

# Classification of routinely processed anaplastic large cell tumours with a small panel of antibodies. An immunohistochemical study with clinical follow-up

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**Summary.** A proportion of anaplastic large cell tumours is difficult to classify on sections of routinely processed, paraffin-embedded tissue. Differentiation into large cell lymphoma, carcinoma, melanoma or sarcoma is important in order to assess prognosis and proper treatment. Although the use of immunohistochemistry has been reported in the differentiation between some of these types of neoplasms, no antibody panel, which can directly differentiate all of them, has been described. In the present study we evaluated the value of a panel of 5 antibodies for the classification of 29 anaplastic large cell tumours, which could not be classified by experienced pathologists using conventional histological and histochemical techniques. The panel, which can be used on routinely fixed paraffin-embedded tissue, consisted of 5 different antibodies directed against keratin, vimentin, the human milk-fat globule membrane antigen MAM-6, a melanoma associated antigen and common leucocyte antigen.

The use of this panel directly resulted in a definite diagnosis in 95% of the cases and provided valuable information for the diagnosis in the remaining cases. The diagnosis was confirmed by additional marker studies and electron microscopy. Moreover, clinical follow-up, including treatment data, was in accordance with the diagnosis based on the panel.

**Key words:** Immunohistochemistry - Anaplastic tumours - Large cell tumours - Differential diagnosis

## Introduction

A number of publications have appeared about the diagnostic value of immunohistochemistry with monoclonal antibodies in the classification of anaplastic large cell tumours (Warnke et al., 1983; Battifora et al., 1984; Baumal et al., 1984; Borowitz et al., 1984; Gatter et al., 1984, 1985; Lauder et al., 1984; Bosq et al., 1985). In this way e.g. lymphoma could be distinguished from carcinoma or sarcoma (Borowitz et al., 1984), carcinoma from lymphoma (Gatter et al., 1984; Lauder et al., 1984; Bosq et al., 1985) and melanoma from carcinoma or lymphoma (Gatter et al., 1985). All these studies have in common that they were retrospective, did not cover the whole range of possibilities for these tumours (carcinoma, sarcoma, melanoma or lymphoma), and that the results were only rarely evaluated by clinical follow-up. Moreover, in most cases the antibodies used had to be applied on frozen sections.

In the last years we have tested a large number of (predominantly monoclonal) antibodies for their relevance in tumour diagnosis. This has resulted in the composition of a panel of 5 antibodies, of which 3 are monoclonal, for the classification of anaplastic large cell tumours and applicable on routinely fixed and processed tissue. The panel is composed in such a way that the obtained positive and negative reaction patterns reinforce each other to achieve a definite diagnosis. It consists of antibodies against keratin (Nagle et al., 1982; Ramaekers et al., 1983; van Muyen et al., 1984), vimentin (Denk et al., 1983; Gabbiani et al., 1983; Osborn et al., 1983; Runger-Brändle et al., 1983), common-leucocyte antigen (CLA) (Warnke et al., 1983; Lauder et al., 1984; Meijer et al., 1985), a melanoma associated antigen (Duinen et al., 1984; Mackie et al., 1984; Vennegoor et al., 1985), and the human milk-fat globule membrane antigen (HMFGM) MAM-6 (Hilkens et al., 1984).

In this paper we describe, in a prospective study,

our results with this panel of antibodies on 29 anaplastic large cell tumours, which could not be classified by experienced surgical pathologists with conventional histology. After establishing the (initial) diagnosis by means of the pane, additional immunohistochemical marker studies and electron microscopy were performed to verify it. Moreover, the diagnostic value was evaluated by means of clinical follow-up including treatment results. It will be shown that the use of our antibody panel resulted in a definite diagnosis in the vast majority of the tumours while in only one case additional marker studies were needed.

## Materials and methods

### *Patients*

During the period of 1 April 1983 to 30 November 1985 experienced surgical pathologists were unable to classify 29 anaplastic large cell tumours, obtained from 15 females and 14 males, between 47 and 82 years of age. The tissue specimens were either derived from patients of our hospital, or were sent to our laboratory as a diagnostic problem. Pleiomorphic cells were found to be the main cell type in 21 tumours, round cells in 3 tumours, and spindle cells in 5 tumours. The differential diagnoses, based on conventional histology, comprised 21 cases of carcinoma or large cell lymphoma, 2 cases of carcinoma or sarcoma, and 6 cases of carcinoma, sarcoma or melanoma (Table 1).

### *Tissue processing*

If fresh material was obtained, representative tissue samples were (a) snap frozen in liquid nitrogen (b) fixed in 2% phosphate buffered glutaraldehyde (pH 7.4) for EM, (c) fixed in Burckhardt's fixative and embedded in methacrylate (Te Velde et al., 1977) for plastic sections and (d) fixed in 4% neutral buffered formalin or a formol sublimate mixture (Bosman et al., 1977) and subsequently embedded in paraffin wax (mpt 52-54°C) for conventional histology. In 4 cases only formalin-fixed and paraffin-embedded material was available.

For conventional histology sections were stained with haematoxylin-eosin, Giemsa and PAS with and without diastase digestion. In addition a silver impregnation stain and an elastica stain according to van Gieson were made.

### *Immunohistochemistry*

4- $\mu$ m section of the paraffin-embedded tissue were mounted on poly-L-lysine coated glass slides (Huang et al., 1983), and stained by the indirect immunoperoxidase technique. Endogenous peroxidase activity was blocked with 0.5% H<sub>2</sub>O<sub>2</sub> in methanol. For the detection of cytokeratins and vimentin in formalin-fixed, paraffin-embedded tissue, tissue sections were pretreated with 0.1% pronase for 15 min at 37°C (Merkel et al., 1981). Cryostat sections were also stained by the indirect immunoperoxidase technique as previously described (van der Valk et al., 1983). In each staining series a known positive control

section was included. Phosphate buffered saline (PBS), non-immune ascites fluid or normal rabbit immunoglobulin were used as negative controls.

### *Antibodies*

The antibodies used in the panel and their specificities are described in Table 2.

The antibodies that were subsequently used to verify the diagnosis are listed in Table 3.

Goat anti-mouse Ig-horseradish peroxidase (HRP) (TAGO) or rabbit anti-mouse Ig-HRP (DAKO) were used in the first step. Swine anti-rabbit Ig-HRP (DAKO) was used, when polyclonal rabbit antibodies were used in the first step.

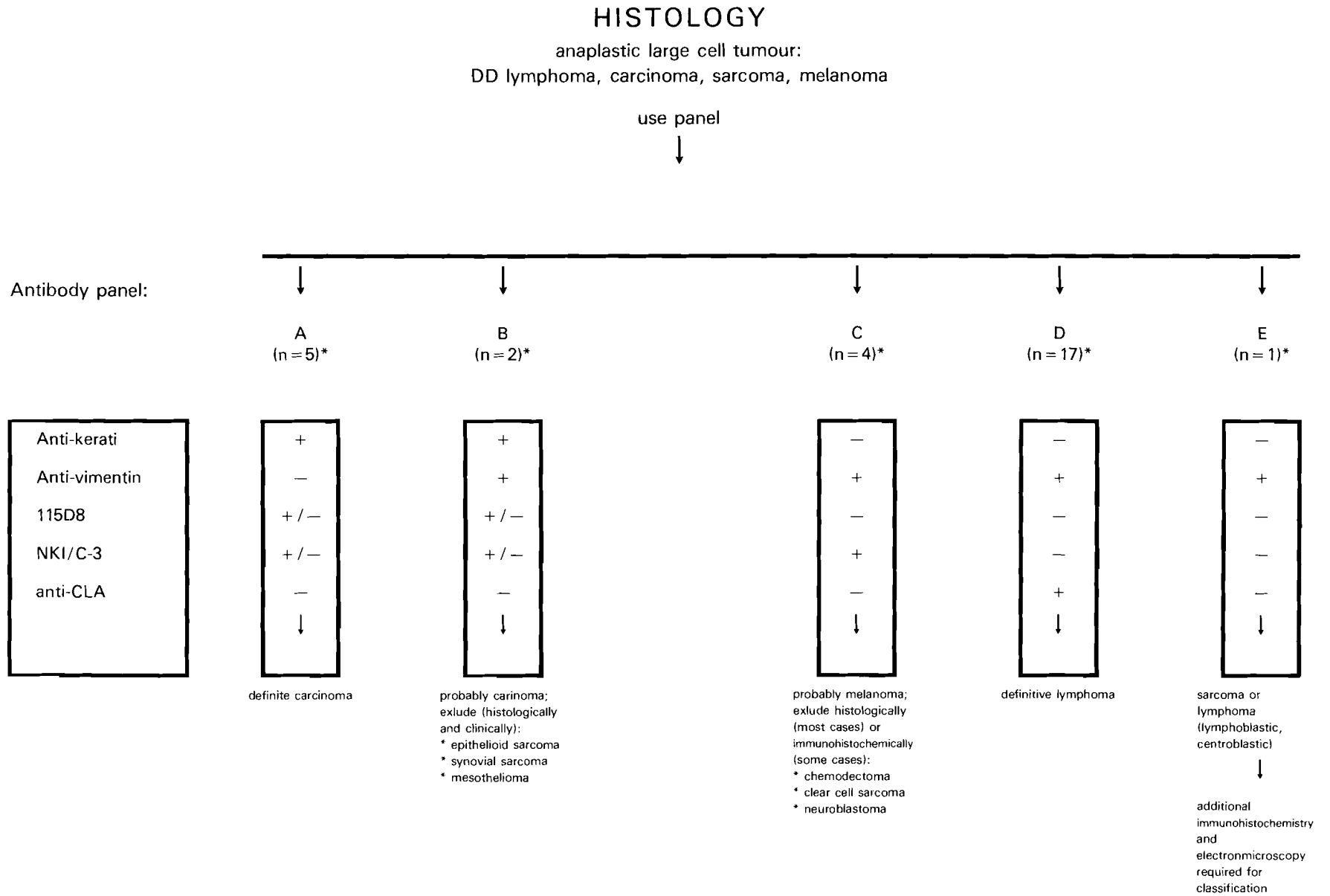
## Results

The staining combinations which can be met in practice with our antibody panel are given in Fig. 1 and indicated A, B, C, D and E. Their relevance to reach a definite diagnosis is also given.

In table 2, the biopsy location, the differential diagnosis based on conventional histology, the immunohistochemical reaction patterns obtained with the panel, the diagnosis based on it, the verification by additional data as well as clinical follow-up are shown.

In 21 cases the differential diagnosis made on routinely processed paraffin sections concerned carcinoma versus lymphoma (no. 1-21 of Table 1). In 17 out of these 21 cases combination D was found (anti-keratin-; anti-vimentin +; 115D8 -; NKI/C-3 -; anti-CLA +), indicating that the tumours was lymphomatous by nature (see also Fig. 2). Subsequently, the diagnosis was confirmed by the presence of markers for B cell lineage (14 cases) or monocytic lineage (3 cases). EM was compatible in all cases. Clinical follow-up showed partial or complete tumour regression under poly-chemotherapy (CT) sometimes in combination with local radiation therapy (RT) or RT alone. Five of these patients died within 3-10 months with systemic involvement. In one of these cases (no. 18) combination E was found (only anti-vimentin +). With this combination a large cell lymphoma or sarcoma is possible. Since on histology sarcoma was not considered, a large cell lymphoma was highly probable. As the tumour cells in addition reacted positive with the markers for histiocytic/monocytic lineage (FK24, MY7, MO2, and lysozyme, AAT and ACT), and markers for B cell (leu 12) and T cell (leu 4, TA1) lineage were negative, a true histiocytic lymphoma was indicated. EM showed cells without desmosomes, abundant cytoplasm with varying numbers of lysosomes and phagosomes, and irregular nuclei. Clinical follow-up showed poor tumour regression under CT and local RT. The patient died after 2 months. In 3 other of these 21 cases (no. 19, 20, 21) combination A was found (only anti-keratin and 115D8 positive) indicating that the tumour was carcinomatous by nature (see also Fig. 3). EM and clinical follow-up confirmed this. One of these patients died after 17 months (no. 19) with lymph node and cerebral metastases.

Fig. 1. Classification of anaplastic large cell tumours by a panel of antibodies



\* indicates the number of cases present in this study with this combination

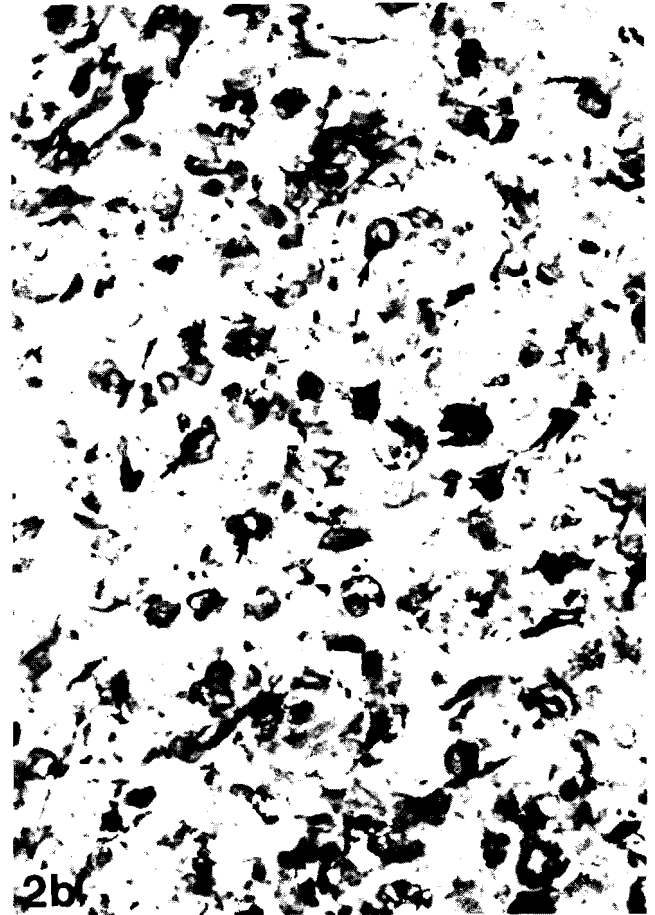
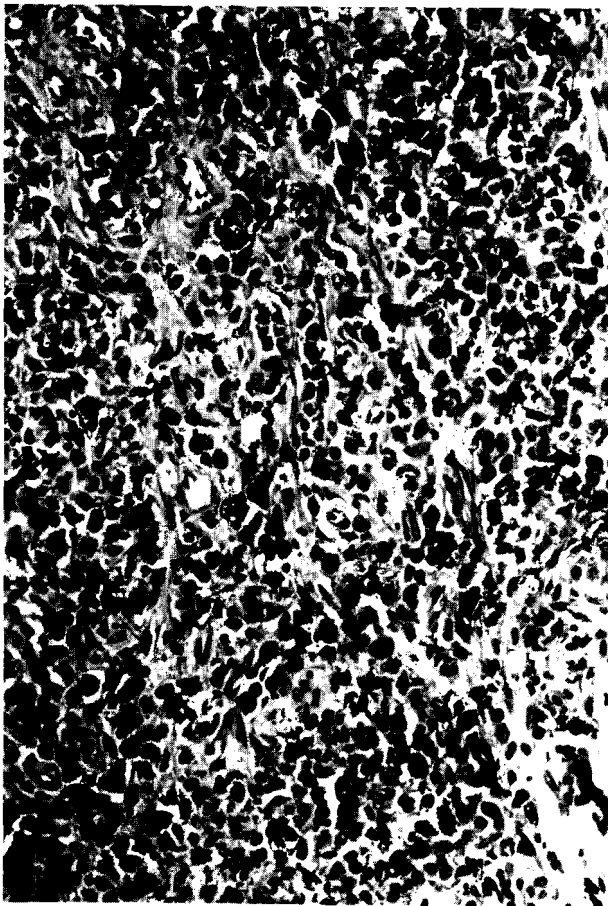
*Immunohistochemistry of anaplastic large cell tumours*

In 2 cases (no. 22 and 23) on conventional histology the differential diagnosis of carcinoma versus sarcoma was made. In both cases combination B was found (anti-keratin and anti-vimentin +, 115D8 + 0R-), indicating that the tumour might be a carcinoma. Since mesothelioma, epithelioid sarcoma and synovial sarcoma, which could also be possible with this combination, could be excluded on conventional histology and clinical data, the diagnosis carcinoma was made. In both cases this diagnosis was confirmed in the laryngectomy specimens by conventional histology, and (in no. 23) by EM of the original biopsy.

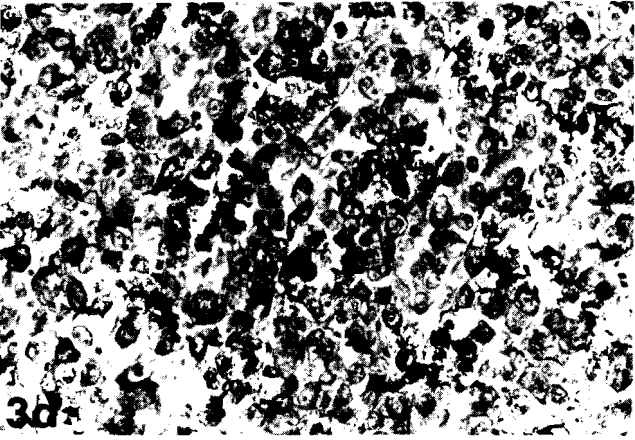
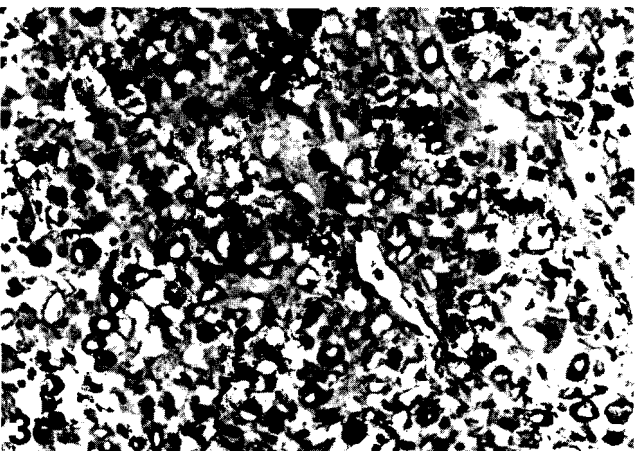
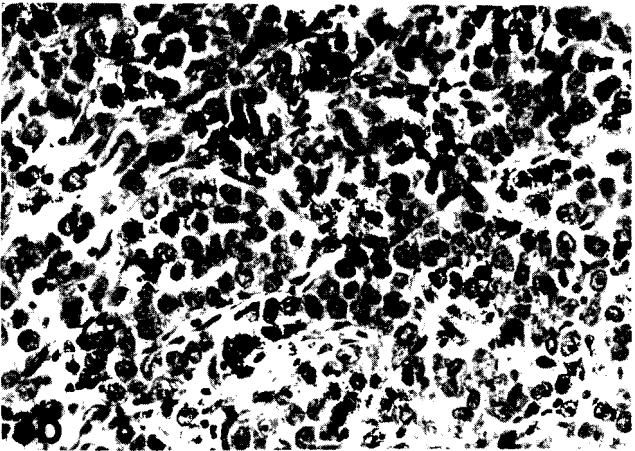
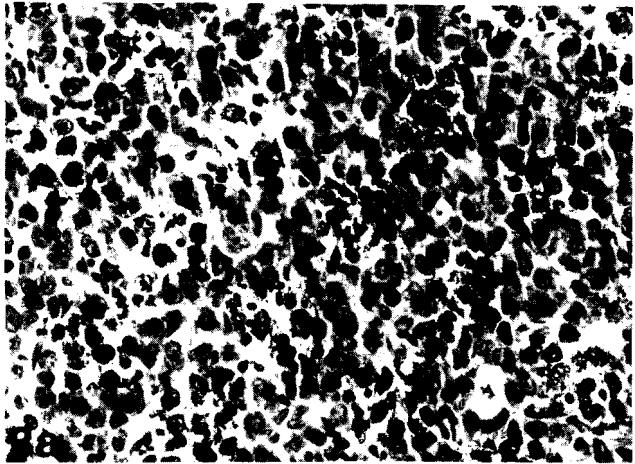
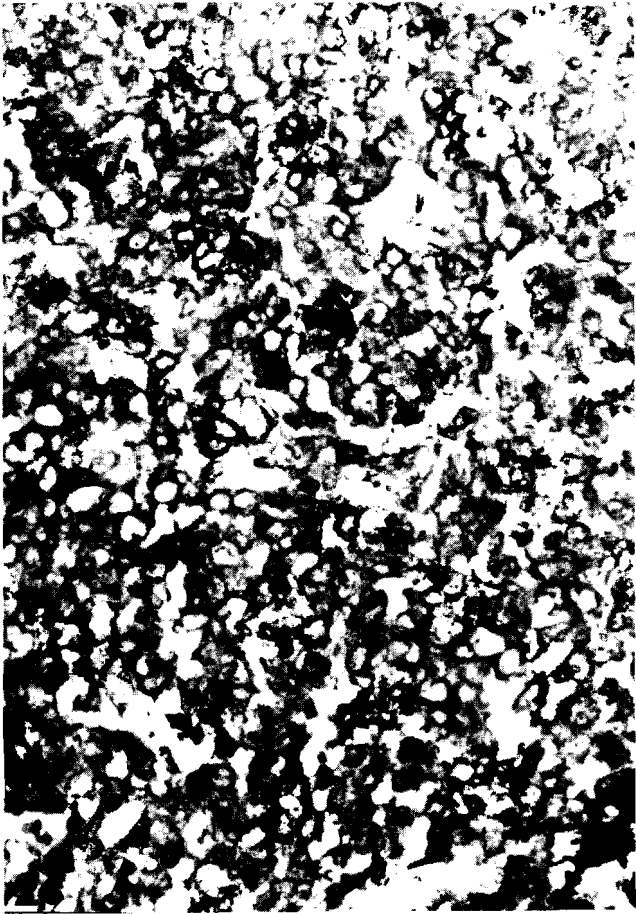
In 6 cases the differential diagnosis of carcinoma, sarcoma or melanoma was made (no. 24-29). In 2 out of

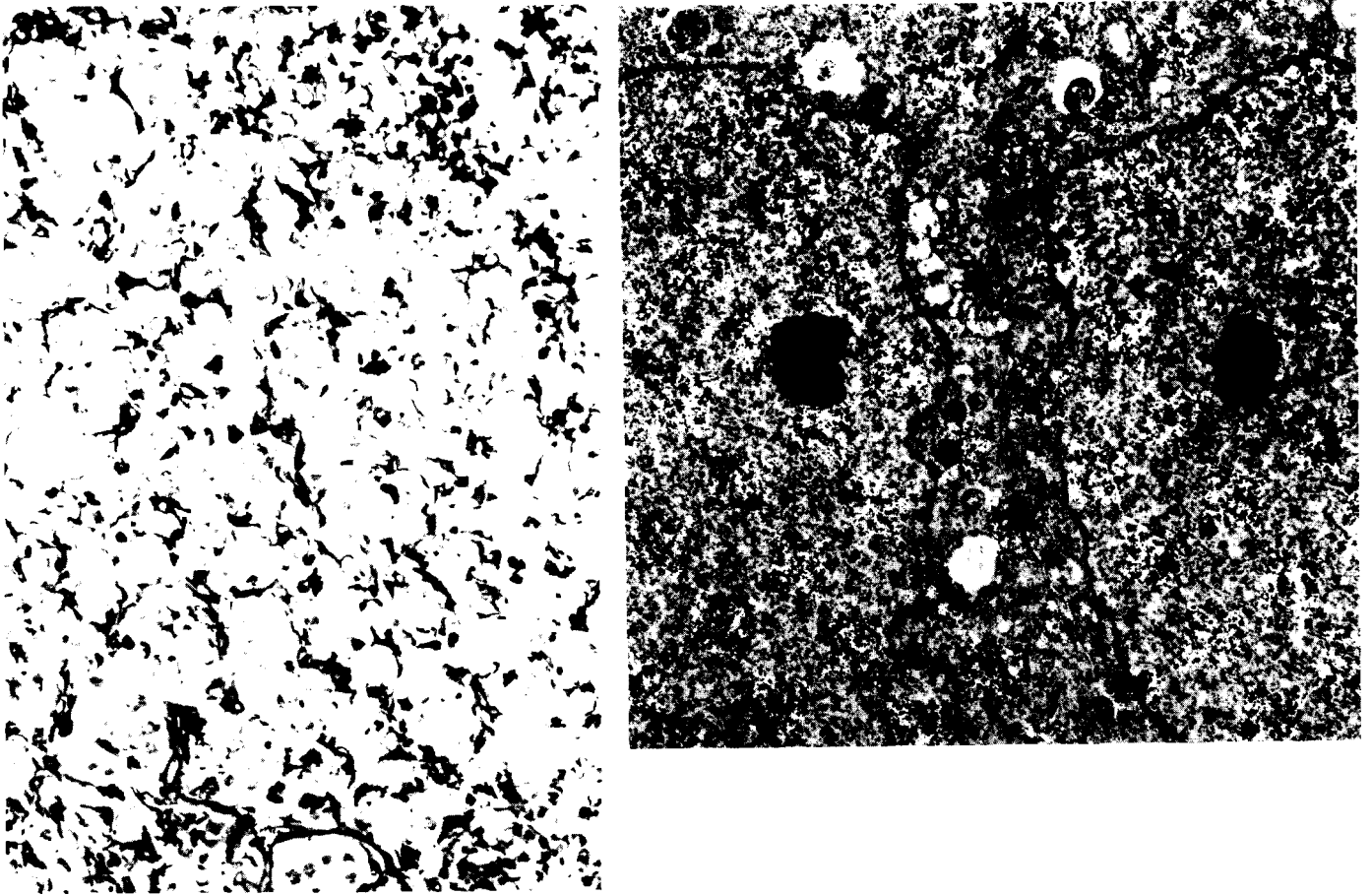
these 6 cases (no. 24,25) combination A was found (anti-keratin and 115DI +; NKI/ C-3 + or-), indicating a carcinoma. This was confirmed by EM in both cases.

In the other 4 cases combination C was found (anti-vimentin and NKI/C-3 +), indicating a melanoma. Other histogenetically related tumours such as chemodectomas, neuroblastomas and carcinoids could be excluded with conventional histology. Additional immunohistochemistry showed a positive reaction for S-100 protein in the tumour cells. EM was confirmative in all 4 cases. Follow-up tissue specimens showed focally melanin in tumour cells in the Schmorl staining.



**Fig. 2.** Undifferentiated large cell tumour finally classified as centroblastic lymphoma (case no. 8). **a.** A high power view of the tumour shows its anaplastic nature and focal tendency to grow in sheets. H.E. x 330. **b.** Vimentin staining on paraffin section shows a positive reaction in part of the tumour cells (see arrowheads). x 528. **c.** Immunostaining with anti-CLA on a cryostat section shows a positive reaction in the tumour cells. x 528





**Fig. 3.** Undifferentiated large cell tumour finally classified as poorly differentiated squamous cell carcinoma (case no. 20). (a,b.) A high power view of the tumour shows its anaplastic nature, and tendency to grow in sheets. H.E. x 264. (c,d.) Immunostaining with anti-keratin (C) shows a positive reaction in the tumour cells on a cryostat section and with 115D8 (D)

on a paraffin section. x 264. (e) Immunostaining with anti-vimentin on a cryostat section shows a positive reaction in fibroblasts and lymphocytes. The tumour cells do not react. x 208. (f.) Electron microscopy shows a detail of two cells with several desmosomes. No other distinguishing characteristics. x 7,500

**Table 1.** Clinical and diagnostical data of the patients

NO	AGE (YEARS)	SEX <sup>1</sup>	BIOPSY LOCATION	ANTI- <sup>2</sup> KERATIN	ANTI- <sup>2</sup> VIMENTIN	ANTI- <sup>2</sup> CLA	11508 <sup>2</sup>	NKI/C-3 <sup>2</sup>	DIAGNOSIS MADE AFTER PANEL	VERIFICATION BY: <sup>3</sup> ADDITIONAL TECHNIQUES	CLINICAL FOLLOW-UP	DEFINITE DIAGNOSIS
DD CARCINOMA/LYMPHOMA <sup>4</sup>												
1	54	M	breast (right)	—	3+	3+	—	—	lymphoma	EM: comp lymph, no ev for ca IH: leu 12, IgM, $\lambda$ , HLA-DR	Skin: multiple lesions. CT-I → CR. Free of disease. + 4 mo.	Centroblastic lymphoma
2	79	F	tonsil (left)	—	3+	3+	—	—	lymphoma	EM: comp lymph, no ev for ca IH: leu12, IgM, $\lambda$ , HLA-DR	Localizations in LN, pleura, spleen. CT-I → + (3 mo).	centroblastic lymphoma
3	78	F	tonsil (left)	—	3+	3+	—	—	lymphoma	EM: comp lymph, no ev for ca IH: IgM, $\lambda$	Staging procedure: negative. RT → CR. Free of disease. + 6 mo.	centroblastic lymphoma
4	70	M	kidney (left)	—	3+	3+	—	—	lymphoma	EM: comp lymph, no ev for ca IH: IgM, $\lambda$	Referred patient, diagnosis elsewhere carcinoma. CT-II → PR. Revision diagnosis in lymphoma on original biopsy. Localization in retroperitoneal LN 3 mo later. CT-I/RT → CR. Free of disease. + 22 mo.	centroblastic lymphoma
5	75	M	nasopharynx	—	3+	3+	—	—	lymphoma	EM: comp lymph, no ev for ca IH: leu12, IgM, $\lambda$ , HLA-DR	Staging procedure: localization in cervical LN. CT-I/RT → CR. Free of disease. + 4 mo.	centroblastic lymphoma
6	70	M	tongue	—	3+	3+	—	—	lymphoma	EM: comp lymph, no ev for ca IH: leu 12, IgG, $\lambda$ , HLA-DR	Staging procedure: negative. RT → CR. Free of disease. + 5 mo.	centroblastic lymphoma
7	74	F	pubic os	—	3+	3+	—	—	lymphoma	EM: comp lymph, no ev for ca IH: leu 12, IgM, $\lambda$ , HLA-DR	Several pelvic localizations. CT-I/RT → CR. Free of disease. + 16 mo.	centroblastic lymphoma
8	82	F	nasal cavity (right) <sup>5</sup>	—	3+	3+	—	—	lymphoma	EM: comp lymph, no ev for ca IH: leu12, IgM, $\lambda$ , HLA-DR	Staging procedure: negative. RT → CR. After 16 mo localization in retroperitoneal, inguinal LN. CT-I → + (3 mo.)	centroblastic lymphoma
9	70	M	nasopharynx	—	1+	2+	—	—	lymphoma	EM: comp lymph, no ev for ca IH: leu12, IgM, $\lambda$ , HLA-DR	Staging procedure: negative. CT-I/RT → CR. Free of disease. + 6 mo.	centroblastic lymphoma
10	74	F	maxilla (right)	—	3+	3+	—	—	lymphoma	EM: comp lymph, no ev for ca IH: leu12, IgG, $\lambda$ , HLA-DR	Staging procedure: localization in cervical LN. CT-I/RT → CR. Free of disease. + 14 mo.	centroblastic lymphoma
11	74	F	nasopharynx	—	2+	2+	—	—	lymphoma	EM: comp lymph, no ev for ca IH: leu12, IgG, $\lambda$ , HLA-DR	Staging procedure: localizations in cervical, inguinal LN. CT-I/RT → CR. Free of disease. + 14 mo.	centroblastic lymphoma
12	82	F	buttock (right)	—	3+	3+	—	—	lymphoma	EM: comp lymph, no ev for ca IH: leu12, IgM, $\lambda$ , HLA-DR	Other localization in pleural cavity. CT-I → PR → cerebral metastasis → + (10 mo).	centroblastic lymphoma
13	64	M	nasopharynx	—	3+	3+	—	—	lymphoma	EM: comp lymph, no ev for ca IH: leu12, IgM, $\lambda$ , HLA-DR	Other localization in cervical LN. CT-I → CR. Free of disease. + 8 mo.	centroblastic lymphoma
14	68	F	cervical lymph node	—	2+	2+	—	—	lymphoma	EM: comp lymph, no ev for ca IH: leu12, IgM, $\lambda$ , HLA-DR	Undifferentiated tumour of the tongue 3 years before. Treated elsewhere as carcinoma by resection. Revision diagnosis in lymphoma on original specimen. Other localization in axillar LN. CT-I → + (5 mo).	centroblastic lymphoma
15	68	F	cervical lymph node	—	3+	3+	—	—	lymphoma	EM: comp THL, no ev for ca IH: FK 24, my7, MO2, Lys, AAT, ACT, HLA-DR	Other localization in mediastinal LN. CT-I/RT → + (5 mo).	true histiocytic lymphoma
16	78	F	nasal cavity (right)	—	3+	3+	—	—	lymphoma	EM: comp THL, no ev for ca IH: FK 24, my7, MO2, AAT, ACT, HLA-DR	Other localizations in cervical LN and EM. CT-I → CR. Free of disease. + 20 mo.	true histiocytic lymphoma
17	75	AM	tonsil (left)	—	3+	3+	—	—	lymphoma	EM: comp THL, no ev for ca IH: FK 24, hy7, MO2, Lys, AAT, ACT, HLA-DR	Other localization in cervical LN. CT-I/RT → CR. Free of disease. + 15 mo.	true histiocytic lymphoma
18	58	M	maxilla (right)	—	3+	—	—	—	lymphoma/sarcoma	EM: comp THL, no ev for ca IH: FK 24, my7, MO2, lys, AAT, ACT, HLA-DR	staging procedure: Negative. CT-I/RT → + (12 mo).	true histiocytic lymphoma
19	66	M	axillar lymph node	3+	—	—	1+	—	carcinoma	EM: adeno differentiation	Adeno carcinoma of the stomach 3 years before. Localization in mediastinal, retroperitoneal LN. RT → progressive → cerebral metastasis → + (17 mo).	poorly differentiated adeno carcinoma

20	57	F	cheek mucosa <sup>6</sup>	3+	-	-	3+	-	carcinoma	EM: comp with carcinoma	Resection specimen: poorly differentiated squamous cell carcinoma.	poorly differentiated squamous cell carcinoma
21	66	F	liver (metastasis)	2+	-	-	1+	-	carcinoma	EM: adeno differentiation	Resection of undifferentiated rectal carcinoma 3 years before. Recurrent liver metastasis after 8 months. CT-III - PR. + 11 mo.	poorly differentiated adeno carcinoma
DD CARCINOMA/SARCOMA <sup>4</sup>												
22	72	M	larynx	2+	3+	-	-	-	carcinoma?	EM: ND	Resection specimen: poorly differentiated squamous cell carcinoma.	poorly differentiated squamous cell carcinoma
23	47	M	larynx	1+	2+	-	3+	-	carcinoma?	EM: comp with carcinoma	Resection specimen: poorly differentiated squamous cell carcinoma. positive cervical LN. RT - CR. Free of disease. + 16 mo.	poorly differentiated squamous cell carcinoma
DD CARCINOMA/SARCOMA/MELANOMA <sup>4</sup>												
24	53	M	axillar lymph node	3+	-	-	1+	-	carcinoma	EM: adeno differentiation	Localizations in axillar, cervical, retroperitoneal LN; thoraxwall, pleural effusion. Clinical a primary breast carcinoma is suspected. CT-IV - PR. + 16 mo.	poorly differentiated adeno carcinoma
25	57	M	nasal cavity (left)	1+	-	-	1+	1+	carcinoma	EM: adeno differentiation	Resection specimen: poorly differentiated adeno carcinoma. After 2 years local recurrence + localization in cervical LN. CT-V - CR. Free of disease. + 26 mo.	poorly differentiated adeno carcinoma
26	82	F	oesophagus	-	3+	-	-	1+	melanoma?	EM: few premelanosomes IH; S 100 protein	Local recurrence: focally pigmented melanoma. resection. + 26 mo.	melanoma
27	67	M	nasal cavity (right)	-	3+	-	-	1+	melanoma?	EM: few premelanosomes IH; S 100 protein	Local recurrence: focally pigmented melanoma. After 2 mo positive cervical LN - resection. + 18 mo.	melanoma
28	68	F	nasal cavity (left)	-	3+	-	-	3+	melanoma?	EM: few premelanosomes IH; S 100 protein	Undifferentiated to our of the nasal cavity. Treated elsewhere melanoma as carcinoma by resection. Revision diagnosis in probably melanoma on original specimen. Local recurrence: focally pigmented melanoma. Localization in cervical LN. RT. Free of disease. + 30 mo.	melanoma
29	54	F	ethmoid	-	3+	-	-	2+	melanoma?	EM: few premelanosomes IH; S 100 protein	Undifferentiated tumour of the ethmoid. Treated as carcinoma by resection. After 2 year local recurrence. Revision of original specimen in probably melanoma. Local recurrence: focally pigmented melanoma. After 3 mo. metastasis in liver, mesenterial LN. + 4 mo.	melanoma

1) M = male, F = female  
2) Staining results are expressed as: -, no positive tumour cells; 1+ < 25% 2+, 25-50%; 3+ > 75% of the tumour cells stained  
3) EM: electron microscopy

IH: immunohistochemistry; only positive results are given.  
Comp lymph: no ev for ca; compatible with lymphoma, no evidence for carcinoma.  
Comp THL: no ev for ca; compatible with true histiocytic lymphoma, no evidence for carcinoma.  
Adeno differentiation: cells with desmosomes, intr- or intercellular lumens with microvilli, mucous vacuoles.  
Carcinoma: cells with many desmosomes; no further signs of differentiation.

Lys: lysozyme.  
AAT: alpha<sub>1</sub>-anti-trypsin.  
ACT: alpha<sub>2</sub>-antichymotrypsin.  
BM: bone marrow.  
ND: not done.  
RT: radiation therapy.  
CR: complete remission.  
PR: partial remission.  
LN: lymph node.  
CT-I: adriamycin, oncovin, endoxan, prednisolone.  
CT-II: endoxan, adriamycin.  
CT-III: 5-fluorouracil (5-FU).  
CT-IV: 5-fluorouracil (5-FU).  
CT-V: 5-AZA-2-deoxycytidine.  
+ (...mo): survival in months after diagnosis.  
+ (...mo): died ... months after diagnosis.

4) Original differential diagnosis made on conventional histology  
5) see also Fig. 2  
6) see also Fig. 3



*Immunohistochemistry of anaplastic large cell tumours***Table 2.** Panel of antibodies used for the differentiation of anaplastic large cell tumours

ANTIBODY	SPECIFICITY	SOURCE REFERENCE
Anti-keratin (P)	cytokeratins, broad spectred.	(a)
Anti-vimentin (P)	vimentin, mol. wt 57 KD. present on mesenchymal cells and sarcoma.	(b)
115D8 (M)	MAM-6: human milk-fat globule membrane antigen, mol. wt 400 KD. Present on nearly all carcinomas	Hilkens et al., 1984
NKI/C-3 (M)	a melanoma-associated antigen. Recognizes a hterogenous glycoprotein, mol. wt 25-110 KD. Also present in cells of neuroectodermal origin and mesotheliomas.	(c) Vennegoor et al, 1985
Anti-CLA (M)	clones PD 7/26, 2BII, reecognizes a determinant with a mol. wt of 200 KD. Present on nearly all lymphoid tumours (not all lymphoblastic tumours rect).	(a)

P: polyclonal; M: monoclonal; CLA, common leucocyte antigen

(a) purchased from DAKOPATTS, Copenhagen, Denmark

(b) purchased from Eurodiagnostics, Apeldoorn, The Netherlands

(c) obtained from the Netherlands Cancer Institute, Amsterdam, The Netherlands

**Table 3.** Antibodies used to verify the final diagnosis

ANTIBODY	SPECIFICITY	SOURCE
leu4 (M)	human T lymphocyte antigen	a
TA1 (M)	human T lymphocyte antigen	b
leu12 (M)	human B lymphocyte antigen	a
anti-ig, $\kappa$ , $\lambda$ (P)	human immunoglobulins, K and $\lambda$ light chains of human immunoglobulins present on human B lymphocytes	c
HLA-DR (M)	(OKIa) B lymphocytes, activated T lymphocytes, monocytes, histiocytes, Langerhans cells, veiled cells, interdigitating cells.	d
Fk 24 (M)	(OKM1) monocytes, histiocytes, granulocytes, subpopulation of T cells and most null lymphocytes	e
My 7 (M)	normal and leukemic human myeloid cells. The antigen is expressed on monocytes, histiocytes, granulocytes	f
MO 2 (M)	human myeloid antigen. Found on monocytes, histiocytes, blast cells of myelomonocytic leukemia	f
Anti-lysozyme (P)	histiocytes, myeloid cells, lacrimal and salivary glands	c
Anti-AAT (P)	histiocytes, yolk sac tumours, foetal liver, hepato cellular carcinoma	c
Anti-ACT (P)	histiocytes	c
Anti-desmin (P)	muscle cells: smooth, skeletal, cardiaque	g
Anti-myoglobin (P)	muscle cells: skeletal, cardique	h
Anti-neurofilament (P)	mwt 70 KD, neurons	k
Anti-F VIII Rag (P)	endothelial cells, megakaryocytes	c
Anti-S 100 protein (P)	supportive cells of nervous tissue, melanomas, Langerhans cells, veiled cells, interdigitating cells	c

(M): Monoclonal antibody; (P): Polyclonal antibody, mwt: molecular weight

a. purchased from Beacton and Dickinson, Monoclonal Centre, U.K.

b. obtained from Drs. J.H. Kersey and T.W. Lebien, University of Minesota, U.S.A.

c. purchased from Dakopatts, Copenhagen, Denmark

d. purchased from Ortho Pharmaceutical Corp. Immunobiology div., U.S.A.

e. obtained from dr F. Koning, University Hospital Leiden, the Netherlands

f. purchased from Coulter Clone, U.K.

g. purchased from Eurodiagnostics B.V., the Netherlands

h. purchased from Cappel Lab., U.S.A.

k. purchased from Monosan, SANBIO b.V., the Netherlands.

**Discussion**

In this study we have presented the results obtained with a panel of 5 antibodies applied on 29 initially unclassifiable anaplastic large cell tumours. Only based on this panel we were able to achieve a definite diagnosis in 22 cases (76%). When the clinical data and histology were included in the assessment, even 28 cases (95%) could be classified directly. In only one case additional techniques were needed.

Although reports have appeared dealing with the use of immunocytochemistry in the differential diagnosis of some types of these tumours, e.g. carcinoma or melanoma (Gatter et al., 1985), carcinoma or lymphoma (Gatter et al., 1984; Lauder et al., 1984; Bosq et al., 1985), our antibody panel has the advantage that the whole range of anaplastic large cell tumours is covered. This is important because sometimes more than two possibilities have to be considered in the differential diagnosis of these tumours. Another feature of our study, compared with other reports, is that it is a prospective one and was evaluated by clinical follow-up.

The panel is composed in such a way that (1) the obtained positive and negative reaction patterns reinforce each other, and (2) its antibodies all have a specificity for certain tumour types (see Table 2) although none of them is decisive on its own. The least specific antibody in this panel is NKI/C-3 since it is not only present in melanoma cells, but also in histogenetically related cells such as chemodectomas, neuroblastomas, carcinoids and mesotheliomas (Duinen et al., 1984; Mackie et al., 1984; Vennegoor et al., 1985). However, at the moment no melanoma-associated antibodies with higher specificity, which can be used on paraffin sections, are available. S-100 protein, another antigen known to be present in melanoma cells, and also preserved in paraffin sections is even less specific (Kahn et al., 1983). Other more specific melanoma-associated monoclonal antibodies like HMW 653.40S (Natali, 1981) can only be used on frozen section.

The various immunohistochemical reaction patterns, which can be met in practice, are shown in Figure 1, and will be discussed now.

Reaction pattern A (keratin +; vimentin—; 115D8 + or—; NKI/C-3 + or—; CLA—), leads to a definite of keratin, and the absence of CLA and vimentin in the tumour cells. The presence of the HMFGM antigen MAM-6, as detected by 115D8, gives additional support to the epithelial nature of the cells. 115D8 is included in the panel as it can be used on routinely processed material, without significant loss of antigenicity (Hilkens et al., 1984). Especially if the tissue is not properly fixed, the demonstration of this antigen is often decisive in the final diagnosis.

Reaction pattern B (keratin +; vimentin +; 115D8 + or—; NKI/C-3 + or—; CLA—) makes a carcinoma probable since keratin is not present in most sarcomas. However, as this marker pattern can also be found in mesotheliomas, synovial sarcomas, and epithelioid

sarcomas, these tumours have to be excluded first (Chase et al., 1984; Corson et al., 1984). In the large majority of the cases this can be done on conventional histology and clinical details. This reaction pattern, in which markers characteristic for mesenchym (vimentin) and epithelium (keratin) are both present, is rather particular, and is considered to be rare in carcinomas. (Ramaekers et al., 1983; Rungger-Brändle et al., 1983). However, coexpression of vimentin and keratin is described in thyroid carcinomas (Miettinen et al., 1984; Henzen-Logmans et al., 1987) and pulmonary carcinomas (Jasani et al., 1985; Mullink et al., 1986). If 115D8 reacts positively it further strengthens the epithelial nature of the tumour cells (Hilkens et al., 1984).

In reaction pattern C (keratin—; vimentin +; 115D8 —; NKI/C-3 +; CLA—) a positive reaction for NKI/C-3 makes the diagnosis of melanoma especially likely (Duinen et al., 1984; Mackie et al., 1984; Vennegoor et al., 1985). But this reaction pattern can also be found in histogenetically related tumours such as carcinoids, chemodectomas, clear cells sarcomas and neuroblastomas (Vennegoor et al., 1985). They are often easily excluded by means of conventional histology alone. If necessary the presence of neurofilament can be helpful to exclude neuroblastoma (Carlei et al., 1984). Clear cell sarcomas of the soft tissues have a similar reaction pattern to melanomas, which is in accordance with their kinship. (Chung et al., 1983). Anti S-100 protein, and other melanoma-associated antibodies such as HMW 653.40S can be used for additional support of this diagnosis (Natali et al., 1981; Wen et al., 1983; Ruiter et al., 1986).

Reaction pattern D (Keratin —; vimentin + 115D8 —; NKI/C-3 — CLA +) leads to a definite diagnosis of lymphoma. Although CLA is present on leucocytes, in practice the presence of this antigen in anaplastic large cells tumours supports the diagnosis lymphoma. Additional markers are necessary to characterize the lymphoid tumours into a B, T or histiocytic cell type (Stein et al., 1984).

Reaction pattern E (keratin —; vimentin +; 115D8 —; NKI/C-3 — CLA —) Makes a sarcoma probable. Additional markers are needed for subtyping (see Roholl et al., 1985). Since, in our experience, some lymphoblastic and centroblastic lymphomas do not stain for CLA with the monoclonal antibody we used (DAKO-LC) (Meijer et al., 1985) these tumours have to be excluded. They can easily be excluded histologically by their monotonic cell pattern (most cases) or by the use of additional lymphoma markers.

In summary, in a prospective study with clinical follow-up, we could extend previous accounts of the use of immunohistochemistry for the classification of anaplastic large cell tumours (Borowitz et al., 1984; Gatter et al., 1984; Lauder et al., 1984). Moreover, when used as a small selected panel of antibodies, the whole range of these tumours can be covered and will directly result in a definite diagnosis of the vast majority of cases. In only a minority or for subtyping (lymphomas!), additional markers have to be used.

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