Leader

Fine needle aspiration of lymph nodes

I D Buley

Needle aspiration of lymph nodes was used at the beginning of the 20th century to identify parasites in sleeping sickness, secondary syphilis, filariasis, and bubonic plague. In 1921, Guthrie at the Johns Hopkins Hospital described systematic diagnosis of lymph node aspirates using air dried films and Romanowsky staining.[1] This began the modern development of this technique and I intend to summarise its role, now, at the end of the 20th century.

Role
Fine needle aspiration (FNA) enables rapid, safe, relatively painless, and inexpensive sampling of lymph nodes. It does not require hospital admission or anaesthesia and does not

Table 1 *The role of fine needle aspiration of lymph nodes*

1	Diagnosis of reactive lymphadenopathy and recognition of some specific conditions. Follow up and subsequent biopsy is necessary where a cause for the change is not apparent and where resolution does not occur.
2	Diagnosis of metastatic malignancy and indication of the possible primary site(s).
3	Diagnosis of lymphoid malignancy followed by a biopsy for confirmation and accurate subtyping unless the patient is unfit for surgery.
4	In known malignancy, staging and monitoring for relapse or the effects of treatment. Obtaining material for research studies.
5	Diagnosis of infectious disease.

Cellular Pathology,
John Radcliffe
Hospital, Headington,
Oxford OX3 9DU, UK
I D Buley

leave a scar. Its role is summarised in table 1. The advent of HIV infection makes FNA particularly attractive for surgeons called to take biopsies of enlarged lymph nodes in HIV positive patients. Such aspirates pose particular problems of technique, specimen preparation, and interpretation, with a differential diagnosis that includes unusual infective conditions, reactive changes, Kaposi's sarcoma, and non-Hodgkin's lymphomas[2-5] (fig 1).

Technique and preparation
The basic technique of fine needle aspiration is well described elsewhere.[6] A 22 gauge or smaller needle should be used for the patient's comfort and to ensure an evenly spread preparation with good cell visualisation and without thick, apparently cohesive, tissue fragments which may be misleading in lymph node aspirates. The use of the ubiquitous 21 gauge "green" needle should be discouraged. Non-aspiration fine needle cytology, where lesions are probed without an aspirating syringe, produces good quality but small specimens. Giemsa staining is particularly valuable in the assessment of lymphoid cells, facilitating recognition of the variety of cell types in reactive conditions and lymphomas. Availability of surplus material for microbiological study or immunocytochemistry is particularly valuable in lymph node aspirates, increasing the prospect of avoiding unnecessary surgery or further investigations.

Enthusiasm for use of special techniques should be tempered by an appreciation of the reasons for a cytological approach to lymphadenopathy and a recognition that such techniques can be applied more reliably to larger tissue samples where biopsy is necessary.[7] The preparation technique used in my own institution is summarised in table 2. The use of a fixative containing transport medium to make spare material for immunocytochemistry or as the sole means of producing conventional stained cytospin preparations may be advantageous where there is a particular infectious hazard. Such indirect preparations avoid the aerosolisation of the unfixed specimen where preparations are made by extrusion from the needle directly onto the slide.

Reactive conditions
The most common reactive pattern is follicular hyperplasia (fig 2). Aspirates are cellular, dispersed, and polymorphic with a full range of
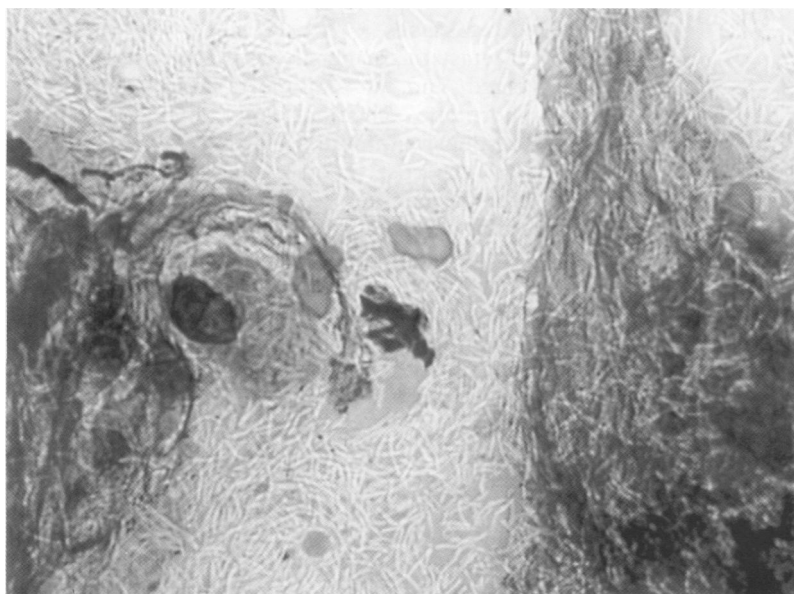

Figure 1 Negative image of numerous mycobacteria on a background of necrosis in a lymph node aspirate from a patient with AIDS. (High power, Giemsa staining.)

Table 2   *Preparation: lymph node fine needle aspiration material*

Two aspirates—Dry and wet fixed smears for Giemsa and Papanicolau staining. Wash the needle and syringe after each aspirate carefully with transport medium. Consider sending material for culture
Transport medium—4.5% human albumin
Contents of transport tube are centrifuged at 1500 rpm for 5 min. Washed by decanting the supernatant and resuspending in 3 ml of Tris buffered saline and then 4 drops of cell suspension are cytocentrifuged at 500 rpm for 5 min. A single cytospin preparation is Giemsa stained and the remaining seven preparations are allowed to dry and then frozen, in foil, back to back at −20°C

reactive cell types. Lymphocytes, centrocytes, centroblasts, immunoblasts, lymphoplasmacytoid cells, plasma cells, occasional neutrophil and eosinophil polymorphs, histiocytes, and a few endothelial cells can be discerned. Prominent tingible-body macrophages are scattered within the smear, providing helpful evidence of germinal centre sampling. Fragments of cytoplasm may be seen in the background. These "lymphoglandular bodies" are characteristic of lymphoid cytoplasm, either benign or malignant. Occasional epithelioid histiocytes with
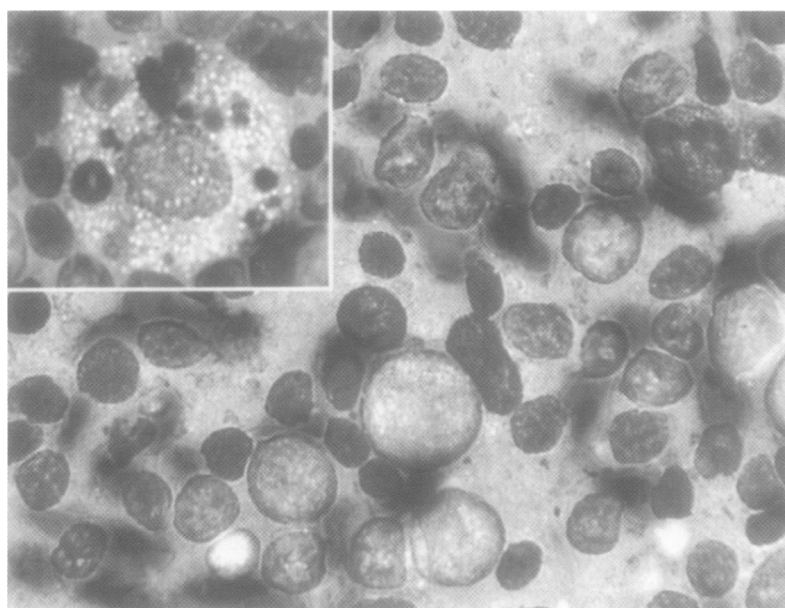

Figure 2   *The characteristic polymorphic population of lymphoid cells, including a tingible-body macrophage, seen in follicular hyperplasia. (High power, Giemsa staining.)*
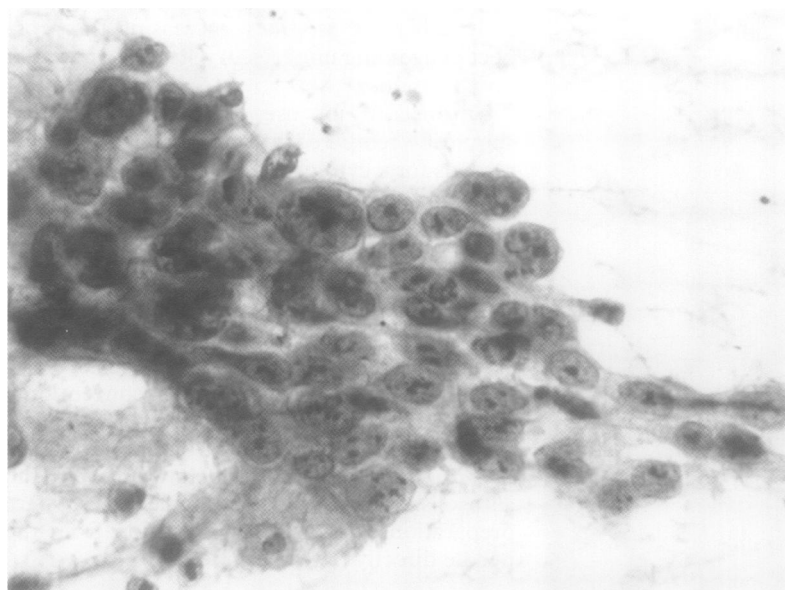

Figure 3   *A cervical lymph node aspirate from a male aged 41 years yielding loosely cohesive malignant cells with prominent nucleoli and delicate cytoplasm. Only after the suggestion of a germ cell tumour was made was the finding of a painful and slightly enlarged left testicle made. Subsequent histology confirmed the diagnosis of seminoma. (Papanicolau stain, high power.)*

characteristic "footprint" shaped nuclei are seen in follicular hyperplasia but where there are larger and numerous aggregates of these cells they raise the possibility of toxoplasmosis. An aspirate from a paracortical response is also polymorphic but lacks tingible-body macrophages, and large immunoblasts are prominent, particularly in infectious mononucleosis. Features of follicular hyperplasia or a paracortical response should lead to other investigations to define the cause. If a cause is not found and the lymphadenopathy persists, biopsy should be carried out to avoid a false negative diagnosis, particularly of lymphocyte predominant Hodgkin's disease and low grade non-Hodgkin's lymphomas.

Large groups of histiocytes including multinucleate forms imply a granulomatous condition; mycobacterial infection should be considered and is likely if necrosis is also present. Ziehl Neelsen staining and culture of aspirated material should be carried out. If the cause remains unclear a granulomatous FNA should lead to biopsy in order to diagnose mycobacterial infection, exclude a granulomatous response to a malignancy within the node, or substantiate a diagnosis of sarcoidosis.

Biopsy of persistent reactive lymphadenopathy where the cause has not been defined after needle aspiration and other investigations will allow the diagnosis of rarer lymph node pathologies such as sinus histiocytosis with massive lymphadenopathy,[8] histiocytic necrotising lymphadenitis,[9] cat scratch disease,[10] and Kimura's disease.[11] The cytology of these lesions may be distinctive but since they are uncommonly seen they may not be so easily diagnosed on a prospective basis.

Metastases

Metastatic malignancy is now actively managed and it is important to identify the hormone, chemotherapy, and radiotherapy responsive tumours. This necessitates cytological expertise, good clinical information, and the recognition of the value of biopsy in difficult cases. FNA is valuable in the initial diagnosis of lymph nodes containing metastatic malignancy. In known malignancy it is useful for staging and in monitoring for relapse or the effects of treatment. Overall, the sensitivity and specificity for the diagnosis of metastatic malignancy should be of the order of 98%.[12] [13]

The first consideration is whether the aspirate is genuinely from a lymph node, as there is a risk of misdiagnosis particularly in aspirates from the neck. The problem of distinguishing degenerative atypia in a branchial cyst aspirate from a necrotic well differentiated squamous carcinoma metastasis in the neck is well known. In a genuine lymph node aspirate, extrinsic cells also need not necessarily indicate
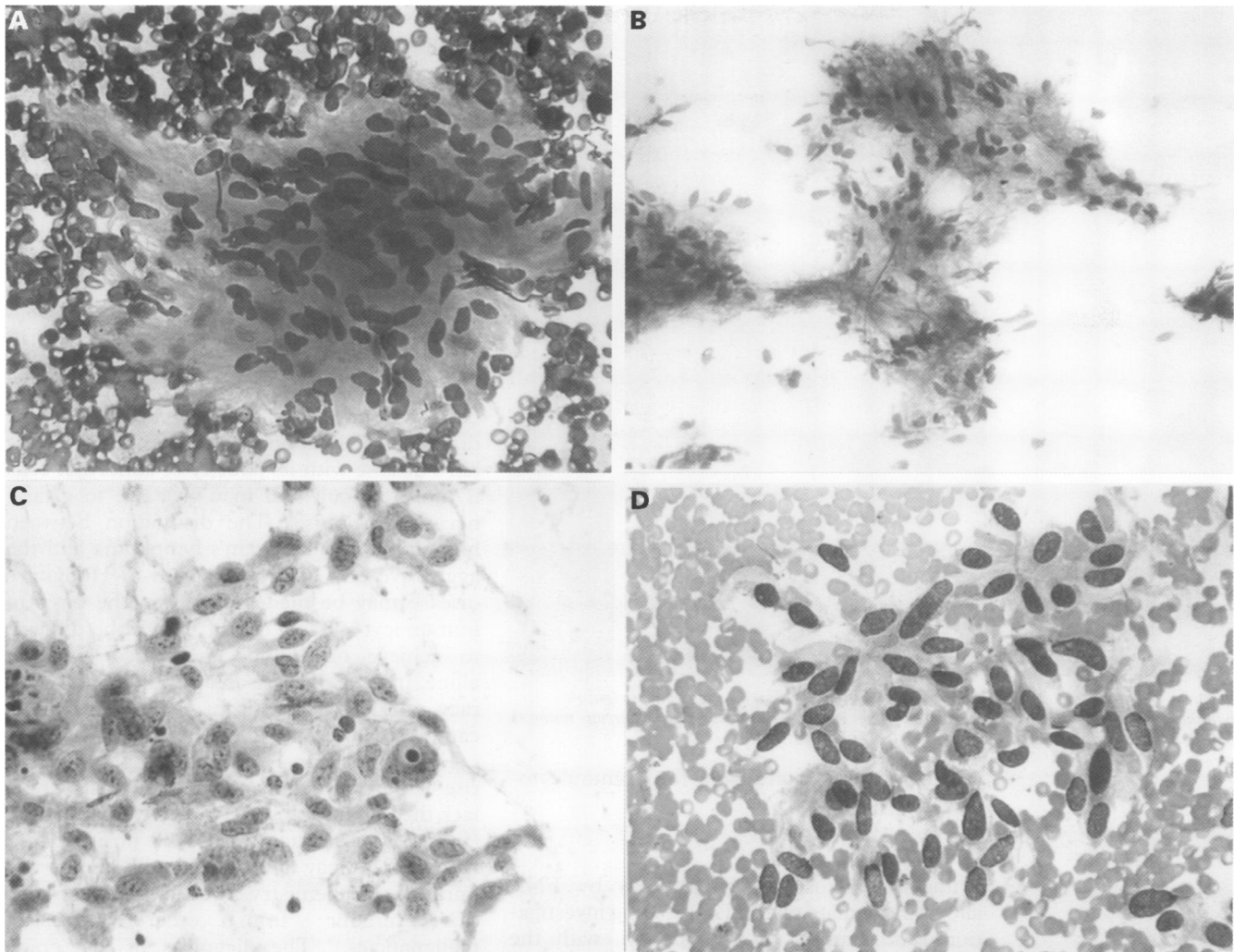
Figure 4 Spindle cells in "lymph node" aspirates. (A) Neurilemoma (Giemsa stain, high power). (B) Palisaded myofibroblastoma showing ill defined spindle cells clustered around fibrillary material. With this stain the perinuclear accumulations of actin are indistinct (Giemsa stain, high power). (C) Malignant melanoma characterised by brown pigment in both malignant cells and accompanying melanophages (Papanicolau stain, high power). (D) Metastatic medullary carcinoma of the thyroid characterised by a polymorphic appearance elsewhere, fragments of amyloid, focal red granularity in the cytoplasm, and immunocytochemical positivity for calcitonin (Giemsa stain, high power).

malignancy. They may be benign cells arising from adjacent tissues, benign epithelial inclusions particularly in axillary or parotid lymph nodes, or even benign melanocytic inclusions.

Where there is genuine malignancy, broad categorisation into subtype is generally straightforward using conventional cytological criteria. This facilitates a directed approach to the subsequent investigation of the primary site. The possibility of a metastatic germ cell tumour must always be considered as this is a poorly differentiated neoplasm which is usually responsive to chemotherapy and radiotherapy (fig 3). Immunocytochemistry may enable refinement of subtyping, particularly in anaplastic malignancy. Care must be taken with immunocytochemistry on cytological material as this is subject to diagnostic pitfalls which centre on the lack of adequate cytological control material in most laboratories.[7]

The diagnosis of metastatic melanoma may pose particular problems since the cytological appearances in melanoma are very variable. Melanin pigment is seen in less than two thirds of cases[14] with the carcinoma-like, spindle cell, and lymphoma-like variants predominantly being amelanotic in cytological material. Other variants include undifferentiated, myxoid, and clear cell forms.

A spindle cell proliferation in an aspirate which purports to be from a lymph node poses an interesting cytological differential diagnosis (fig 4). The lump may be mimicking a lymph node clinically and this is commonly the case with neurilemomas. The significance of the characteristically painful aspirate may elude the aspirator. The spindle cells have long narrow nuclei, typically with pointed ends. Nuclear palisading and undulation may be seen, and background myxoid material may be present. A genuine lymph node aspirate may give a similar appearance when a palisaded myofibroblastoma is aspirated.[15] Kaposi's sarcoma should be considered in the appropriate clinical context. In more frankly malignant aspirates, particularly in neck nodes, a spindle cell squamous carcinoma is likely. Spindle cell melanoma and metastatic medullary carcinoma of the thyroid should also be considered before contemplating the possibility of metastatic sarcoma, which very rarely first presents with a lymph node metastasis. An unusual possibility is a follicular dendritic cell sarcoma[16] but diagnosis is likely to be retrospective unless
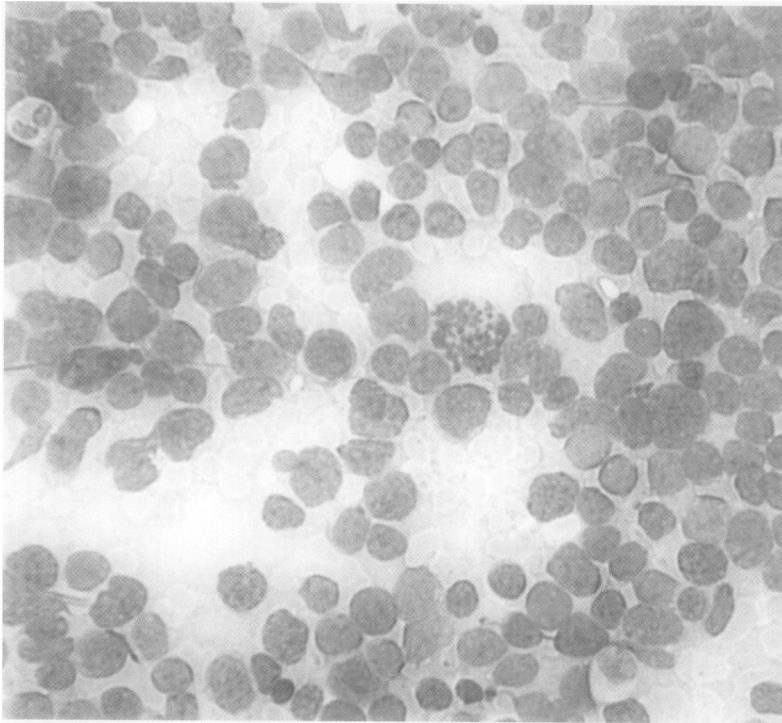
Figure 5   The characteristic relative monomorphism of a high grade non-Hodgkin's lymphoma. This lymphoblastic lymphoma shows medium sized cells with numerous mitoses. (High power, Giemsa staining.)

ample material is available for immunocytochemistry.

Lymphomas

Primary diagnosis of lymphomas by FNA allows immediate planning of staging investigations and biopsy. In my opinion, with the present state of knowledge, biopsy at first diagnosis is always desirable. While the accuracy of FNA with supporting immunocytochemistry is sufficient, in experienced hands, to define current treatments, the diagnosis of lymphoma begins a treatment and follow up programme which may last the patient's lifetime. The opportunity to obtain tissue at the outset of the disease should not be missed. It enables confirmation of the diagnosis, accurate subtyping, and preservation of archival material, which may be of use if there are further advances in treatment. On occasion an acute clinical situation may necessitate treatment based on a fine needle aspirate diagnosis alone. This tends to occur in the context of guided needle aspiration before emergency radiotherapy for vena caval obstruction by mediastinal disease, for spinal cord compression in vertebral lymphoma, or in very ill patients with widespread lymphoma. FNA alone is an acceptable means of diagnosing recurrent lymphoma and in assessing whether high grade transformation has occurred.

The problems that may limit the accuracy of cytological lymphoma diagnosis include the loss of architecture common to most cytological specimens and a confusing mixture of malignant and reactive elements. This may be seen in partial lymph node involvement and is intrinsic to some lymphomas such as Hodgkin's disease, T-cell-rich B cell lymphoma, and in some T cell lymphomas including Lennert's lymphoma. Lymphomas may be difficult to

diagnose histologically and this is mirrored cytologically. Ancillary techniques, including immunocytochemistry, quantitative immunophenotyping using flow cytometry, and molecular techniques to determine clonality and cell lineage, all enhance accuracy of diagnosis in the minority of cases which are difficult morphologically. The problem of defining a diagnostic ratio of κ to λ light chain staining in the relevant cell population in a lymphoid aspirate is especially facilitated by quantitative immunophenotyping techniques.[17]

Hodgkin's disease is generally easy to diagnose, relying on the recognition of characteristic Hodgkin's and Reed-Sternberg cells on the appropriate reactive background. The diagnosis of lymphocyte predominant Hodgkin's disease with its paucity of abnormal cells is more difficult and may give rise to a false negative diagnosis. The distinction between high grade non-Hodgkin's lymphoma and the aggressive end of the spectrum of Hodgkin's disease may be made mainly on the basis of immunocytochemistry. This might be difficult to achieve on limited cytological material alone. In spite of these areas of difficulty, accuracy of FNA diagnosis of Hodgkin's disease can be greater than 90%.[18]

High grade non-Hodgkin's lymphomas give rise to frankly malignant lymphoid cells which lack the polymorphism of a reactive population (fig 5). The diagnosis of CD30 positive Ki-1 lymphomas may also be heavily reliant on immunocytochemistry in order to distinguish this tumour from other anaplastic malignancies.[19] The diagnosis of low grade non-Hodgkin's lymphoma also relies on recognition of a monotonous or restricted population of lymphoid cells and is enhanced in B cell non-Hodgkin's lymphomas by the ability to demonstrate light chain restriction. Low grade T cell non-Hodgkin's lymphomas are relatively uncommon and may be difficult to distinguish from an unusual paracortical reaction. Nonetheless the cytological findings are sufficiently atypical to precipitate a request for biopsy.

The level of accuracy which can be expected with experience and the use of ancillary techniques is exemplified by one study which accurately distinguished reactive lymphoid hyperplasia from malignant lymphoma in 97% of cases.[20] An antibody panel which includes a T and B cell marker, κ and λ light chain staining, and antibodies against CD15, CD30, and epithelial membrane antigen is adequate to facilitate safe practice. Clearly further immunostaining, for example ALK in anaplastic large cell lymphoma, and other techniques including gene rearrangement studies are feasible on cytological material but may be more appropriately carried out on the subsequent biopsy specimen.

1 Grunz EH, Spriggs AI. *History of clinical cytology: a selection of documents*, 2nd ed. Darmstadt: G-I-T-Verlag Ernst Giebeler, 1983:149.

2 Reid AJC, Miller RF, Kocjan GI. Diagnostic utility of fine needle aspiration cytology in HIV infected patients with lymphadenopathy. *Cytopathology* 1998;9:230–9.

3 Martin-Bates E, Tanner A, Suvarna SK, *et al.* Use of fine needle aspiration cytology for investigating lymphadenopathy in HIV positive patients. *J Clin Pathol* 1993;46:564–6.

4 Ellison E, Lapuerta P, Martin SE. Fine needle aspiration (FNA) in HIV positive patients: results from a series of 655 aspirates. *Cytopathology* 1998;9:222–9.

5 Buley ID. Fine needle aspiration (FNA) in HIV⁺ patients [review]. *Cytopathology* 1998;9:219–22.

6 Orell S R, Sterrett GF, Walters M, *et al. Manual and atlas of fine needle aspiration cytology*, 2nd ed. Edinburgh: Churchill Livingstone 1992:8–23.

7 Buley ID. Update on special techniques in routine cytopathology. *J Clin Pathol* 1993;46:881–6.

8 Schmitt FC. Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease). Cytomorphologic analysis on fine needle aspirates. *Diagn Cytopathol* 1992;8:596–9.

9 Greenberg ML, Cartwright L, McDonald DA. Histiocytic necrotising lymphadenitis (Kikuchi's disease): cytological diagnosis by fine needle biopsy. *Diagn Cytopathol* 1993;9: 444–7.

10 Donnelly A, Hendricks G, Martens S, *et al.* Cytologic diagnosis of cat scratch disease (CSD) by fine needle aspiration. *Diagn Cytopathol* 1995;13:103–6.

11 Jayaram G, Peh KB. Fine needle aspiration cytology in Kimura's disease. *Diagn Cytopathol* 1995;13:295–9.

12 Prasad RRA, Narasimhan R, Saukavan V, *et al.* Fine needle aspiration cytology in the diagnosis of superficial lymphadenopathy. An analysis of 2418 cases. *Diagn Cytopathol* 1996;15:382–6.

13 Steel BL, Schwartz MR, Ramzy I. Fine needle aspiration biopsy of lymphadenopathy in 1103 patients. Role, limitations and analysis of diagnostic pitfalls. *Acta Cytol* 1994;39: 76–81.

14 Nasiell K, Tani I, Skoog L. Fine needle aspiration cytology and immunocytochemistry of metastatic melanoma. *Cytology* 1997;2:137–47.

15 Dei Tos AP, Libera DD, Bittesini L. Aspiration biopsy cytology of intranodal myofibroblastoma. Case report with immunocytochemical analysis. *Diagn Cytopathol* 1995;13: 134–8.

16 Ryley NG, Bastert J, Ferguson DJP, *et al.* Follicular dendritic cell sarcoma of lymph node—report of FNA cytological appearances. *Cytopathology* (in press)

17 Jeffers MD, Milton J, Herriot R, *et al.* Fine needle aspiration cytology in the investigation of non-Hodgkin's lymphoma. *J Clin Pathol* 1998;51:189–96.

18 Fulciniti F, Vetrami A, Zeppa P, *et al.* Hodgkin's disease: diagnostic accuracy of fine needle aspiration; a report based on 62 consecutive cases. *Cytopathology* 1994;5:226–33.

19 Noorduyu LA, Van Heerde P, Meyer CJLM. Cytology of Ki-1 (CD30) positive large cell lymphoma. *Cytopathology* 1990;1:297–304.

20 Stewart CJR, Duncan JA, Farquharson M, *et al.* Fine needle aspiration cytology diagnosis of malignant lymphoma and reactive lymphoid hyperplasia. *J Clin Pathol* 1998;51:197–203.