Fine needle aspiration cytology in the diagnosis of uncommon types of lymphoma

F Mayall, A Darlington, B Harrison

Aims: Fine needle aspiration (FNA) cytology is an accepted means of diagnosing and typing common forms of lymphoma, particularly small lymphocytic lymphoma, follicular lymphoma, and large B cell lymphoma. However, its usefulness for diagnosing less common forms of lymphoma is not clearly established and this study was designed to examine this.

Methods: The study reviewed the FNAs of suspected lymphomas collected over a period of approximately five years.

Results: FNA samples were available for 138 definite lymphomas; most were common forms of B cell lymphoma. However, there was also one Burkitt lymphoma (BL), two Burkitt-like large B cell lymphomas, 15 classic Hodgkin lymphomas (HLs), two nodular lymphocyte predominant Hodgkin lymphomas, four mantle cell lymphomas, two mediastinal (thymic) large B cell lymphomas (MCLs), 11 peripheral T cell lymphomas (PTCLs), and five T cell rich large B cell lymphomas (TCRBLCs).

Conclusions: FNA diagnosis of BL was possible with immunoflow cytometry (IFC), cell block immunohistochemistry (IHC), and cell block fluorescent in situ hybridisation for c-myc alteration. It was difficult to make a definite diagnosis of HL and MCL on FNA alone. Both tend to be sclerotic tumours and FNA tends to yield scanty neoplastic cells. The FNA diagnosis of PTCL depended on cell block IHC; IFC was not usually useful. TCRBCL did not show light chain restriction on IFC of FNA samples, probably because of frequent reactive B cells in the tumour. Thus, HL, MCL, and TCRBCL are often difficult to diagnose accurately on FNA cytology, even when using IFC and cell block IHC.

In the past 10 years, fine needle aspiration (FNA) cytology has become accepted as a means of diagnosing and typing common forms of lymphoma, particularly small lymphocytic lymphoma, follicular lymphoma, and large B cell lymphoma. These types of lymphoma alone make up approximately 70% of all lymphomas. The usefulness of FNA cytology in the diagnosis of these lymphomas is reliant on immunoflow cytometry (IFC) and cell block immunohistochemistry (IHC). It is also reliant on the pathologist making “near patient” provisional assessment of the nature of the specimen and then collecting appropriate specimens. However, the usefulness of FNA cytology in the diagnosis of less common forms of lymphoma is not clearly established, and our study set out to examine this issue.

METHODS

The method was similar to that described in a previous study from our department. The study was set in a large tertiary referral hospital in rural New Zealand. We reviewed the FNAs of suspected lymphomas collected over a period of approximately five years. Most of these were either benign or were common forms of lymphoma that were not the focus of our present study. The cases selected for further examination were those with a final diagnosis of Burkitt lymphoma, Burkitt-like large B cell lymphoma, classic Hodgkin lymphoma, nodular lymphocyte predominant Hodgkin lymphoma, mediastinal (thymic) large B cell lymphoma, peripheral T cell lymphoma, and T cell rich large B cell lymphoma.

The FNAs were performed by one of three pathologists with an interest in FNA cytology, except for image guided FNAs, in which the needle was given to one of these pathologists for preparation of the slides after the radiologist had aspirated the lesion. Early on in the study, the pathologist frequently visited the patients on the wards to perform FNAs, but this was often unsatisfactory because the patient was unavailable or distracted. Later in the study, the patients were brought to the outpatients’ clinic in the pathology department. The FNAs performed by the pathologists were done with a needle only technique, using a 23 or 25 gauge 1.5 inch (38 mm) needle. The slides were air dried, and then centrifuged again for two minutes. The leucocytes were then resuspended in 2 ml of phosphate buffered saline (PBS, phosphate buffered saline) 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 slides were then taken back to the laboratory to be mounted and examined again. The specimen in heparin RPMI was taken to the haematology laboratory, transferred to a 5 ml tube, and centrifuged for two minutes at approximately 400 g. The supernatant was discarded and the cells were resuspended in 2 ml of ammonium chloride lysis solution. The cells were gently vortexed and 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 screen filter. An approximate cell count was performed.

Abbreviations: FNA, fine needle aspiration; FISH, fluorescent in situ hybridisation; IFC, immunoflow cytometry; IHC, immunohistochemistry; PBS, phosphate buffered saline

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and a panel of directly conjugated monoclonal antibodies was 
selected (Immunotech, Westbrook, Maine, USA). The cells 
and the antibodies were incubated for 20 minutes in the dark 
at room temperature, after which they were washed once in 
PBS, centrifuged, and washed again. They were then 
analysed with the flow cytometer. Material for k and l light 
chain analysis was treated differently. The centrifuged 
sample in heparin RPMI was incubated with prewarmed 
PBS for 20–30 minutes at 37°C, to remove cytocytic 
immunoglobulin, and then centrifuged. The supernatant 
was discarded and if red cells were present then they were 
lysed with ammonium chloride as above. Cells were then 
incubated with antibodies (CD19 and anti-k or CD19 and 
anti-l) as above.

A "first run" panel of antibodies evolved as our 
experience developed and with the acquisition of a new 
immunoflow cytometer approximately a quarter of the way 
through our study. In some cases we performed a "second 
rung" with a more specialised panel to investigate a specific 
diagnosis. The number of antibodies used was sometimes 
restricted by the scarcity of cells. At the start of our study, a 
Coulter PROFILE II was used, but later the laboratory 
obtained a Coulter EPICS® XL (Beckman Coulter, Fullerton, 
California, USA).

In our laboratory we have adopted light chain ratio limits 
from previous studies. \( ^{4–6} \) A k to l ratio of greater than 3 or a l 
to k ratio of greater than 2 were accepted as evidence of 
monoclonality.

For some cases, cell blocks for immunohistochemistry were 
collected as in our earlier paper. \( ^{7} \) These were usually taken 
after the initial FNA showed overtly malignant cytology, as 
described previously. \( ^{7} \) Recently we have adopted a variation 
of this method when possible. This involves placing the drop 
of sample on the upturned lid of the specimen container 
instead of washing it into the formalin. The lid is left 
upturned for a few minutes to allow the sample to start to 
clot. The lid is then turned over and screwed down. The drop 
of sample on the lid is then left to fix in the formalin vapour 
for a least six hours. It is then prised off the lid with a scalpel 
blade and is treated as if it were a routine biopsy histology 
specimen. This "clot block" gives rise to a higher density of 
cellular material than is obtained from a cell block formed 
from agar, shows fewer artefacts, and requires less prepara-
tion.

Cell block fluorescent in situ hybridisation (FISH) was 
performed on cell blocks from three cases of possible Burkitt 
lymphoma to examine possible c-myc gene rearrangements. 
The sections for FISH were prepared in the same way as for 
cell block immunohistochemistry. They were then tested with 
an 8q24 c-myc break gene rearrangement probe.

RESULTS

During our five year study period, a total of 138 definite 
lymphomas were diagnosed that had FNA samples available. 
Most of these 138 tumours were common forms of B cell 
lymphoma. However, there was also one case of Burkitt 
lymphoma, two of Burkitt-like large B cell lymphoma, 15 of 
classic Hodgkin lymphoma, two of nodular lymphocyte 
predominant Hodgkin lymphoma, four of mantle cell 
lymphoma, two of mediastinal (thymic) large B cell 
lymphoma, 11 of peripheral T cell lymphoma, and five of T 
cell rich large B cell lymphoma. Table 1 shows the details of 
these cases. The first three cases in table 1 were suspected 
Burkitt lymphomas and had cell block FISH performed, but 
this demonstrated an abnormality only in case 2.

DISCUSSION

The case of Burkitt lymphoma and the two cases of Burkitt-
like large B cell lymphoma were similar in that they all had 
morphology consistent with Burkitt lymphoma and strongly 
expressed CD10 and CD77 on IFC. Only in case 2 did cell 
block FISH show a 8q24 c-myc breakpoint gene rearrange-
tment to confirm the diagnosis. The use of cell block FISH 
instead of conventional cytogenetics has the advantage that 
the cytogenetics can be performed as an afterthought. In 
practice, it is rarely necessary to obtain cytogenetic informa-
tion to type common forms of lymphoma, so that the use of 
the limited amount of sample material in this way is not 
wartanted. Cytogenetics becomes an expensive investigation 
if done unselectively.

Of the 15 cases of classic Hodgkin lymphoma only one 
yielded inadequate cells for cytological examination. It was 
thought that five were probably Hodgkin lymphoma.

"The use of cell block fluorescent in situ hybridisation 
instead of conventional cytogenetics has the advantage 
that the cytogenetics can be performed as an after-
thought" \( ^{8} \)

Five were considered to be "possible lymphoma". Five 
were considered to be reactive on cytology and even on 
review did not show convincing Reed-Sternberg cells (fig 1). 
Seven yielded insufficient cells for IFC. All of the cases for 
which IFC was performed showed a predominant population 
of T cells, except for case 4. This patient had had chronic 
lymphocytic leukaemia for many years and had had 
developed synchronous classic Hodgkin lymphoma. A 
predominant population of T cells was also found in the two 
cases of nodular lymphocyte predominant Hodgkin 
lymphoma. Of these two cases, one was thought to be reactive 
on FNA cytology and it was thought that the other was probably 
Hodgkin lymphoma. The potential of Hodgkin lymphoma to 
yield misleading "reactive" FNA cytology diagnoses has been 
discussed in previous papers, as has the difficulty in 
obtaining sufficient cells from these cases for IFC. In a 
recent study Chhieng et al reviewed FNAs of 89 cases of 
Hodgkin lymphoma. \( ^{10} \) Based on a comparison of the 
histological diagnosis and original cytological diagnosis, 43 
cases had a positive diagnosis of Hodgkin lymphoma, 20 had 
a suspicious or atypical diagnosis, 13 had a benign diagnosis 
(falsenegative cases), and 10 were non-diagnostic. Three 
additional cases had a malignant diagnosis other than 
Hodgkin lymphoma. Chhieng et al appear to have made 
definite diagnoses of Hodgkin lymphoma on FNA cytology.

Figure 1 A Diff-Quik® stained fine needle aspiration of a classic 
Hodgkin lymphoma. Typically, Reed-Sternberg cells are either absent or 
are only scanty, and even then typical bilobate nuclear morphology is 
often not seen clearly.
Table 1 Details of the uncommon forms of B cell lymphoma encountered in our study

<table>
<thead>
<tr>
<th>Case</th>
<th>Site</th>
<th>Method</th>
<th>Initial diagnosis</th>
<th>Percentage of cells positive (numbers indicate CD numbers)</th>
<th>Final diagnosis</th>
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<td></td>
<td></td>
<td></td>
<td>Percentage of cells positive (numbers indicate CD numbers)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Para-aortic</td>
<td>I</td>
<td>Large cell lymphoma</td>
<td>17 5 6 10 79 91 79 3 2 73 94</td>
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<td>2</td>
<td>Mesenteric</td>
<td>I</td>
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<td>1 8 1 1 99 99 99 2 24 99</td>
<td>BJBC</td>
</tr>
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<td>M</td>
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<td>1 4 3 1 1 96 90 98 3 98 96</td>
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</tr>
<tr>
<td>4</td>
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<td>M</td>
<td>THL</td>
<td>30 23 99 6 0 68 71 54 71 28</td>
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</tr>
<tr>
<td>5</td>
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<td>M</td>
<td>Reactive</td>
<td>Inadequate for flow cytometry</td>
<td>65 1 67 0 No Yes</td>
</tr>
<tr>
<td>6</td>
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<td>65 1 67 0 No Yes</td>
<td>BL (on biopsy)</td>
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<tr>
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</tr>
<tr>
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</tr>
<tr>
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<td>M</td>
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</tr>
<tr>
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<td>11</td>
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<td>Inadequate cells</td>
<td>90 77 20 2 11 9 8 9 9</td>
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</tr>
<tr>
<td>12</td>
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<td>I</td>
<td>Lymphoma</td>
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<td>BL (on biopsy)</td>
</tr>
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</tr>
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<td>34 23 4 0 Yes No</td>
<td>BL (on biopsy)</td>
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<tr>
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<td>?Lymphoma</td>
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<tr>
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<tr>
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<td>BL (on biopsy)</td>
</tr>
<tr>
<td>22</td>
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<td>23</td>
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<td>M</td>
<td>Lymphoma</td>
<td>2 5 5 100 6 1 99 99 14 99 99</td>
<td>No No</td>
</tr>
<tr>
<td>24</td>
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<td>Lymphoma</td>
<td>4 2 100 1 1 97 99 12</td>
<td>No No</td>
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<td>Lymphoma</td>
<td>4 2 100 1 1 97 99 12</td>
<td>No No</td>
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<tr>
<td>26</td>
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<td>Lymphoma</td>
<td>4 2 100 1 1 97 99 12</td>
<td>No No</td>
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<tr>
<td>27</td>
<td>Perihepatic mass</td>
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<td>Lymphoma/carcinoma</td>
<td>80 62 78 10 17 12</td>
<td>Yes No</td>
</tr>
<tr>
<td>28</td>
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<td>M</td>
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<td>76 42 78 31 1 2 1 1</td>
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</tr>
<tr>
<td>29</td>
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<td>M</td>
<td>Lymphoma</td>
<td>68 63 49 60 11 0 18 14 14 11 15</td>
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<tr>
<td>30</td>
<td>Right neck</td>
<td>M</td>
<td>Malignant</td>
<td>93 43 83 41 0 6</td>
<td>No No</td>
</tr>
<tr>
<td>31</td>
<td>Right neck</td>
<td>M</td>
<td>Lymphoma</td>
<td>94 78 89 1 92 0 1 1</td>
<td>No No</td>
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<tr>
<td>32</td>
<td>Left neck</td>
<td>M</td>
<td>Malignant</td>
<td>70 0 16</td>
<td>No No</td>
</tr>
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<td>No No</td>
</tr>
<tr>
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<td>M</td>
<td>Malignant</td>
<td>68 17 51 60 40 17</td>
<td>No No</td>
</tr>
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<td>35</td>
<td>Left inguinal</td>
<td>M</td>
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<td>70 42 26 3 30 30</td>
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<td>37</td>
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<td>M</td>
<td>Lymphoma/carcinoma</td>
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<td>M</td>
<td>Probable carcinoma</td>
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<td>M</td>
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<td>Lymphoma</td>
<td>89 75 48 83 26 27 14 11 5 25 17</td>
<td>No No</td>
</tr>
<tr>
<td>42</td>
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<td>M</td>
<td>Lymphoma</td>
<td>79 55 76 18 6 28 30 6 30 35</td>
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</tr>
</tbody>
</table>

BL, Burkitt lymphoma; BJBC, Burkitt-like large B cell lymphoma; CLL, chronic lymphocytic leukaemia; FNA, fine needle aspiration; I, image guided FNA; M, manually guided FNA; MCL, mantle cell lymphoma; MBCL, mediastinal (thymic) large B cell lymphoma; NLPHL, nodular lymphocyte predominant Hodgkin lymphoma; PTCL, peripheral T cell lymphoma; TCRBCL, T cell rich B cell lymphoma.
In our laboratory, we would not usually make a definite diagnosis of Hodgkin lymphoma without a biopsy, particularly since the recognition of nodular lymphocyte predominant Hodgkin lymphoma as a separate entity to classic Hodgkin lymphoma. This also appears to be the view of Young and Al-Saleem. An exception would be in the event of recurrent disease that had previously biopsy confirmed. Another exception would be if the patient’s clinical condition was so poor that a biopsy was not possible before treatment. The only case of Hodgkin lymphoma in our study that did not have a biopsy was a patient with Ann Arbor stage IV disease. Chemotherapy was initiated immediately after the FNA. Cell block IHC can assist in the diagnosis of Hodgkin lymphoma and the distinction between classic Hodgkin lymphoma and nodular lymphocyte predominant Hodgkin lymphoma. However, in practice, it is often not possible to obtain a sufficiently cellular sample for a cell block. The reason for this is probably that classic Hodgkin lymphoma is usually a sclerotic tumour, and also the neoplastic cells are only a minor component of the (usually) scanty sample.

### Take home messages

- Fine needle aspiration (FNA) diagnosis of Burkitt lymphoma was possible with immunoflow cytometry (IFC), cell block immunohistochemistry (IHC), and fluorescence in situ hybridisation for c-myc alteration.
- The definitive diagnosis of Hodgkin lymphoma and mediastinal (thymic) large B cell lymphoma was difficult on FNA alone.
- The FNA diagnosis of peripheral T cell lymphoma depended on cell block IHC, and IFC was not useful.
- T cell rich large B cell lymphomas did not show light chain restriction on IFC of FNA samples, probably because of frequent reactive B cells in the tumour.
- Thus, although FNA is accurate for diagnosing and typing common forms of lymphoma, Hodgkin lymphoma, mediastinal (thymic) large B cell lymphoma, and T cell rich large B cell lymphoma are often difficult to diagnose accurately on FNA cytology, even with the use of IFC and cell block IHC.

A “small cell” lymphoma with an immunophenotype of CD5+, CD19+, and CD23+ strongly suggests a mantle cell lymphoma. Typically, mantle cell lymphoma has rather characteristic nuclear features, including monotonous nuclei with finely stippled chromatin.

### Figure 2

A Diff-Quik® stained fine needle aspiration of a mediastinal (thymic) large B cell lymphoma. This type of lymphoma yielded very scanty cells that often exhibited artefacts and were difficult to interpret. In one patient, five aspirations were required before a definite diagnosis of malignancy could be made.

### Figure 3

A paraffin wax embedded section of a T cell rich large B cell lymphoma that has been immunostained for CD20. There are approximately as many malignant (large) B cells as there are benign (small) B cells. If the ratio of malignant to benign B cells is less than approximately 1.5, then light chain restriction will not be detectable in the B cell population as a whole.
straightforward strategy for finalising the diagnosis than using a scanty sample for IFC with an extended panel of markers in the anticipation that abnormal expression might be found.

There were five cases of T cell rich large B cell lymphomas. All of these showed no evidence of light chain restriction. An examination of the IFC results for these cases shows that the proportion of B cells in these cases (CD19 or CD20 positive) ranged from 1% to 30%. However, on examining the CD20 immunostain on the corresponding histological material it is evident that many of the CD20 positive cells were in fact small benign B cells. Although relatively inconspicuous because of their small size, these benign B cells are often numerous (fig 3). A simple calculation shows that it is not possible to achieve significant light chain restriction (above a $\lambda$ to $\kappa$ ratio of 2 or a $\kappa$ to $\lambda$ ratio of 3) if the ratio of malignant B cells to benign B cells is less than approximately 1.5. It seems likely that the benign B cells “swamp” any light chain restriction in the neoplastic B cells, rendering it undetectable. In our laboratory, less than 0.5% of B cell lymphomas fail to show light chain restriction on IFC that is technically adequate, if T cell rich large B cell lymphomas are excluded. It has been suggested that light chain restriction can be demonstrated in T cell rich large B cell lymphoma by selectively gating the larger B cells. However, this first requires the diagnosis of T cell rich large B cell lymphoma to be suspected. In addition, when this strategy was attempted on the stored data from our cases it was possible to demonstrate light chain restriction in only one of the five cases (case 38; $\kappa$ to $\lambda$ ratio of 30).

“It is not possible to achieve significant light chain restriction if the ratio of malignant B cells to benign B cells is less than approximately 1.5.”

In conclusion, FNA diagnosis of Burkitt lymphoma was possible with IFC, cell block IHC, and FISH for c-myc alteration. It was difficult to make a definite diagnosis of Hodgkin lymphoma and mediastinal (thymic) large B cell lymphoma on FNA alone. Both tend to be sclerotic tumours and FNA tends to yield scanty neoplastic cells. The FNA diagnosis of peripheral T cell lymphoma depended on cell block IHC. IFC was not usually useful. T cell rich large B cell lymphomas did not show light chain restriction on IFC of FNA samples, probably because of frequent reactive B cells in the tumour. FNA is established as an accurate method of diagnosing and typing common forms of lymphoma. However, our study shows that Hodgkin lymphoma, mediastinal (thymic) large B cell lymphoma, and T cell rich large B cell lymphoma are often difficult to diagnose accurately on FNA cytology, even with the use of IFC and cell block IHC.

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REFERENCES