphoid hyperplasia in the clinically appropriate setting supports conservative management of many patients with lymphadenopathy, reducing the requirement for lymph node biopsy.

In this study of 549 consecutive lymph node aspirates, 54% of satisfactory specimens showed a lymphoid rather than metastatic process. Approximately one quarter of these samples were from cases of lymphoma, which accounted for 22% (67 of 302) of all malignant aspirates in the series. Therefore, lymphoid specimens represented a substantial proportion of all cases in our routine practice. However, the relative importance of lymphoma in FNA practice is variable, being dependent both on the patient population and local referral policy. Hsu *et al* reported only 13 lymphomas comprising 1.8% of all malignant aspirates in a series of 735 lymph node aspirates from patients in Hong Kong.¹³ In contrast, Prasad and colleagues found that lymphomas accounted for 31.6% of malignant lymphadenopathy in a large series of Indian patients.¹⁴ These represented 11.8% of all lymph node aspirates compared with 12.2% in our study.

Sixty one of 67 malignant lymphomas were identified correctly in this study, a sensitivity of 91%. Other recent reports have also shown that FNA cytology is an accurate diagnostic procedure in most cases of lymphoma.¹⁴⁻²² However, metastatic lymph node disease can be established by aspiration cytology with even greater sensitivity, illustrating the relative difficulty in the assessment of lymphoid proliferations. The apparent accuracy of lymphoma diagnosis is also partly dependent on the proportion of primary to recurrent lymphomas in FNA cytology series. In general, evaluation of recurrence is more straightforward owing to the raised clinical suspicion of lymphoid malignancy and the comparison that can be made with previous cytology or biopsy material. Patients with recurrent lymphoma accounted for 84% of cases in the study by Sneige and colleagues,¹⁸ approximately half the cases reported in two further studies,^{20, 21} 27% of cases in this series, but only 15% of cases described by Pilotti and colleagues.¹⁵

There were six false negative errors in this series. Three were follicular lymphomas and the false negative rate in this diagnostic category was therefore 19% (3 of 16). The smear preparations from these cases showed a mixed lymphoid pattern with histiocytes, and even on review it was considered that the morphological appearances favoured a reactive rather than a neoplastic process. Immunocytochemistry and PCR were misleading in two of these cases because they suggested a reactive lymphoid process. Inconclusive immunoglobulin light chain expression was also noted in occasional

FNA specimens of B cell lymphomas by Sneige *et al*, who suggested that partial nodal involvement by lymphoma might be responsible^{18, 23}; this was apparent histologically in one of our cases. Follicular lymphomas may present particular difficulty in FNA specimens because the neoplastic element itself is polymorphous (centrocytes and centroblasts), and there may be a significant population of reactive T lymphocytes and, less commonly, histiocytes. McNeely²⁴ reviewed 14 histologically confirmed follicular lymphomas in which FNA cytology had been performed before biopsy; four cases had been misinterpreted as reactive lymphoid hyperplasia. Ten of 16 follicular lymphomas were misdiagnosed on aspiration cytology in the series of Pilotti and colleagues.¹⁵ Other authors have documented similar difficulties in the diagnosis of follicular lymphoma or, more generally, lymphomas of mixed cell type.^{21, 22} Therefore, it is important that pathologists and clinicians are aware that negative FNA results do not exclude lymphoma in patients with unexplained lymph node enlargement, and that early repeat sampling by FNA or lymph node biopsy should be considered depending on the clinical findings.² Thus, four of the false negative cases in our series were subject to excision biopsy one to four weeks after FNA. In our practice, clinically suspicious lymph nodes are sampled initially by FNA, usually at the patient's first outpatient visit. Smears from the aspirate are examined at the clinic using the Diff-Quick staining method and a provisional diagnosis is offered. If the appearances indicate a lymphoid rather than a metastatic process, a clinical decision to obtain either a core biopsy or arrange formal excision biopsy of the node can be made.

Two erroneous reports in this study involved cases of Hodgkin's disease and were primarily a result of inadequate sampling. In one case, the apparently benign initial aspirate probably delayed further investigation of the patient's lymphadenopathy. Hodgkin's disease may be misinterpreted in cytology samples as Reed-Sternberg cells and their variants may be relatively sparse or masked by the dominant polymorphous infiltrate, which can mimic a reactive nodal hyperplasia.^{22, 23} The presence of epithelioid granulomas, as in one of our cases, may also suggest an infective aetiology.¹⁴ Sampling error is a particular hazard in the nodular sclerosing Hodgkin's disease subtype, possibly because the fibrosis interferes with cell yield.^{12, 25} Proportionately, Hodgkin's disease produced more diagnostic errors than non-Hodgkin's lymphoma in this study (one false positive and two false negative errors out of 17 cases). The sensitivity of FNA in the diagnosis of Hodgkin's disease has been approximately 80% in most studies, although only nine of 30 cases were correctly identified in one recent series.¹⁴

There were three false positive fine needle aspirates. One case of metastatic small cell carcinoma mimicked a small cell lymphoma cytologically, a well established diagnostic pitfall, which often requires immunocytochemical analysis for resolution.¹¹ In this case, immunocytochemistry

preparations were technically unsatisfactory. The two further false positive cases were reported as suspicious rather than diagnostic of lymphoma. Each showed a polymorphous lymphoid population but with apparently disproportionate numbers of large blast cells, mimicking recurrent Hodgkin's disease in one patient. Although over-interpretation of reactive lymphoid aspirates might cause inappropriate concern, in our view it is better to advise biopsy in aspirates exhibiting an atypical lymphoid pattern, particularly if ancillary studies have shown equivocal results.

The use of special techniques in diagnostic cytology specimens is well established and, as in histopathology, has a particularly important role in the assessment of lymphoproliferative disorders.²⁻⁴ Immunocytochemistry is the most widely used technique, being of value in the assignment of undifferentiated neoplasms to the lymphoid category and, more specifically, in documenting the phenotype of suspected neoplastic lymphoid cells to B, T, null, anaplastic, or Hodgkin's disease related subtypes. This study confirmed the value of immunocytochemistry in FNA specimens. The technique was used in 33% of all cases and contributed to the diagnosis in 87% of the tested samples. In two cases, immunocytochemistry was essential to distinguish lymphoma from metastatic carcinoma. Immunocytochemical staining was helpful in demonstrating mixed B and T cell populations and polytypic immunoglobulin light chain expression in many reactive lymph nodes, and in showing the dominant B cell phenotype of most B cell lymphomas. In addition, Reed-Sternberg cell related antigens, such as CD15 and CD30, were demonstrated in many Hodgkin's disease samples. Immunoglobulin light chain restriction was shown in only one third of the B cell lymphomas tested in this series and, therefore, immunocytochemistry did not prove monoclonality in most of these tumours. Likewise, Pilotti and colleagues¹⁵ found immunocytochemistry of limited value in the distinction between reactive lymphoid hyperplasia and low grade B cell lymphoma. Immunocytochemistry can also produce equivocal or misleading results in cases with a small clonal population, as in T cell rich B cell lymphomas.¹⁸⁻²⁶ However, Robins and colleagues²⁷ and Oertel and colleagues²⁸ demonstrated light chain restriction in more than 90% of B cell lymphomas in FNA samples using immunocytochemical methods.

In situ hybridisation for detection of light chain mRNA offers increased specificity and, in our experience, also increased sensitivity compared with immunocytochemistry for the demonstration of light chain restriction in B cell lymphoma.⁹ The technique contributed to the final diagnosis in 47 of 61 (77%) tested specimens in this series. In general, ISH preparations were easier to interpret than those stained immunocytochemically because there was no background staining, which caused interpretative difficulty with the latter, and ISH was also the most specific technique in our study, producing only one potentially misleading result. However, 13 specimens were

non-contributory in that no light chain expression was detected.

The use of PCR to detect immunoglobulin heavy chain gene rearrangement also offers genotypic analysis of B cell proliferations. PCR has been used to detect monoclonality in excised lymph nodes from patients with B cell lymphomas²⁹ and, more recently, the technique has been applied to cytological material, including serous fluid and FNA specimens.⁸⁻³⁰⁻³¹ In this study, PCR was the least valuable technique, contributing to the diagnosis in 62% of tested cases. A higher cell yield was required for PCR than for immunocytochemistry or ISH and there was a relatively high unsatisfactory rate, surprisingly, given the theoretical sensitivity of PCR. More importantly, PCR was potentially misleading in 16% of cases, including two false positive and five false negative results. The former cases, which otherwise showed a reactive pattern on cytomorphology, led us to advise lymph node excision biopsies in both patients. As previously documented, one biopsy showed reactive changes with features suggestive of toxoplasmosis.⁸ The lymphadenopathy resolved spontaneously in the second patient and, therefore, biopsy was not performed. It seems possible that inadvertent sampling of a dominant clone within a reactive lymph node might have produced the apparent monoclonal gene rearrangement pattern in these cases. The false negative cases in the series may also have resulted from sampling error, although the subsequently excised node showed partial involvement by lymphoma in only one case. Perhaps more importantly, the sensitivity of PCR analysis of immunoglobulin gene rearrangements may be low when a limited number of primers is used.³² This is particularly relevant in follicular lymphomas, which accounted for three of the five false negative PCR cases.

In summary, this study of 277 FNA cytology specimens showed an overall diagnostic accuracy of 97% using cytomorphology in conjunction with appropriate ancillary investigations in the analysis of reactive and neoplastic lymphoid proliferations. However, a small proportion of cases were misdiagnosed, either because of sampling error or misinterpretation. It is emphasised that lymph node FNA cytology is complementary to histological assessment and that biopsy is advisable in cases with apparent clinical discrepancy.

- 1 Skoog L, Lowhagen T, Tani E. Lymph nodes. In: Gray W, ed. *Diagnostic cytology*. Edinburgh: Churchill Livingstone, 1995:481-514.
- 2 Katz RL. Cytologic diagnosis of leukemia and lymphoma. Values and limitations. *Clin Lab Med* 1991;11:469-99.
- 3 Katz RL, Caraway NP. FNA lymphoproliferative diseases: myths and legends. *Diagn Cytopathol* 1995;12:99-100.
- 4 Sneige N. Diagnosis of lymphoma and reactive lymphoid hyperplasia by immunocytochemical analysis of fine-needle aspiration biopsy. *Diagn Cytopathol* 1990;6:39-43.
- 5 Lubinsky J, Chosia M, Huebner K. Molecular genetic analysis in the diagnosis of lymphoma in fine needle aspiration biopsies. 1 Lymphomas versus benign lymphoproliferative disorders. *Acta Cytol* 1988;10:391-8.
- 6 Williams ME, Frierson HF, Tabbarah S, et al. Fine needle aspiration of non-Hodgkin's lymphoma. Southern blot analysis for antigen receptor, bcl-2, and c-myc gene rearrangements. *Am J Clin Pathol* 1990;93:754-9.
- 7 Wan JH, Sykes PJ, Orell SR, et al. Rapid method for detecting monoclonality in B cell lymphoma in lymph node aspirates using the polymerase chain reaction. *J Clin Pathol* 1992;45:420-3.

- 8 Jeffers MD, McCarriston J, Farquharson MA, *et al.* Analysis of clonality in cytologic material using the polymerase chain reaction. *Cytopathology* 1997;8:114–21.
- 9 Stewart CJR, Farquharson MA, Kerr T, *et al.* Immunoglobulin light chain mRNA detected by in situ hybridisation in diagnostic fine needle aspiration cytology specimens. *J Clin Pathol* 1996;49:749–54.
- 10 Skoog L, Tani E. The role of fine-needle aspiration cytology in the diagnosis of non-Hodgkin's lymphoma. *Diagn Oncol* 1991;1:12–8.
- 11 Leong ASY, Stevens M. Fine-needle aspiration biopsy for the diagnosis of lymphoma: a perspective. *Diagn Cytopathol* 1996;15:352–7.
- 12 Pontifex AH, Haley L. Fine-needle aspiration cytology in lymphomas and related disorders. *Diagn Cytopathol* 1989; 5:432–5.
- 13 Hsu C, Leung BSY, Lau S-K, *et al.* Efficacy of fine-needle aspiration and sampling of lymph nodes in 1,484 Chinese patients. *Diagn Cytopathol* 1990;6:154–9.
- 14 Prasad RPA, Narasimhan R, Sankaran V, *et al.* Fine-needle aspiration cytology in the diagnosis of superficial lymphadenopathy: an analysis of 2,418 cases. *Diagn Cytopathol* 1996;15:382–6.
- 15 Pilotti S, Di Palma S, Alasio L, *et al.* Diagnostic assessment of enlarged superficial lymph nodes by fine needle aspiration. *Acta Cytol* 1993;37:853–66.
- 16 Das DK, Gupta SK, Datta BN, *et al.* FNA diagnosis of non-Hodgkin's lymphoma and its subtyping under working formulation of 175 cases. *Diagn Cytopathol* 1991;7:487–98.
- 17 Cartagena N, Katz RL, Hirsch-Ginsberg C, *et al.* Accuracy of diagnosis of malignant lymphoma by combining fine-needle aspiration cytology with immunocytochemistry and in selected cases, Southern blotting of aspirated cells: a tissue-controlled study of 86 patients. *Diagn Cytopathol* 1992;8:456–64.
- 18 Sneige N, Dekmezian RH, Katz RL, *et al.* Morphologic and immunocytochemical evaluation of 220 fine needle aspirates of malignant lymphoma and lymphoid hyperplasia. *Acta Cytol* 1990;34:311–22.
- 19 Qizilbash AH, Elavathil LJ, Chen V, *et al.* Aspiration biopsy cytology of lymph nodes in malignant lymphoma. *Diagn Cytopathol* 1985;1:18–22.
- 20 Carter TR, Feldman PS, Innes DJ, *et al.* The role of fine needle aspiration cytology in the diagnosis of lymphoma. *Acta Cytol* 1988;32:848–53.
- 21 Daskalopoulou D, Harhalakis N, Maouni N, *et al.* Fine needle aspiration cytology of non-Hodgkin's lymphomas. A morphologic and immunophenotypic study. *Acta Cytol* 1995;39:180–6.
- 22 Steel BL, Schwartz MR, Ramzy I. Fine needle aspiration biopsy in the diagnosis of lymphadenopathy in 1,103 patients. Role, limitations and analysis of diagnostic pitfalls. *Acta Cytol* 1995;39:76–81.
- 23 Sneige N, Dekmezian R, El-Naggar A, *et al.* 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* 1991;67:1003–7.
- 24 McNeely TB. Diagnosis of follicular lymphoma by fine needle aspiration biopsy. *Acta Cytol* 1992;6:866–8.
- 25 Ramzy I, Rone R, Schultenover SJ, *et al.* Lymph node aspiration biopsy. Diagnostic reliability and limitations—an analysis of 350 cases. *Diagn Cytopathol* 1985;1:39–45.
- 26 Galindo LM, Havlioglu N, Grosso LE. Cytological findings in a case of T-cell rich B-cell lymphoma: potential diagnostic pitfall in FNA of lymph nodes. *Diagn Cytopathol* 1996;14:253–7.
- 27 Robins DB, Katz RL, Swan F, *et al.* Immunotyping of lymphoma by fine-needle aspiration. A comparative study of cytospin preparations and flow cytometry. *Am J Clin Pathol* 1994;101:569–76.
- 28 Oertel J, Oertel B, Kastner M, *et al.* The value of immunocytochemical staining of lymph node aspirates in diagnostic cytology. *Br J Haematol* 1988;70:307–16.
- 29 Ashton-Key M, Diss TC, Isaacson PG, *et al.* A comparative study of the value of immunohistochemistry and the polymerase chain reaction in the diagnosis of follicular lymphoma. *Histopathology* 1995;27:501–8.
- 30 Trainor KJ, Brisco MJ, Wan JH, *et al.* Gene rearrangement in B- and T-lymphoproliferative disease detected by the polymerase chain reaction. *Blood* 1991;78:192–6.
- 31 Chen Y-T, Mercer GO, Chen Y. Polymerase chain reaction-based detection of B-cell monoclonality in cytologic specimens. *Arch Pathol Lab Med* 1993;117:1099–103.
- 32 Segal GH, Jorgensen T, Scott M, *et al.* Optimal primer selection for clonality assessment by polymerase chain reaction analysis: II. Follicular lymphomas. *Hum Pathol* 1994;25:1276–82.