

# Immunoflow cytometry and cell block immunohistochemistry in the FNA diagnosis of lymphoma: a review of 73 consecutive cases

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## Abstract

**Aims**—To review the results of 73 consecutive fine needle aspirations (FNAs) that were collected by a pathologist and analysed by immunoflow cytometry. Material for a cell block was also collected from some of these lesions.

**Methods**—The setting was a large general hospital in rural New Zealand. The FNAs were performed by a pathologist, or a radiologist for image guided localisations. Material for immunoflow cytometry was collected into RPMI and, when required, material for a cell block was collected into formalin.

**Results**—Of the 73 samples collected by FNA nine were inadequate. Light chain restriction could be demonstrated in most FNA samples from B cell lymphomas (28 of 30 adequate samples). The exceptions were two cases of T cell rich B cell lymphoma. Artefactual light chain restriction was seen occasionally in T cell lymphomas, presumably as a result of autoantibodies binding to the cell surfaces. It was possible to subtype most (18 of 30 adequate samples) B cell lymphomas as chronic lymphocytic leukaemia (CLL), follicle centre cell lymphoma (FCCL), or mantle cell lymphoma. The CD4 to CD8 ratio was not usually restricted in T cell lymphomas and coexpression of CD4 and CD8 was not usually found. Loss of pan-T cell antigens was seen in some T cell lymphomas. Four of the six T cell lymphomas and three of the four non-lymphoid malignancies were diagnosed with the aid of cell block immunohistochemistry. Only one of the four cases of Hodgkin's lymphoma showed Reed-Sternberg cells in the FNA smears.

**Conclusions**—It is not always possible to characterise lymphomas as fully with FNA and immunoflow cytometry as is possible with biopsy histology and a full battery of modern investigations. Nevertheless, in the setting of a large rural general hospital immunoflow cytometry on FNA samples is a highly effective method of diagnosing and typing B cell lymphomas. Immunoflow cytometry is of little use for T cell lymphomas or Hodgkin's lymphomas. We advocate the use of cell block immunohistochemistry in preference to immunoflow cytometry for cases in which the cytological appearance of the specimen is overtly malignant but

## the differential diagnosis includes non-lymphoid malignancy.

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In the past, the cytological diagnosis of lymphomas from fine needle aspiration (FNA) samples has been particularly difficult. Usually, one obtains a sample that is either obviously malignant but equivocally lymphoid or, conversely, a sample that is obviously lymphoid but equivocally malignant. However, recently FNA diagnosis of lymphoid lesions has been made easier by the arrival of immunoflow cytometry in most large pathology laboratories. Immunoflow cytometry has been used mainly for the analysis of haematological diseases, but increasingly it is being used by cytologists. Early studies of FNAs and immunoflow cytometry used a limited range of antibodies and were not able to perform dual staining. Recently, there have been substantial advances in the sophistication of the methods and equipment used for flow cytometry. It is accepted that FNA cytology with immunolabelled flow cytometry can, in some circumstances, serve as a replacement for open biopsy and conventional histology and immunohistochemistry.<sup>1 2</sup> However, FNA with immunoflow cytometry is not always successful. Scanty cellularity in the sample can prevent a satisfactory analysis, and even with an adequate sample the results might be misleading. In particular, non-lymphoid malignancies can be hard to distinguish from lymphoid lesions if the sample also contains reactive lymphoid cells; B cell lymphomas sometimes do not exhibit light chain restriction; and T cell lymphomas can have a large population of reactive B cells.

The aim of our study was to review the results of 73 consecutive FNAs that were collected by a pathologist and analysed by immunoflow cytometry. Material for a cell block was also collected from some of these lesions. The setting was a large general hospital in rural New Zealand. The FNAs were performed over a two year period. In this time, approximately 800 FNAs of non-breast lesions were performed together with approximately 1400 breast FNAs.

## Methods

The FNAs were performed by one of two mobile pathologists with an interest in FNA cytology, except for image guided FNAs, in

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Table 1 Antibodies and their fluorochrome labels

Antibody	Fluorochrome
CD2	FITC
CD3	FITC
CD4/CD8	FITC/PE
CD5	PE
CD10	FITC
CD19	FITC
CD20	FITC
CD23	FITC
FMC7	FITC
Sm IgM	FITC
CD19/ $\kappa$ chain	PE/FITC
CD19/ $\lambda$ chain	PE/FITC

FITC, fluorescein isothiocyanate; PE, phycoerythrin.

which case one of these pathologists prepared the slides after the radiologist had aspirated the lesion. The FNAs performed by the pathologists were done with a needle only technique, using a 23 or 25 gauge 1.25 inch (3 cm) needle. The slides were air dried, often aided by a small hair dryer, and Diff-Quik® stained (Dade Behring Diagnostics, Newmarket, Auckland, New Zealand). The slides were then examined with a portable Olympus CHK microscope and a hand written report was issued within a few minutes. If this examination of the specimen suggested that the lesion might be lymphoid and lymphoma seemed possible, then a second FNA was performed, still using a needle only technique, to collect a second sample. This sample was collected quickly and the needle was washed through with 3 ml of heparin RPMI (12.8 mg of heparin ammonium in 45 ml of RPMI) within a few seconds of being collected. The reason for the urgency was that a clotted sample reduces the yield of cells for flow cytometry. Early in our experience with collecting specimens for flow cytometry we washed needles through with sterile heparin saline before the FNA was performed, but we found that this was not necessary. The slides were taken back to the laboratory to be mounted and examined again. The specimen in RPMI was taken to the haematology laboratory, transferred to a 5 ml tube and centrifuged for two minutes at approximately 400  $\times g$ . The supernatant was discarded and the cells were resuspended in 2 ml of ammonium chloride lysis solution. The cells were gently vortexed, incubated at room temperature for 10 minutes, and then centrifuged again for two minutes. The leucocytes were then resuspended in 2 ml of phosphate buffered saline (PBS). If debris was present, it was removed with a nylon Swiss screen filter. An approximate cell count was performed and a panel of directly conjugated monoclonal antibodies was selected (table 1) (Immunotech Inc, Westbrook, Maine, USA). The cells and the antibodies were incubated for 20 minutes in the dark at room temperature. These were then washed once in PBS, spun down, and then washed again. They were then analysed with the flow cytometer. Material for  $\kappa$  and  $\lambda$  light chain analysis was treated differently. The centrifuged sample in RPMI was incubated with prewarmed PBS for 20–30 minutes at 37°C, to remove the cytophilic immunoglobulin, and then centrifuged. The supernatant was discarded and if red blood cells were present they were lysed with ammo-

nium chloride as above. Cells were then incubated with antibodies (against CD19/ $\kappa$  light chain or CD19/ $\lambda$  light chain) as above.

The “first run” panel of antibodies evolved as our experience developed and with the acquisition of a new immunoflow cytometer approximately half way through our study. At the start of the study, a Coulter PROFILE II was used but later the laboratory obtained a Coulter EPICS® XL (Beckman Coulter Inc, Fullerton, California, USA). In some cases we performed a “second run” with a more specialised panel to investigate a specific diagnosis. The panel of antibodies used was sometimes restricted by the scarcity of cells. In these cases, antibodies against CD19/ $\kappa$  light chain and CD19/ $\lambda$  light chain were given priority.

In our laboratory we have adopted light chain ratio limits from previous studies.<sup>2–4</sup> A  $\kappa$  to  $\lambda$  ratio of greater than 3 or a  $\lambda$  to  $\kappa$  ratio of greater than 2 was accepted as evidence of monoclonality.

For some cases, cell blocks for immunohistochemistry were collected as described previously.<sup>5</sup> These were usually taken after the initial FNA showed overtly malignant cytology but it was not certain that the lesion was lymphoid in nature.

## Results

Table 2 shows our results—the cases are set in chronological order within each diagnostic category. It can be seen that the range of cell markers expanded during the course of our study. The panel of markers was sometimes tailored a little in view of the initial diagnosis, so as to focus on a particular issue and to avoid wasting cells.

## Discussion

Nine of the 73 samples (12%) had inadequate cells for analysis. Four of these were from benign lymph nodes. It was felt that adequate samples were easier to obtain from malignant lesions simply because of their larger size. Young *et al* found that five of 107 (4.7%) of their specimens were inadequate in a similar study.<sup>2</sup> A highly cellular lymphoma aspirate might exhibit uniform negativity on immunoflow cytometry if the cells are necrotic, as in case 3.

Twenty eight cases exhibited light chain restriction (defined above), allowing an unequivocal diagnosis of B cell lymphoma to be made on the FNA sample alone. On review of the notes at the end of our study, we could find no evidence that any of these diagnoses were incorrect. Case 48 had been thought to be suspicious of lymphoma with a  $\kappa$  to  $\lambda$  ratio of 1.95. However, a review of the notes about a year after the FNA was performed revealed no clear evidence that the patient had developed lymphoma. In particular, there was no evidence of lymphoma on a computed tomography scan. Case 73 also had a marginal light chain ratio but did not have clinically evident lymphoma eight months later.

Five of the 33 B cell lymphomas did not exhibit light chain restriction. For three case this was because of inadequate cellularity. The

Table 2 Immunoflow cytometry results from 73 suspected lymphomas together with cell block and biopsy histology

Case	Site	Method	Initial diagnosis	Percentage of cells positive (numbers indicate CD numbers)																			Final diagnosis	
				2	3	4	5	8	10	19	20	23	IgM	FM7	19/k	19/l	5/19	4/8						
<b>B cell lymphoma</b>																								
1	Abdominal mass	I	Susp. of lymphoma	4	2	1	12	13	1	1	3	98	97	75	85	98	<1	<1	<1	<1				Diffuse large B cell lymphoma
2	Base of neck	M	Benign/lymphoma	Inadequate			99			4		45			2	8	8	8	8				CLL	
3	L neck	M	Necrotic lymphoma	Inadequate																				FCCL (from previous history)
4	Sternal mass	I	Myeloma	Inadequate																				Myeloma (on cell block histology)
5	R groin node	M	Susp. of CLL				12	13	1			74												CLL
6	L posterior neck	M	Susp. of CLL				99					90												CLL
7	R post neck	M	Susp. of FCCL									91												Probable FCCL
8	R paravertebral	I	Susp. of CLL				6	2	5	3	2	83												CLL
9	L neck	M	Probable carcinoma				98	41	90	48		1												T cell rich B cell lymphoma (on biopsy histology)
10	R neck	M	"High grade" lymphoma	Inadequate																				Diffuse large B cell lymphoma (on biopsy histology)
11	R neck	M	Necrotic lymphoma	6	4	8	4	8	4	91	42	88	8	50	86	8	8	8	8					FCCL
12	R inguinal	M	Lymphoma	16		16				81	84	84	4	81	74	1	1	1						FCCL
13	L inguinal	M	Susp. of lymphoma	9	3	1	3	92	99	34		34	17	34	77	1	1	1						Diffuse large B cell lymphoma
14	R axillary node	M	Lymphoma	20	21	19	21	3	78	78	80	16	69	78	77	1	2	2						FCCL
15	Base of neck	M	Lymphoma	19	18	12	28	5	<1	81	<1	<1	81	29	3	3	81	81	81					Diffuse large B cell lymphoma
16	L neck	M	Lymphoid cells	54	70	13	27	31	39	7	22	22	7	7	7	7	22	<1	<1					B cell lymphoma, exact type uncertain
17	Abdominal mass	M	Lymphoma	24	13	27	8	68	58	79		3	1	3	39	3	56	<1	<1					FCCL
18	R neck	M	Susp. of lymphoma	58	34	3	3	39	43	1		3	3	8	19	3	3	3	3					B cell lymphoma, exact type uncertain
19	L neck	M	Susp. of lymphoma	21	15	99	5	5	<1	80	55	79	58	8	2	2	79	99	99					CLL
20	R breast	M	Lymphoma	2	5	100	6	1	99	99	14	99	99	99	2	2	99	99	99					Mantle cell lymphoma
21	L supraclavicular	M	Possible lymphoma	10	13	3	70	85	87	3	83	87	3	53	80	1	6	6	6					FCCL
22	L neck	M	Possible CLL	41	33	99	10	<1	36	37	1	3	45	3	3	45	57	57	57					CLL
23	L supraclavicular	M	Previous CLL	4	2	100	1	1	97	99	12	98	5	95	5	5	95	97	97					CLL
24	L parotid	M	Lymphoma	29	15	24	11	56	64	61	12	73	77	77	55	2	2	2	2					Mantle cell lymphoma
25	R neck	M	Lymphoma	18	13	20	7	20	79	87	11	80	12	73	12	16	16	16	16					FCCL
26	R neck	M	Lymphoma	13	20	7	20	7	20	79	87	11	80	12	73	12	16	16	16					FCCL
27	R neck	M	Lymphoma	18	13	20	8	78	25	81	4	78	73	72	4	66	<1	<1	<1					B cell lymphoma, exact type uncertain
28	L neck	M	Lymphoma	17	13	16	2	74	82	83	81	84	82	82	82	1	1	1	1					FCCL
29	Abdominal mass	I	Lymphoma	41	43	29	88	24	33	56	14	33	14	10	1	36	38	38	38					FCCL (from previous history)
30	R submandibular	M	Lymphoma	66	39	60	25	1	31	26	4	20	27	27	16	4	4	4	4					B cell lymphoma, exact type uncertain
31	R inguinal	M	Infarcted tissue	87								9			90	26	26	26	26					B cell lymphoma, exact type uncertain
32	R neck	M	Scanty cells																					T cell rich B cell lymphoma (on biopsy histology)
33	R neck	M	Lymphoma	68	17	51	60	40	<1	7					6	6	6	6	6					T cell rich B cell lymphoma (on biopsy histology)
<b>T cell lymphoma</b>																								
34	L neck	M	Malignant	68	17	51	60	40	<1	7														Peripheral T cell lymphoma (on cell block histology)
35	L neck	M	Malignant	70																				Peripheral T cell lymphoma (on cell block histology)
36	R calf	M	Lymphoma	98	5	96	95	11	1	15					10	13	13	13	13					Peripheral T cell lymphoma (on cell block histology)
37	R back	M	Malignant	22	2	1	1	5	17	22				53	1	1	1	1	1					Peripheral T cell lymphoma (on cell block histology)
38	Perihepatic mass	I	Lymphoma/ca.	80	62	78	10	<1	7	12					5	5	5	5	5					Peripheral T cell lymphoma (on biopsy histology)
39	L inguinal	M	Lymphoma/ca.	70	42										5	5	5	5	5					Peripheral T cell lymphoma (on cell block histology)
<b>Hodgkin's lymphoma</b>																								
40	L supraclavicular	M	Inadequate cells	Inadequate																				Hodgkin's lymphoma (on biopsy histology)
41	Mediastinum	I	Reactive	90	77	87	20	2	11	9	23	9	9	9	6	5	5	5	5					Hodgkin's lymphoma (on biopsy histology)
42	L neck	M	Reactive	69											15	20	20	20	20					Hodgkin's lymphoma (on biopsy histology)
43	L neck	M	Hodgkin's lymphoma	Inadequate																				Hodgkin's lymphoma
44	Mediastinum	I	Thymoma/lymphoma	19	53	94	99	90	5	2	24				3	3	3	3	3					Thymoma (on cell block and biopsy histology)
45	Paravertebral mass	I	Carcinoma/lymphoma	67	43										18	11	11	11	11					Adenocarcinoma (on biopsy histology)
<b>Non-lymphoid tumours</b>																								
46	R breast	M	Carcinoma/lymphoma	No lymphoid cells																				Breast carcinoma (on cell block histology)
47	R supraclavicular	M	Carcinoma/lymphoma	No lymphoid cells																				Small cell carcinoma (on cell block histology)
<b>Benign</b>																								
48	L parotid mass	M	Sialadenitis	60	34	57	13	1	46	47					41	21	21	21	21					Susp. B cell lymphoma (but no overt lymphoma a year later)
49	R neck	M	Benign	Inadequate																				Benign

Table 2 Immunofluorescence cytometry results from 73 suspected lymphomas together with cell block and biopsy histology

Case	Site	Method	Initial diagnosis	Percentage of cells positive (numbers indicate CD numbers)																	Final diagnosis
				2	3	4	5	8	10	19	20	23	IgM	FMC7	19/κ	19/λ	5/19	4/8			
50	R neck	M	Benign	49	38	57	67	18	4	19	14	25	9	16	16	3		Granulomatous inflammation			
51	L axilla	M	Benign	Inadequate														Benign			
52	L parotid	M	Probably benign	30			31			46				23	17			Toxoplasmosis (clinically)			
53	L neck	M	Benign	38	38	23		14	2	42	56	40		33	25			Benign			
54	R neck	M	Benign	Inadequate														Benign			
55	R infraclavicular	M	Benign	Inadequate														Benign			
56	R neck	M	Benign	23				6	<1	76		33		13	22			Benign			
57	R neck	M	Benign	58	70	55		51		14	37	2		27	14			Benign			
58	L neck	M	Benign	86						25				18	11			Benign			
59	R neck	M	Benign	61				13	15	40				19	19			Benign			
60	R neck	M	Benign	89	61		88	25	1	12	14	8		6	5	4		Benign			
61	Periportal node	I	Benign	90	63			26	2	12	12	4	10	2	3	1		Benign			
62	L neck	M	Benign						<1	23	15			11	10			Benign			
63	R supraclavicular	M	Benign	48			44	15	<1	54	58	21	45	29	19	1		Benign			
64	L neck	M	Benign	84	58		81	20	<1	19	19	7	11	16	10	2		Benign			
65	R neck	M	Benign	44	30	39		7	<1	60	60	36		6	25			Benign			
66	L neck	M	Benign	58	36	53		15	1	44	45	11	34	20	9	1		Benign			
67	Salivary gland	M	Benign	20	10	18		6	77	81				78	45			Benign			
68	R inguinal	M	Benign	70	52		68	16	<1	32	17	28	28	13	12			Benign			
69	L axilla	M	Benign	76	62	82		29	6	22	34	15	41	38	38			Benign			
70	L parotid	M	Benign	53	53		75	16	<1	9	11	10	11	22	12	6		Benign			
71	R neck	M	Benign	53	54		50	12	<1	41	41	1	29	23	20	>5		Benign			
72	Mediastinal mass	I	Benign	60			66	20	3	36	41	30	34	8	9	10		Benign encysted serous fluid			
73	L neck	M	Possible lymphoma	21					<1	88			22	47	23			Susp. B cell lymphoma (but no overt lymphoma 1.5 years later)			

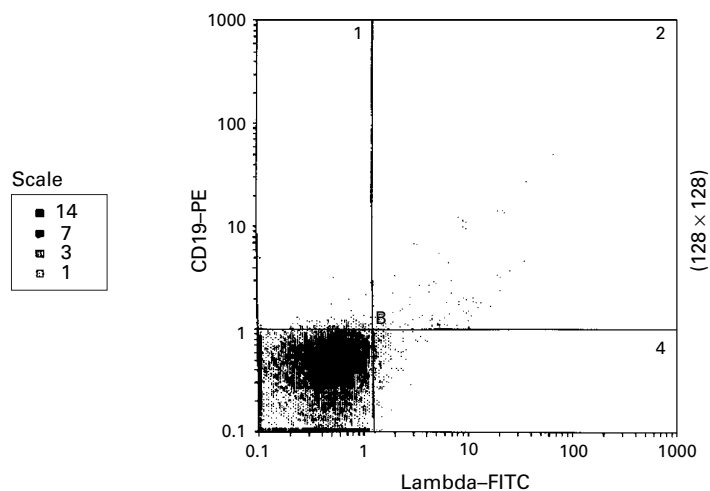
Ca., carcinoma; CLL, chronic lymphocytic leukaemia; FCCL, follicle centre cell lymphoma; FNA, fine needle aspiration; I, image guided FNA; L, left; M, manually guided FNA; R, right; susp., suspicious.

other two (cases 9 and 33) were particularly confusing because the cytology showed a malignant tumour with highly atypical cells mixed with benign looking lymphoid cells. The flow cytometry showed no evidence of light chain restriction. Biopsies were taken and the flow cytometry results from these were similar to those from the FNA. However, paraffin wax embedded sections and immunohistochemistry showed that the large highly atypical cells were CD45 and CD20 positive and negative for cytokeratin, S100, and CD3. Thus, a diagnosis of T cell rich B cell lymphoma was made. We assumed that the neoplastic B cells were not expressing light chains strongly or were "swamped" by a population of reactive B cells.

Except for cases 9 and 33, the lowest light chain ratio of any B cell lymphoma was a  $\lambda$  to  $\kappa$  ratio of 3.1 (case 16). Case 37 was remarkable in that it showed artefactual light chain restriction in a T cell lymphoma (fig 1). We assume that this was as a result of specific binding of  $\kappa$  to the surface of the tumour cells, perhaps as an autoimmune response to the tumour cells. The lymphoma occurred in a patient with AIDS. On biopsy, the tumour cells were strongly positive for CD3 but negative for CD20. Fortunately, this artefact did not confuse the diagnosis, because coexpression of light chain with CD19 was not found.

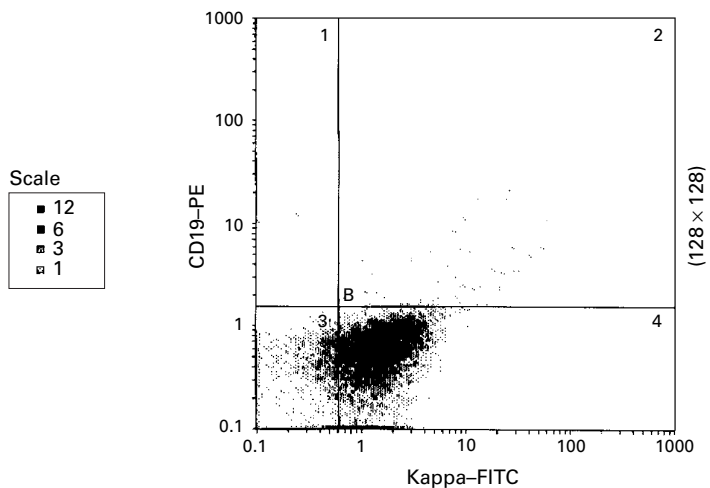
For 18 cases it was possible to make a diagnosis of B cell lymphoma and also subtype the lymphoma as either CLL (chronic lymphocytic leukaemia/small lymphocytic lymphoma), FCCL (follicle centre cell lymphoma), or mantle cell lymphoma. Most of the other cases that exhibited light chain restriction were called "B cell lymphoma, exact type uncertain" or "diffuse large B cell lymphoma" if they were composed of overtly malignant large cells. Uncertainty as to the exact type of B cell lymphoma was not considered sufficient reason to perform a biopsy. This was usually because the patient had other medical problems that were a more immediate risk to the patient's health or because exact typing would not alter treatment.

Four of the six T cell lymphomas were diagnosed with a combination of cytology, flow cytometry, and cell block immunohistochemistry, but for two it was not possible to collect a cell block. Both of these then had biopsies. The participation of the pathologist in the collection of the specimen and the immediate assessment of the cytology meant that the need for cell block histology had been anticipated. The ratio of CD4 to CD8 (or CD8 to CD4) was examined but this was not usually useful in the recognition of T cell lymphoma. The benign lesions in cases 59 and 65 had CD4 to CD8 ratios of 4.15 and 4.29, respectively. Only two of the six T cell lymphomas had CD4 to CD8 (or CD8 to CD4) ratios greater than these. Jeffers *et al* describe one case of T cell lymphoma in their series of 46 FNA sampled lesions.<sup>6</sup> This lymphoma showed subset restriction, with more than 90% of T cells expressing CD8. Coexpression of CD4 and CD8 was examined for four of the six T cell lymphomas but was only found in case 34. Loss of pan-T cell anti-



Stats: Normalised, Listgating: Disabled

Hist	Region ID	%	Count	Mn X	Mn Y	PkPosX	PkPosY	PkCnt	FPCVX	FPCVY
2	B1 B	1.5	117	0.528	1.14	0.576	1.02	6	58.52	22.34
	B2 B	1.1	87	5.27	2.08	5.36	1.10	3	94.44	85.63
	B3 B	94.8	7437	0.404	0.367	0.102	0.102	78	67.36	62.07
	B4 B	2.6	203	1.51	0.478	1.27	0.619	9	22.37	56.50



Stats: Normalised, Listgating: Disabled

Hist	Region ID	%	Count	Mn X	Mn Y	PkPosX	PkPosY	PkCnt	FPCVX	FPCVY
2	B1 B	0.0	2	0.158	11.0	0.102	10.2	1	43.51	7.20
	B2 B	1.0	62	7.04	2.78	2.43	1.58	3	105.79	66.65
	B3 B	13.2	840	0.366	0.381	0.576	0.619	13	47.82	60.52
	B4 B	85.8	5474	1.49	0.497	1.10	0.619	25	51.37	60.29

Figure 1 A scatter plot showing  $\kappa$  light chain restriction in a T cell lymphoma from a patient with AIDS (case 37). There was no coexpression of CD19 and  $\kappa$ , exemplifying the importance of dual staining in assessing light chain restriction.

gens was seen in cases 34 and 37. Zardawi *et al* have stated that the loss of one or more pan-T cell antigen allows a firm diagnosis of T cell lymphoma to be made (in the correct clinical context).<sup>4</sup> It should be remembered that immature thymic T cells exhibit loss of pan-T cell antigens, as in case 44.

The cytological diagnosis of Hodgkin's lymphoma depends on the presence of typical

Reed-Sternberg cells. Flow cytometry does not contribute except to exclude a monoclonal B cell population. There were no definite Reed-Sternberg cells in the FNA samples of three of our four cases. Because a diagnosis of lymphoma was suspected clinically, the nodes were biopsied. One case did show abundant Reed-Sternberg cells in the FNA sample and a diagnosis was made cytologically. The node

was excised before treatment and the diagnosis was confirmed. We would not advocate the diagnosis of Hodgkin's lymphoma being made on FNA alone except in exceptional circumstances. Young *et al* studied the cytology and flow cytometry of 107 aspirates of suspected lymphoid lesions.<sup>2</sup> Three of these were Hodgkin's lymphoma but none showed Reed-Sternberg cells in the FNA samples. Flow cytometry showed a polyclonal population.

There were four cases that had a final diagnosis of a non-lymphoid neoplasm. The initial differential diagnosis favoured a non-lymphoid tumour in each case but lymphoma was also thought to be possible. In one of these patients, a core biopsy was performed by a radiologist once the cytology had been examined. Cell blocks were taken for the others.

There were 28 cases that seemed to be benign on cytology and flow cytometry. However, two of these were clinically suspicious and had biopsies that showed them to be Hodgkin's lymphoma. None of the other patients had a biopsy and none had evidence of lymphoma when the notes were reviewed at the end of our study.

Before the widespread availability of immunoflow cytometry, immunofluorescent cytochemistry on cytopsin preparations was used in some large laboratories for the characterisation of lymphomas from FNA samples. This has the advantage that it can be performed with fewer cells and it is not difficult to appreciate the relation between the size of the cells and their antigen expression. The latter makes it easier to diagnose some conditions such as T cell rich B cell lymphomas. Immunocytochemistry has some major disadvantages. The technique is highly labour intensive and requires specially trained technical staff. Dual expression of markers is less easy to demonstrate. A result would usually take considerably longer than the four hour turn around time that we have for immunoflow cytometry. The number of cells that can be counted and the number of markers that can be used are restricted by the time it takes to perform manual counting. A higher light chain ratio (about 6 : 1) is required to prove monoclonality,<sup>7</sup> although in practice this rarely alters the detection of monoclonality in a sample.<sup>8</sup> For these reasons, immunoflow cytometry is probably the preferred method except in a few specialist centres.

Young *et al* graded the follicle centre cell lymphomas in their study<sup>2</sup> according to the proportion of "transformed cells" in the smear. Their method was validated in a previous study.<sup>9</sup> We did not attempt to do this because it would rarely change the way in which the patient was treated.

Eight of the 47 malignant lesions were diagnosed using cell blocks. These comprised one case of myeloma, four of the six T cell lymphomas, and three of the four non-lymphoid malignancies. This demonstrates the role of cell block immunohistochemistry in the diagnosis of suspected lymphoid neoplasms. Although immunoflow cytometry is highly effective in the diagnosis of B cell lymphomas,

particularly CLL, FCCL, and mantle cell lymphoma, cell block immunohistochemistry is probably more useful for "high grade lymphomas", especially peripheral T cell lymphomas, and also for non-lymphoid neoplasms. If a sample from a suspected lymphoid lesion is overtly malignant on cytological criteria then more information is likely to be obtained from a cell block, but if the sample is equivocally benign/malignant then immunoflow cytometry is probably more useful. Ideally, tissue should be obtained for immunoflow cytometry and a cell block, although in practice this is often not possible.

Optimum diagnostic information might be best obtained by biopsying every suspicious lymph node and performing a battery of sophisticated investigations including oncogene expression, cell kinetic studies, and detailed molecular analysis. A biopsy might be particularly important if spare material is needed for research. However, only a few centres have the budget, facilities, or expertise to do all of this. We would always advocate that an FNA diagnosed lymphoma in an otherwise fit young patient should be followed by a biopsy. Most lymphomas occur in elderly patients and are not curable. In these patients, treatment and survival are often influenced more by coexistent pathology, so that detailed analysis of the lymphoma might supply the clinician with a level of detail that is superfluous. Despite the pitfalls that were apparent in our study, FNA with immunoflow cytometry is a powerful technique for the diagnosis and typing of B cell lymphomas. Close communication between the oncologist and pathologist is needed to ensure that FNA sampling is performed on appropriate cases and that the result and issues of uncertainty are conveyed. In the setting of a large general hospital in rural New Zealand, the speed with which FNA cytology and immunoflow cytometry could be performed (about four hours) was important. It allowed patients who had travelled a long distance to be given their result quickly, and allowed other investigations to be undertaken the same day in preparation for treatment.

## Conclusions

Our study shows that it is almost always possible to obtain an adequate sample for immunoflow cytometry from a fine needle aspirate. Light chain restriction could be demonstrated in almost all FNA samples from B cell lymphomas. One notable exception was T cell rich B cell lymphoma. Artefactual light chain restriction could occasionally be seen in T cell lymphomas, presumably because of autoantibodies binding to the cell surfaces. It was possible to subtype most B cell lymphomas as CLL, FCCL, or mantle cell lymphoma. Immunoflow cytometry is not particularly helpful in the diagnosis of T cell lymphoma or Hodgkin's lymphoma. The CD4 to CD8 ratio was not usually restricted in T cell lymphomas and neither was coexpression of CD4 and CD8 usually found. Loss of pan-T cell antigens was seen in some T cell lymphomas. Four of the six T cell lymphomas and three of the four non-lymphoid malignancies were diagnosed

with the aid of cell block immunohistochemistry. We advocate the use of cell block immunohistochemistry in preference to immunoflow cytometry for cases in which the cytological appearance of the specimen is overtly malignant but the differential diagnosis includes non-lymphoid malignancy.

- 1 Skoog L, Tani E. The role of fine-needle aspiration cytology in the diagnosis of non-Hodgkin's lymphoma. *Diagn Oncol* 1991;**1**:12-18.
- 2 Young NA, Al-Saleem TI, Ehya H, *et al.* Utilization of fine-needle aspiration cytology and flow cytometry in the diagnosis and subclassification of primary and recurrent lymphoma. *Cancer* 1998;**84**:252-61.
- 3 Zander DS, Iturraspe JA, Everett ET, *et al.* Flow cytometry: in vitro assessment of its potential application for the diagnosis and classification of lymphoid processes in cytological preparations from fine-needle aspirates. *Am J Clin Pathol* 1994;**101**:577-86.
- 4 Zardawi IM, Jain S, Bennett G. Flow-cytometric algorithm on fine-needle aspirates for the clinical workup of patients with lymphadenopathy. *Diagn Cytopathol* 1998;**19**:274-8.
- 5 Mayall F, Chang B, Darlington A. A review of 50 consecutive cytology cell block preparations in a large general hospital. *J Clin Pathol* 1997;**50**:985-90.
- 6 Jeffers MD, Milton J, Herriot R, *et al.* Fine needle aspiration cytology in the investigation of non-Hodgkins lymphoma. *J Clin Pathol* 1998;**51**:189-96.
- 7 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.
- 8 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.
- 9 Young NA, Al-Saleem TI, Al-Saleem Z, *et al.* The value of transformed lymphocyte count in subclassification of non-Hodgkin's lymphoma by fine needle aspiration. *Am J Clin Pathol* 1997;**108**:143-51.