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Cytodiagnosis of non-Hodgkin's lymphoma

A morphological analysis of 215 biopsy proven cases[1]

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Summary. A cytomorphological analysis using a large number of variables was applied to 136 fine needle aspirations and 122 imprints from 258 biopsy proven lymphoid lesions, including 203 non-Hodgkin's lymphomas (NHL), 12 true histiocytic neoplasms and 43 lymphoid hyperplasias. Characteristics of the various cell types are described using the Kiel classification predominantly.

Two blindly performed consecutive cytological analyses, indicated as Cyt I and Cyt II respectively, were compared with histology. False-positive conclusions did not occur. False-negative conclusions were present in 3.7% of cases. The intra-observer reproducibility between Cyt I and Cyt II was 93%.

The inter-observer reproducibility between the 4 authors was examined in 50 cases; consensus regarding malignancy, exact NHL type and benign cytology was 88%, 70% and 100% respectively. Cytodiagnosis of NHL is a reliable method with a high sensitivity which can add substantial information to tissue diagnosis in troublesome cases.

Fine needle aspirations, if adequately performed, had the same cellularity, quality and cellular composition as imprints. Aspiration cytology is very useful for the selection of a representative lymph node for surgical biopsy, for the diagnosis of recurrent NHL, for staging the extent of disease, for the diagnosis of cases in which tissue biopsy is not possible, and as a variable for monitoring treatment.

Key words: Cytology of lymphoma – Kiel classification – Non Hodgkin's lymphoma

To our knowledge no reports on cytodiagnosis of biopsy-confirmed non-Hodgkin's lymphomas (NHL) exist in which an extensive number of cases

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Abbreviations: Cyt I: first blind cytological screening; Cyt II: second blind cytological screening; FNA: fine needle aspiration; l.g.b.: lymphoglandular body; LH: lymphoid hyperplasia; MHT: malignant histiocytic tumour; NHL: non-Hodgkin's lymphoma

is examined using multiple variables; in addition, only few reports describe the reproducibility of cytodiagnosis of NHL (Orell 1982). At least 3 important causes may be responsible for the limited data on this subject. Firstly, the changing concepts and controversial ideas about the different classifications of NHL lead to confusion in the nomenclature of NHL cells. Secondly, cytology in NHL is associated with considerable diagnostic difficulties, even more than histodiagnosis in which a marked variation in reliability and reproducibility exists. Thirdly, a surgical biopsy for histodiagnosis is still required in view of the prognostically important (follicular or diffuse) architecture of the NHL.

On the other hand several reports disclose the advantages of cytodiagnosis of lymphoid lesions. Cell material can be easily obtained and prepared. Imprints may reduce artifactual distortion and enhance cellular details (Feinberg 1980; Lennert 1978). Fine needle aspiration is a good method for selection of a representative node for surgical biopsy and for investigation of recurrent disease (Orell 1982); it is sufficiently accurate for diagnosis in case of inoperable patients. Moreover, fine needle aspirations are easily repeatable so that a lesion can be followed as indicator for determining the reaction to chemo- and/or radiotherapy. According to Lopes Cardozo (1975) there is "no better tool than cytology for determining the stage and various locations of malignant lymphomas". In addition, all established diagnostic methods in use for lymphatic tissue investigation, such as immunology (Bom 1978; Marsh 1983) enzyme cytochemistry (van Heerde 1980; Lennert 1978; Marsh 1983) and electron microscopy (van Heerde 1980) can be applied to aspirated or suspended cells.

According to the late Zajicek (1979), one of the great pioneers of aspiration cytology, "the possibility of extending the classification of malignant lymphomas to aspiration smears has not yet been investigated". With this aim in mind it is our purpose to demonstrate the diagnostic value of cytology in 215 histologically proven non-Hodgkin's lymphomas, using a morphological analysis, and to investigate the intra- and inter-observer reproducibility.

Materials and methods

Cytological smears, both imprints and (in vivo) fine needle aspirations, from biopsy proven lymphoid lesions in 280 consecutive untreated patients, were subjected to a cytomorphological analysis. Twenty-two cases were eliminated because of insufficient material (9×) or diagnosis other than NHL (13×). The material consisted of 136 fine needle aspirations (FNA) and 122 imprints. FNA were performed in vivo with a 23 gauge needle attached to a 20 ml disposable syringe held in a pistol grip (Cameco, Sweden). The cell material was smeared directly, air-dried and stained routinely with Giemsa. Usually highly cellular smears were obtained; in cases where the cell yield was too low, the procedure was repeated.

All material was collected from the Netherlands Cancer Institute files, from 1974 to 1981. The male/female ratio was 163:117, the age ranged from 1 to 86 years with a mean of 66.5 years. Forty-three consecutive cases of benign lymphoid hyperplasia were intermingled at random between the malignant cases as a control. Some tricky cases of paracortical virus induced hyperplasias, histologically and cytologically strongly mimicking malignant lymphoma, were included. The malignant cases comprised 203 non-Hodgkin's lymphomas (NHL) and 12 tumours derived from true histiocytes. Patients names were removed from the slides which were coded randomly.

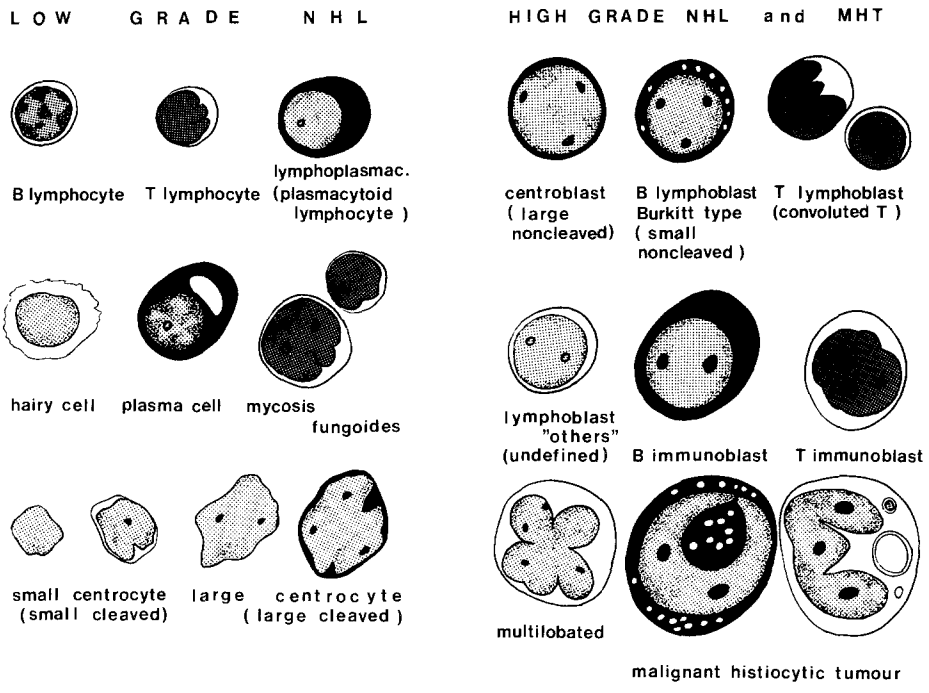


Fig. 1. Schematic drawing of NHL and MHT cells; Lennerts classification. In parentheses Lukes' and Collins' classification if different from Lennerts classification. Lymphoplasmac. = lymphoplasmacytoid

In 83 of the 215 malignant cases the diagnosis was established by immunological, enzyme-histochemical and electron microscopical investigation, as previously described (van Heerde 1980), recently amplified with monoclonal antibodies. All smears were routinely Giemsa stained; in selected cases other stains like PAS, Oil Red O, acid phosphatase and α-naphthylacetate esterase were done. Histological slides were stained with H & E, PAS, reticulin, Giemsa and methylgreen pyronin.

A multivariable cytomorphological analysis was performed. The following variables were tested: quality and cellularity of the smear, degree of (de)differentiation, monotony or heterogeneity of the total cell population, number of normal histiocytes, epithelioid cells, starry sky macrophages, granulocytes, plasma cells, small lymphocytes, number of loose cytoplasmic fragments (so-called "lymphoglandular bodies"), (Conjalka 1979; Söderström 1966) including vacuolated fragments, number of mitotic figures, degree of lysis, chromatin pattern (patchy as in normal lymphocytes, coarse, finely reticular etc.), and finally the number of all categories of abnormal cells. The mean maximum nuclear diameter of diagnostically representative lymphoid cells was determined in comparison with the maximum diameter of a red blood cell or nucleus of a small lymphocyte. Cells not fitting into a well-known entity were categorized as unclassifiable.

The Kiel classification (Lennert 1978) was used since this classification has a cytological base, with clearly defined cell types, and a close relation to immunology. The main cell types are drawn schematically in figure 1 with synonyms in the Lukes-Collins classification (1975).

All cytological slides were investigated blindly by one of us (P.v.H). The above mentioned criteria were determined without any clinical information, nor with knowledge of other findings as histology, immunology, enzyme cytochemical and electron microscopical data.

To analyse the intra-observer reproducibility the same screening was repeated, after a period of several months, again blindly. Afterwards a third review was done in combination with clinical data, histology and – if present – immunological and other diagnostic findings.

In order to test the interobserver reproducibility the cytological smears of 50 cases were examined in the manner described above by the three other authors. In the field of cytodiagnosis of NHL a close cooperation exists between the authors; they are all experienced in the cytology of lymphomas, the Netherlands Cancer Institute being a reference centre for malignant lymphomas.

Results

The results are summarized in the Tables 1 to 6. Some examples of Giemsa-stained cytological pictures are shown in Figs. 2–14. Table 1 demonstrates the definite diagnoses, resulting from the combined (third) cyto-/histo-/immunological, immunohistochemical and electron microscopical examinations, compared with the diagnostic results of the first and second cytological (blindly performed) screening. This table shows that some groups of lymphomas were cytologically underdiagnosed, notably the plasmacytomas (see Fig. 8) and some high grade NHL. In contrast, most low grade NHL, the NHL of the miscellaneous group, and the non-neoplastic lesions were well recognized cytologically. The malignant histiocytic tumours showed a specific cytological picture, described separately (van Heerde 1984). A characteristic morphological feature of malignant histiocytes was the presence of ring-shaped nuclei ("window nuclei"), see Fig. 12. One of the histiocytic tumours appeared to arise from interdigitating cells; this case has been described elsewhere (Feltkamp 1981; van Heerde 1983).

Fine needle aspirations and imprints had virtually the same cellularity, quality and cellular composition.

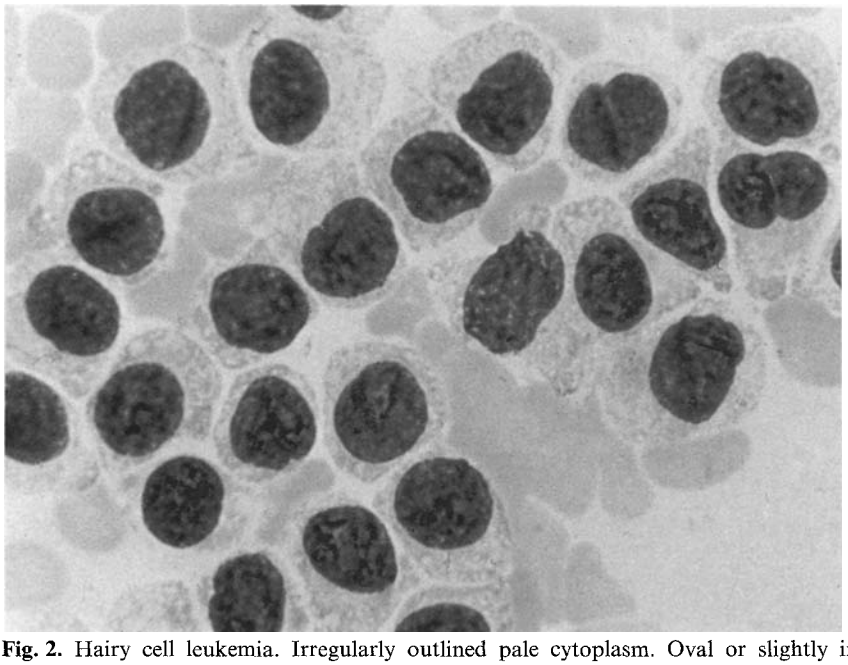


Fig. 2. Hairy cell leukemia. Irregularly outlined pale cytoplasm. Oval or slightly indented uniform nuclei without obvious nucleoli. Giemsa, ×1,200

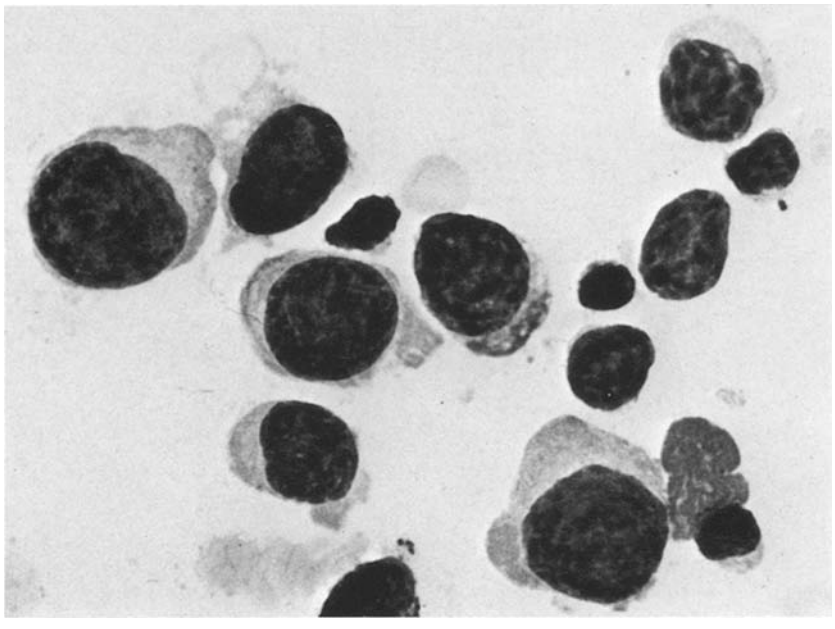


Fig. 3. Mycosis fungoides. Note anisonucleosis, hyperchromatism, pale cytoplasm and some unilateral indentations of the nuclear membrane. Giemsa, × 1,200

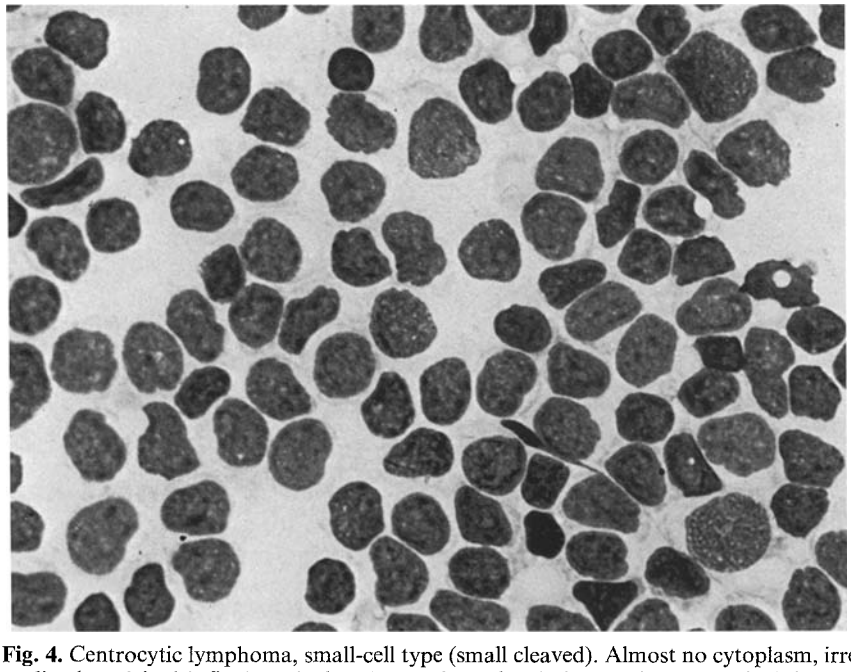


Fig. 4. Centrocytic lymphoma, small-cell type (small cleaved). Almost no cytoplasm, irregularly outlined nuclei with finely reticular chromatin and only inconspicuous nucleoli in some cells. Giemsa, × 1,200

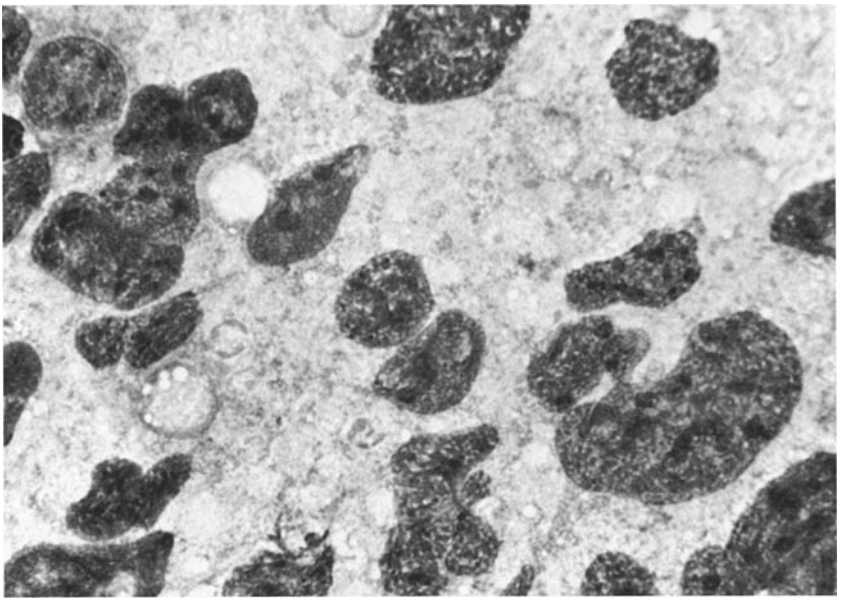


Fig. 5. Centrocytic lymphoma, large cell type (large cleaved cells). Obvious signs of dedifferentiation. Giemsa, ×1,200

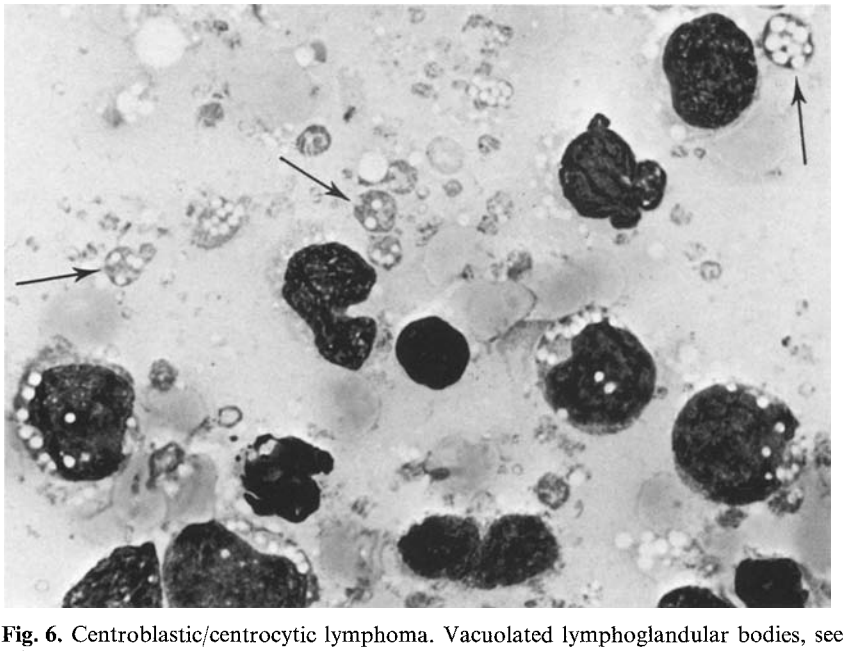


Fig. 6. Centroblastic/centrocytic lymphoma. Vacuolated lymphoglandular bodies, see arrows. Giemsa, ×1,200

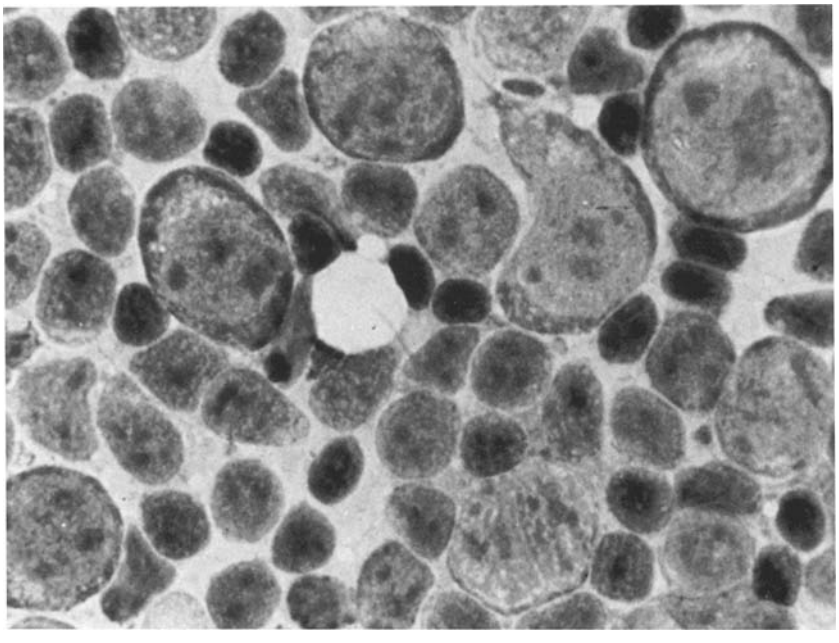


Fig. 7. Centroblastic/centrocytic lymphoma with obvious anisonucleosis. Centroblasts (large noncleaved cells) and small centrocytes (small cleaved cells) and lymphocytes. Giemsa, × 1,200

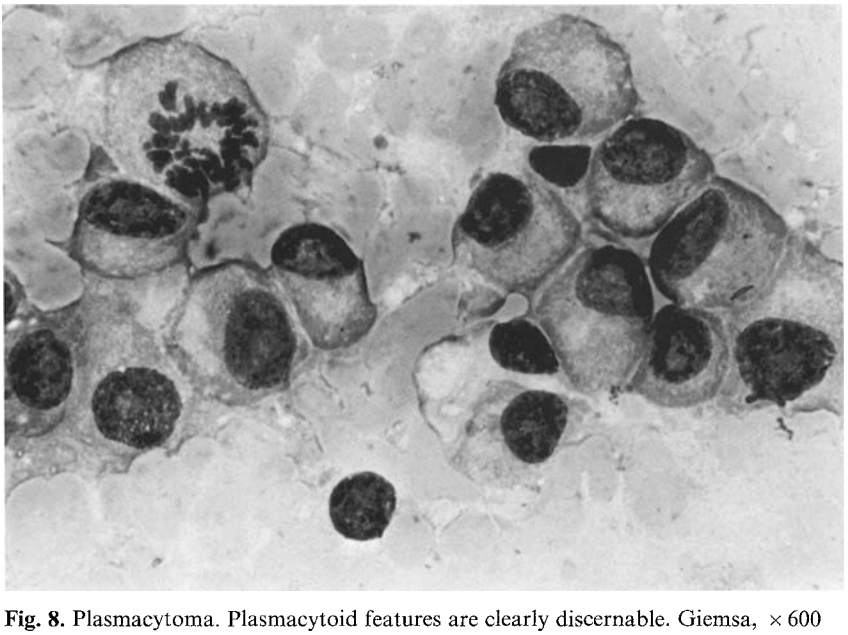


Fig. 8. Plasmacytoma. Plasmacytoid features are clearly discernable. Giemsa, × 600

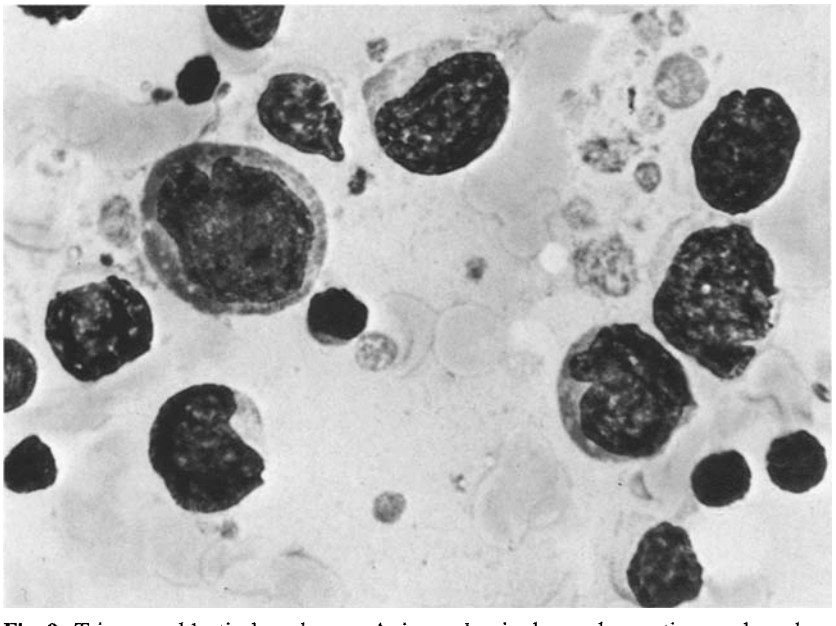


Fig. 9. T-immunoblastic lymphoma. Anisonucleosis, hyperchromatism and nuclear convolution. Lymphoglandular bodies. Giemsa, × 1,200

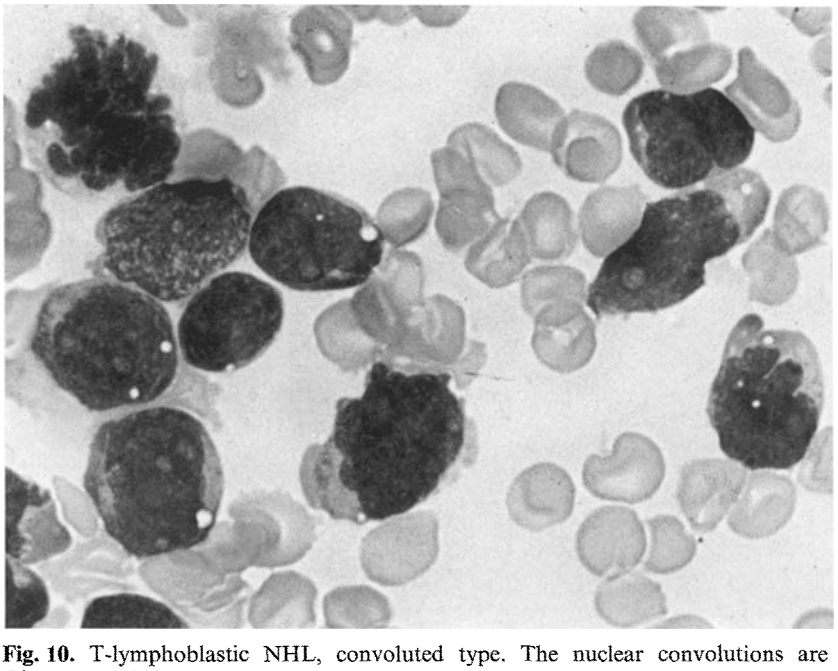


Fig. 10. T-lymphoblastic NHL, convoluted type. The nuclear convolutions are obvious. Giemsa, × 1,200

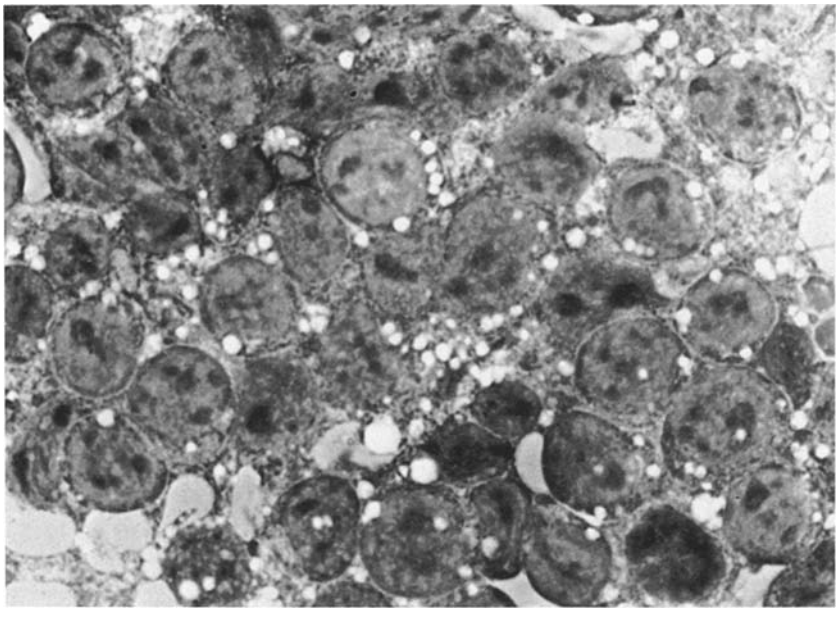


Fig. 11. B-lymphoblastic lymphoma. Monotonous picture; round cells and distinct nucleoli. Giemsa, ×1,200

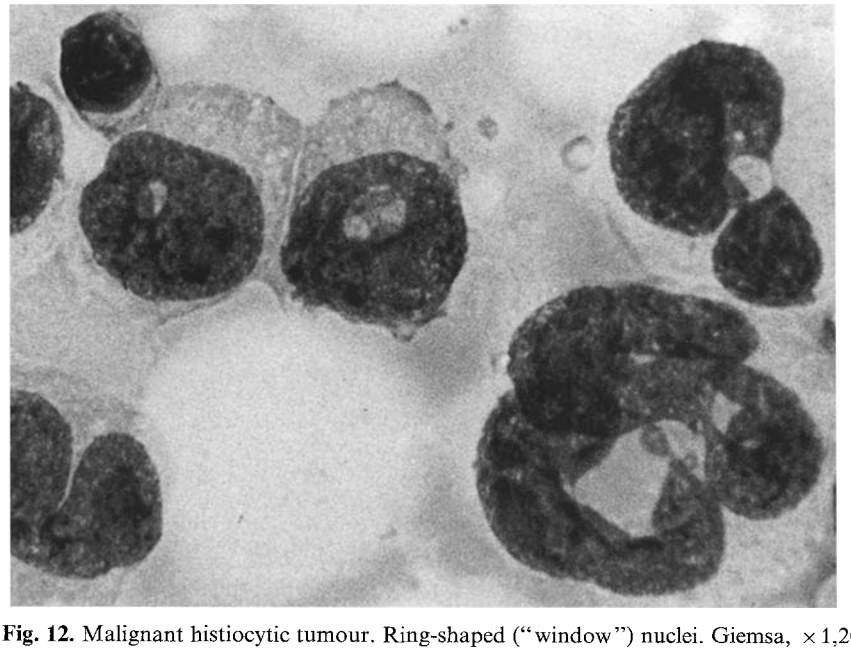


Fig. 12. Malignant histiocytic tumour. Ring-shaped ("window") nuclei. Giemsa, ×1,200

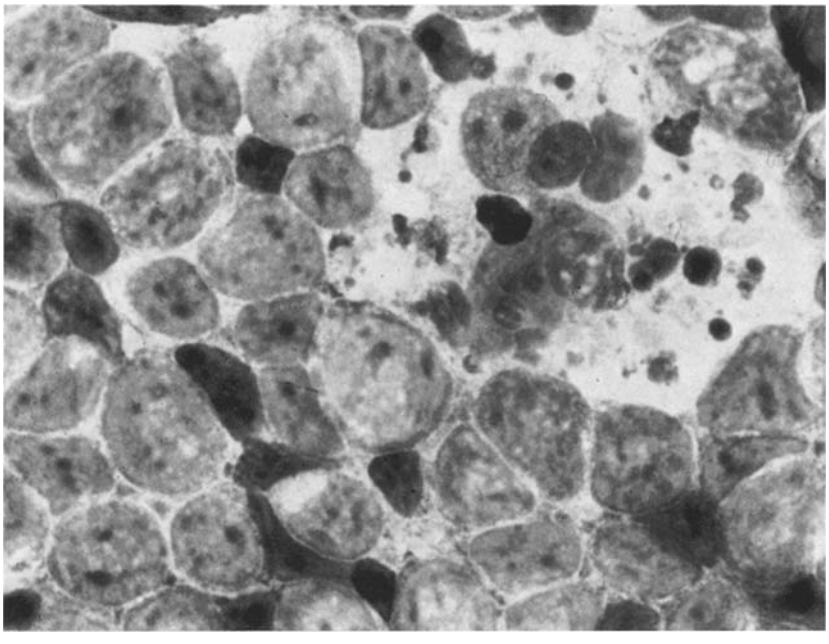


Fig. 13. Lymphoid hyperplasia (follicular in histology). Starry sky macrophage surrounded by centroblasts and some centrocytes. Giemsa, ×1,200

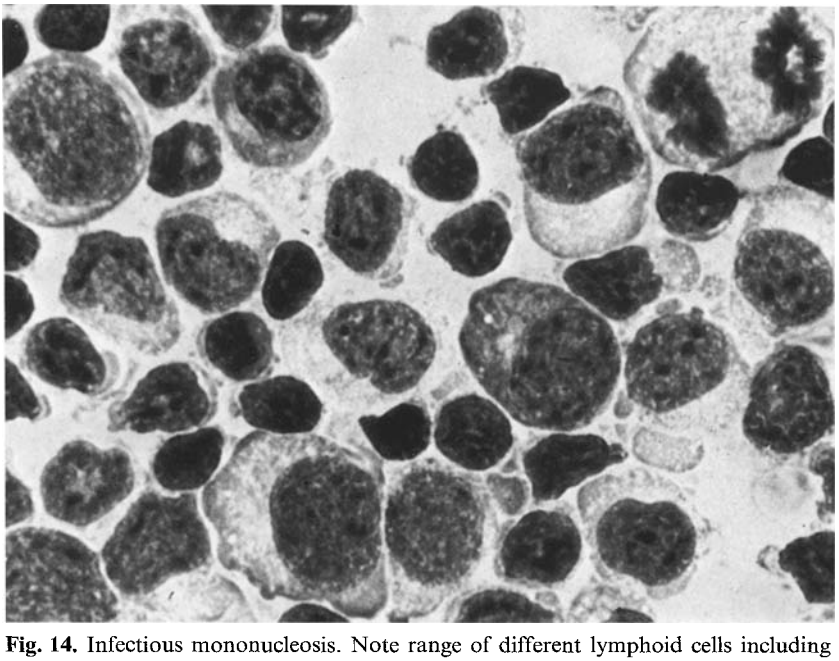


Fig. 14. Infectious mononucleosis. Note range of different lymphoid cells including immunoblasts, immature and mature plasma cells, small lymphocytes and medium-sized lymphoid cells (probably T cells). Giemsa, ×1,200

Table 1. Comparison of diagnoses from first and second blind cytological analysis with definite histological diagnoses in 258 cases. Kiel classification (Lennert 1978)

Definite diagnosis (number)			First cytology screening	Second cytology screening
Lymphocytic (8)	CLL (B)	3	2	3
	Hairy cell leukemia	3	3	3
	Mycosis fungoides	2	3	3
	CLL dd centrocytic	0	2	0
Immunocytic +plasmacytoma (12)	Lymphoplasmacytoid	3	7	4
	Polymorphous immunocytoma	3	4	3
	Immunocytic dd plasmacytoma	1	3	3
	Plasmacytoma	5	1	2
Centrocytic and/or centroblastic (151)	Centrocytic	22	31	33
	Centroblastic/centrocytic	77	67	66
	Large ("anaplastic") centrocytic	35	36	40
	Centroblastic, incl. polymorphous centroblastic	17	8	7
Lymphoblastic (15)	Lymphoblastic B	5	4	3
	Lymphoblastic T	8	5	5
	Lymphoblastic others	2	3	5
Immunoblastic (5)	Immunoblastic B	3	2	2
	Immunoblastic T	2	0	1
	Immunoblastic undefined	0	2	1
Unclassifiable high grade malignant (6)	Unclassifiable high grade malignant	6	8	7
Miscellaneous (18)	Lennert's lymphoma	2	1	2
	Multilobated lymphoma	1	1	1
	Unclassified T-cell lymphoma	3	1	1
	Malignant histiocytic tumour	12	12	12
Dubious/suspect NHL (0)	Dubious/suspect NHL	0	9	7
Lymphoid hyperplasia (43)	Lymphoid hyperplasia	43	43	44

Multiparameter analysis

The degree of dedifferentiation and the mitotic activity in the cytological smears are demonstrated in Table 2. *Dedifferentiated* lymphoid cells differ from normal lymphoid cells in having a finely reticular chromatin pattern, usually irregularly outlined nuclei (see Fig. 5) but occasionally, round nuclei (see Fig. 11), abnormal nucleoli, abnormal cytoplasmic features and in most instances many mitotic figures. The number of *mitoses*, counted in 10 cellular high power fields, – or proportionally more than 10 h.p.f. in less cellular smears –, is also demonstrated in Table 2. A high mitotic activity was found in lymphoblastic NHL, immunoblastic NHL, some large follicle centre cell NHL and malignant histiocytic tumours, not surprisingly showing a nearly

Table 2. Degree of dedifferentiation and number of mitotic figures in 258 cytological slides

Histologically proven diagnosis (number)	Degree of dedifferentiation				Number of mitoses (per 10 cellular high power fields)			
	++	+	±	−	>31	21–30	11–20	0–10
Lymphocytic: CLL, HCL, MF (8)	12.5%	50%	37.5%	0	0	0	12.5%	77.5%
Immunocytoma and plasmacytoma (12)	16%	42%	42%	0	0	8%	0	92%
Centrocytic (22)	25%	42%	29%	4%	0	0	0	100%
Centroblastic/ centrocytic (77)	46%	49%	5%	0	0	1%	10%	89%
Centroblastic and large centrocytic (52)	94%	6%	0	0	10%	10%	26%	54%
Lymphoblastic (15)	93%	7%	0	0	33%	7%	40%	20%
Immunoblastic (5)	100%	0	0	0	40%	0	40%	20%
Unclassifiable high grade malignant (6)	100%	0	0	0	0	17%	50%	33%
Other NHL (miscellaneous) (6)	67%	33%	0	0	0	0	67%	33%
Malignant histiocytic tumour (12)	100%	0	0	0	17%	49%	17%	17%
Lymphoid hyperplasia (43)	0	2%	44%	54%	0	9%	12%	79%

CLL = chronic lymphocytic leukemia; HCL = hairy cell leukemia; MF = mycosis fungoides; ++ = highly dedifferentiated; + = moderately dedifferentiated; ± = slightly dedifferentiated; − = hardly any dedifferentiated cells

similar distribution to the degree of dedifferentiation. Table 3 shows that in all cases of NHL, lymphoid hyperplasia (LH) and malignant histiocytic tumour (MHT), small round cytoplasmic fragments varying in diameter from 2 to 10 microns, the so called *"lymphoglandular bodies"* (l.g.b.) were found (Conjalka 1979; Söderström 1966), see Fig. 9. These were particularly obvious in high grade NHL: in 79% clear vacuoles were present in the l.g.b., see Fig. 6, corresponding to sudanophilic clear vacuoles in the cytoplasm of NHL cells. Vacuolated cytoplasm and vacuolated l.g.b. were not confined to B cells since they were also seen in T-cell lymphomas. Vacuolated l.g.b. were far more obvious in high grade NHL than in low grade NHL. Within the LH only a single case showed distinct vacuolated l.g.b. *Lysis* of individual cells is a well-known phenomenon in peripheral blood and bone-marrow smears from patients with B-cell chronic lymphocytic leukemia.

Table 4 shows that lysis due to fragility of cells was a fairly constant finding in the follicle centre cell lymphomas, notably in the centroblastic and large centrocytic subtypes, and also in other NHL of high grade malignancy. Lysis of cells was much less obvious, commonly absent, in MHT

Table 3. "Lymphoglandular bodies" in 258 cytological slides from NHL, malignant histiocytic tumours and lymphoid hyperplasias

Histologically proven diagnosis (number)	Non-vacuolated lymphoglandular bodies				Vacuolated lymphoglandular bodies			
	++	+	±	−	++	+	±	−
Lymphocytic: CLL, HCL, MF (8)	12.5%	37.5%	50%	0	0	0	50%	50%
Immunocytoma and plasmacytoma (12)	58%	25%	17%	0	0	17%	58%	25%
Centrocytic (22)	29%	58%	13%	0	0	4%	46%	50%
Centroblastic/ centrocytic (77)	51%	43%	6%	0	3%	17%	57%	23%
Centroblastic and large centrocytic (52)	76%	22%	2%	0	4%	38%	52%	6%
Lymphoblastic (15)	46%	27%	27%	0	0	33%	27%	40%
Immunoblastic (5)	80%	0	20%	0	0	60%	20%	20%
Unclassifiable high grade malignant (6)	67%	33%	0	0	0	50%	50%	0
Other NHL (miscellaneous) (6)	33.3%	33.3%	33.3%	0	0	17%	66%	17%
Malignant histiocytic tumour (12)	8%	33%	59%	0	0	8%	67%	25%
Non-neoplastic hyperplasia (43)	12%	79%	9%	0	0	2%	30%	68%

++ = many; + = obvious; ± = few; − = absent

and in LH. Table 4 also shows that small, normal appearing *lymphocytes* were present in almost all cases. Few small lymphocytes (less than 20% of all cells) were seen in hairy-cell leukemia (see Fig. 2), two/thirds of plasmacytomas (see Fig. 8), half of the centrocytic NHL (see Figs. 4 and 5), three quarter of the centroblastic/centrocytic NHL (see Figs. 6 and 7), and nearly all of the centroblastic, large centrocytic, lymphoblastic and unclassifiable NHL, and MHT. On the contrary, the LH were characterized in most cases (89%) by a domination of small lymphocytes. Follicular and diffuse NHL of follicle centre cell origin did not show major differences in the number of small lymphocytes, in contrast to histology (Bom 1978; van Heerde 1980; Lennert 1978) where a relatively high number of small T cells was present in the interfollicular area of follicular NHL.

The *nuclear size* of the predominant or diagnostically important cell types was determined by comparing the maximum nuclear measurement with the maximum diameter of a red blood cell or nucleus of a small lymphocyte (both 7–8 μ, Lopes Cardozo 1975; Spriggs 1981). In Table 4 the mean diameter is noted. A small nuclear diameter was present in most (93%) LH, in a large proportion of the low grade NHL, and in some lymphoblastic NHL. Large to very large nuclei were demonstrated in 42% of centroblastic

Table 4. Lysis of cells, percentage of normal looking small lymphocytes, and mean nuclear size of predominant cell type (mean max. diameter of nucleus compared to diameter of red blood cell or nucleus of small lymphocyte). 258 cases of NHL, malignant histiocytic tumour and non-neoplastic hyperplasia

Histologically proven diagnosis (number)	Lysis of cells			Number of small lymphocytes			Nuclear size / ∅ erythrocyte			
	++	+	±/−	<20%	21–40%	>41%	1–1.5 (8–12 μ)	1.6–2 (13–16 μ)	2.1–2.5 (17–20 μ)	>2.6 (>21 μ)
Lymphocytic: CLL, HCL, MF (8)	12.5%	37.5%	50%	88%	0	12%	75%	12.5%	12.5%	0
Immunocytoma and plasmacytoma (12)	0	42%	58%	42%	42%	16%	50%	50%	0	0
Centrocytic (22)	21%	54%	25%	46%	21%	33%	79%	21%	0	0
Centroblastic/ centrocytic (77)	13%	56%	31%	73%	27%	0	69%	26%	5%	0
Centroblastic and large centrocytic (52)	30%	62%	8%	90%	10%	0	2%	56%	32%	10%
Lymphoblastic (15)	13%	67%	20%	93%	7%	0	20%	73%	7%	0
Immunoblastic (5)	20%	60%	20%	60%	20%	20%	0	40%	60%	0
Unclassifiable high grade malignant (6)	50%	50%	0	83%	17%	0	17%	17%	33%	33%
Other NHL (miscellaneous) (6)	0	67%	33%	33%	67%	0	17%	0	66%	17%
Malignant histiocytic tumour (12)	0	25%	75%	100%	0	0	0	0	25%	75%
Lymphoid hyperplasia (43)	0	19%	81%	2%	9%	89%	93%	5%	2%	0

and large centrocytic NHL, 60% of immunoblastic NHL, 66% of unclassifiable high grade NHL, 83% of the miscellaneous group and all cases of MHT. In spite of a characteristically monotonous cell picture in most NHL, (see Figs. 2, 4 and 11) a marked anisonucleosis was found in immunocytomas, in some follicle centre cell lymphomas (see Fig. 7) and particularly in many T-cell NHL (see Figs. 3 and 9), including T-lymphoblastic lymphomas with convoluted nuclei. In addition to the anisonucleosis, T-cell NHL were often characterized by a marked hyperchromatism (see Figs. 3, 9 and 10), less conspicuous nucleoli, the presence of some convoluted nuclei, only slight basophilia of the cytoplasm, only a few l.g.b. and in many cases scattered eosinophils and epithelioid cells.

Non-neoplastic *non-lymphoid cells* were often seen in T-cell NHL and LH. Starry sky macrophages (see Fig. 13) in combination with centroblasts, centrocytes and small lymphocytes, with or without plasma cells and immu-

Table 5. Summary of some cytomorphological data from the multivariant analysis

	Low grade malignancy		High grade malignancy					Non-neoplastic
	lymphocytic immunocytic	cc cb/cc	cb, large cc immunoblastic	lymphoblastic B	lymphoblastic T	other T-cell NHL	malignant histiocytic	reactive lymph nodes
Dedifferentiation	±/+	+	++	++	++	++	++	−
Mitotic activity	±	±	+	++	++	+(+)	++	±
Lymphoglandular bodies	+	+(+)	++	++	+	+	+	+/±
Vacuolated lymphoglandular bodies	−	±	+	+	±/−	±/−	±	−/(±)
Lysis of cells	+	++	++	+	+	+	±	±
No. of lymphocytes	+	+	±	−	−	±	−	++
Anisonucleosis	−/±	±	+	−	+(+)	++	++	±
Starry sky macrophages	−	−	−/±	+	−	−	−	+
Vacuolated ("window") nuclei	−	−	−	−	−	−	+	−
Monotonous cell picture	+	+	+	++	+	+	+/−	±
Pleomorphic cell picture	+/−	±/−	±/−	−	+	+	−/+	+

cc = centrocytic; cb = centroblastic

noblasts, in the absence of obvious signs of dedifferentiation, were strongly indicative of a LH. Some results from the cytomorphological analysis are summarized in Table 5.

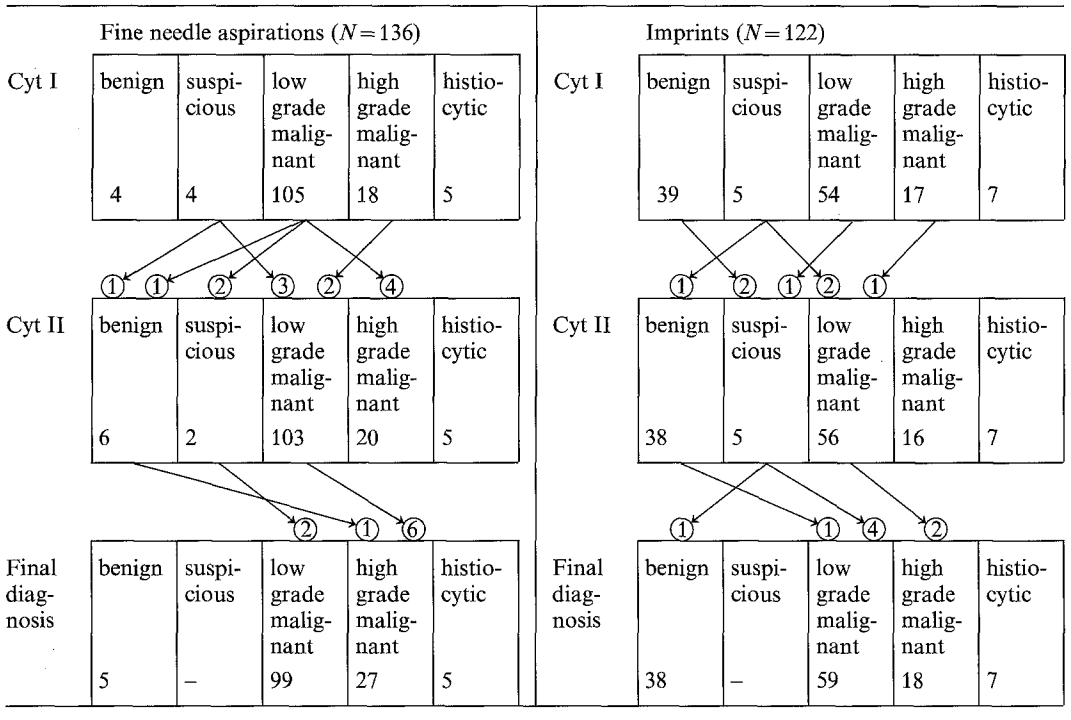
Reproducibility and accuracy

Table 6 shows the differences between the first (Cyt I) and second (Cyt II) blind cytological screening, and between Cyt II and the final diagnosis, both for fine needle aspirations and imprints. For convenience the diagnoses were categorized into 5 groups: benign, suspicious for NHL, low grade malignant NHL, high grade malignant NHL and histiocytic tumours.

1. Comparison of Cyt I and Cyt II. Differences in diagnoses occurred in 9.6% and 5.7% of FNA and imprints respectively; in most instances these were only minor. Different percentages of diagnostic cell types in various parts of the smears explained most of the discrepancies.

2. Comparison of Cyt II and final diagnosis. Differences in diagnoses occurred in 6.6% of both FNA and imprints. Discrepancies in cytological and histological diagnosis according to cell type occurred mainly in the high grade malignancies. In some instances the cytological distinction between lymphoblastic NHL and purely centroblastic NHL was difficult. Some cases of polymorphic centroblastic NHL were cytologically classified

Table 6. Differences between first and second cytological screening (Cyt I and Cyt II) and final diagnosis, both for FNA and imprints



as immunoblastic lymphomas. Some plasmacytomas (see Fig. 8) were cytologically hardly distinguishable from immunocytomas. Within the follicle centre cell lymphomas only minor discrepancies existed. In one case of benign reactive hyperplasia Cyt I was suspicious of centroblastic/centrocytic NHL; in Cyt II, however, a correct diagnosis was given. In 2 cases of benign reactive hyperplasia Cyt I and Cyt II were both dubious. False-positive cytological diagnoses did not occur. False-negative cytology, including cytologically suspect cases, occurred in 3 FNA and 5 imprints, almost all within the centroblastic/centrocytic NHL. If cytologically suspicious cases were not included, only 2 false-negative cases were present.

All (43) LH were cytologically correctly diagnosed as non-neoplastic. So, the specificity (Poe 1980) of the cytodiagnostic method, defined as

$$\frac{\text{true-negative}}{\text{true-negative} + \text{false-positive}},$$

was 100%.

The sensitivity (Poe 1980), defined as

$$\frac{\text{true-positive}}{\text{true-positive} + \text{false-negative}},$$

for FNA was 97.7% when suspicious cases were included, and 99.2% when suspicious cases were excluded.

In 8 cases with uncertain histology because of mechanically damaged tissue, bad fixation, small specimen etc., the cell type could only be determined cytologically.

In most instances the T- or B-cell nature of the NHL could be indicated cytologically with the routine Giemsa stain. From 177 obvious B-cell NHL only in two cases (one B-immunoblastic NHL and one B-lymphoblastic NHL) a T-cell NHL was suspected in Cyt I; in Cyt II all B-cell NHL were correctly diagnosed as such. From 20 cases of T-cell NHL in 4 cases a T-cell origin was not obvious cytologically: 3 T-lymphoblastic NHL and one high grade malignant polymorphous T-cell NHL.

Interobserver reproducibility

To examine the inter-observer reproducibility 50 unselected cases were screened cytologically by all 4 authors by the methods described above. Consensus was defined when at least 3 authors gave the same conclusion as the definitive histological diagnosis.

Consensus about malignancy existed in 88% of cases. Consensus about the exact NHL cell type was reached in 70% of cases; in 4% (2 cases) there was no consensus. Within 8 LH the consensus was 100%.

The intra-observer reproducibility (Cyt I/Cyt II) of each author ranged from 96% (2 authors) to 100% (2 authors) for the categories malignant and reactive lesions together. Differences in cell types between Cyt I and Cyt II occurred in 2 to 16% (mean 8.5%).

False-positive cytological diagnosis by one author only occurred once (Cyt II); this was a case of infectious mononucleosis (see Fig. 14), histologically very much resembling an immunoblastic NHL.

False-negative diagnosis occurred in 0–4% (mean 2%).

Discussion

Aspiration cytology by means of a 23 gauge thin needle is a rapid, technically simple method. Although the growth pattern of a lymphoma cannot be determined cytologically, and therefore in many instances a histological diagnosis is required, aspiration cytology is very useful in the following circumstances: selection of a lymph node for surgical biopsy or as a method to follow the effect of therapy, detection of recurrences, and diagnosis of NHL in inoperable patients. Aspiration cytology may be used in a staging procedure (Lopes Cardozo 1975; Orell 1982) even in sites which are difficult to reach surgically, such as para-aortic nodes. Cellular details are often more obvious in smears than in histological sections (Feinberg 1980). Imprints may reduce artifactual distortions in biopsies which are difficult to interpret (Feinberg 1980). In addition, all the techniques applied to tissue sections such as immunology (Marsh 1983), electron microscopy and enzyme investigation are applicable to aspirated cells. In 8 cases of troublesome

histological diagnosis due to mechanical damage, small tissue specimen or bad fixation, cytodiagnosis was more sensitive than tissue diagnosis. According to York (1982) more than 10% of NHL are unclassifiable in spite of the use of immunological and other markers, a further reason for preparing good cytological slides for additional cytological information. We agree with Orell (1982) that technically perfect cytological slides and experience in the field of lymphomas are required for cytodiagnosis of lymphoid lesions; this is only possible in larger centres with a team of oncological specialists. With good cytological slides differentiation of NHL from undifferentiated carcinomas, malignant melanomas and round-cell sarcomas may be easier than with standard histology, as was shown in our series: 5 cases of undifferentiated carcinoma, previously diagnosed histologically as NHL or probably NHL were detected cytologically and established by further examination.

Routinely Papanicolaou or the Giemsa (Wright) stains are used. In some centres (Feinberg 1980; Spieler 1978) the Papanicolaou stain is preferred; nuclear convolutions and cerebriform nuclei (Rosen 1982) are more easily seen with this stain. With others (Lennert 1978; Lopes Cardozo 1975; Marsh 1983; Schechter 1979) we prefer the Giemsa stain since haematologically familiar features – both nuclear and cytoplasmic details – are more readily visualised using this stain. A large series of other stains is possible on the air dried smears, like DOPA, fat- and enzyme staining. In doubtful cases all available immunological methods can be applied to the aspirated cells. The Kiel classification (Lennert 1978) was used, as by others (Orell 1982; Spieler 1978; Spriggs 1981), because of its cytological base and our positive experience with this immunologically and clinically established classification. In most instances (mean 90%) cytology gave a conclusion identical with the histological diagnosis, especially within the group of the follicle centre cell lymphomas. Twenty five percents of the NHL exhibited a follicular growth pattern in histology. In the Kiel classification the growth pattern is not mentioned as a prognostically important sign. However, in other classification systems, including the recent Working Formulation (1982) the growth pattern (follicular vs diffuse) plays a very important role. So we feel that in all cases where FNA indicates a follicular centre cell lymphoma a tissue biopsy should be performed to determine the growth pattern. Thus the role of FNA is still important since it is a guide for further diagnostic action.

Underdiagnosis was seen in plasmacytomas and a portion of high grade NHL, especially the lymphoblastic subtypes. B-cell- and undefined lymphoblastic types were occasionally difficult to distinguish from centroblastic NHL (Lennert 1978; Spieler 1978; Spriggs 1981). All (12) cases of MHT and all (43) LH were diagnosed as such in both cytological screenings. The degree of dedifferentiation and number of mitotic figures correlated positively with the degree of malignancy; although we agree with Stacher (1980) that number, size and site of nucleoli are prognostically important, we have not investigated the nucleoli separately, since the cell type in the Kiel classification in itself implies a special pattern of nucleoli. The so-called

"lymphoglandular bodies" (Söderström 1966) were present in all cases of NHL, MHT and LH; vacuolated lymphoglandular bodies occurred especially in high grade malignant lymphomas and rarely in LH.

Fragility of cells, manifesting itself as lysis of individual cells, was a common feature in follicle centre cell lymphomas, notably in the large-cell subtypes and in the other high grade malignant lymphomas. It occurred seldom in MHT and LH. The mean nuclear size (see Table 4) which is important in the Lukes-Collins classification (Lukes 1975), correlated positively with the nomenclature in this classification. MHT invariably were characterized by large nuclei, whereas LH showed mainly small nuclei (small lymphocytes).

In spite of the characteristically monotonous cell picture in most NHL, a marked anisonucleosis was found in immunocytomas, in some follicle centre cell lymphomas and particularly in many T-cell NHL, including T-lymphoblastic lymphomas with convoluted nuclei. Lymphoblastic "small non-cleaved" NHL (Lukes 1975) were mostly medium sized.

T-cell NHL were cytologically characterized in most instances by a marked dedifferentiation, an obvious anisonucleosis (Schechter 1979) irregular to convoluted nuclei (Spriggs 1981), only few or absent vacuolated lymphoglandular bodies, mostly pale staining cytoplasm including large "clear cells" (T-immunoblasts) and often some eosinophils, plasma cells and epithelioid cells.

B-cell NHL were generally characterized cytologically by a monotonous cell picture without obvious anisonucleosis, with round or cleaved nuclei (depending on the cell type, see Fig. 1), pale or basophilic cytoplasm (again depending on the cell type), often lysis of cells, generally many l.g.b. (regularly vacuolated) and only a few if any non-lymphoid cells. LH could be distinguished from NHL by the presence of many small lymphocytes with only slight lysis, hardly any dedifferentiation, starry sky macrophages in combination with centroblasts and centrocytes, and a mixture of other lymphoid and non-lymphoid cells. Extreme paracortical hyperplasia, as in viral lymphadenitis, may cause tremendous diagnostic problems, since it may mimic a malignant lymphoma. Cytologically important criteria for benign behaviour include the presence of plasma cells in different degrees of maturation and immunoblastic cells together with other lymphoid cells, and histiocytes without obvious signs of dedifferentiation. Several cases of paracortical hyperplasia, including 2 cases of infectious mononucleosis, were present in the series. In one case an immunoblastic lymphoma was histologically diagnosed in first instance. Thus cytology may be of use in distinguishing pseudolymphoma from malignant lymphoma.

The proportion of hyperplasias (43) and malignancies (215) certainly does not represent the general patient population. This ratio, however, is frequently seen in specialized cancer centres. Therefore the sensitivity is slanted more towards the selection of benign cases in a tumour population than malignant cases in a general population. Apart from this the main purpose of our study was to check the value of objective criteria for typing lymphomas. Therefore in our opinion a reasonable statement can be made

about the sensitivity of the cytodiagnostic method. The cytodiagnosis of malignant lymphomas has been viewed differently, ranging from scepticism (Christopherson 1983; Zajicek 1979) to a high level of confidence in its reliability (Feinberg 1980; Sotto 1982; Spieler 1978; Spriggs 1981). Our cytomorphological analysis of 258 cases has demonstrated a high reliability and reproducibility.

Cytologically false-positive conclusions did not occur; specificity and sensitivity of the cytodiagnostic method were 100% and 98% respectively. The intra-observer reproducibility for the NHL cell type was 96%. The inter-observer reproducibility between the 4 authors for the diagnosis malignant versus benign was 88%, whereas consensus about the exact cell type was reached in 70% of cases; consensus within the benign lesions was 100%; these figures correspond with those of Orell (1982) who mentioned an overall consensus of 83%.

A very high predictability of immunological phenotype of NHL using conventional morphology is claimed by Lukes (1978). In other reports (Jaffe 1982) lower figures are given: 61% for high grade malignant lymphomas and only 50% for T-cell lymphomas. With Giemsa stained smears the immunologic phenotype of NHL could be indicated in 99% of B-cell NHL and in 70% of T-cell NHL. With cytochemistry and/or immunological typing of aspirated cells these errors can be reduced still further.

In *conclusion* cytodiagnosis of NHL is a reliable method which may aid and indeed supply the tissue diagnosis, when performed in oncological centres with sufficient experience. Technically adequate material is obviously of major importance.

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Note from the Managing Editor

An editorial will follow giving comment on the fundamental question of the value of aspiration cytology in lymph node pathology.