Lymphoid proliferations in the orbit: malignant or benign?

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SUMMARY Clinical, pathological, and immunological analysis of 20 patients with ocular adnexal lymphoid disease has demonstrated several parameters which are useful for distinguishing malignant from benign lesions. Patients in the fourth or fifth decade of life presenting with an acute history of pain, oedema, epiphora, double vision, and ptosis, with a mass localised in the lacrimal gland area, are more likely to have a pseudolymphoma or a chronic inflammatory lesion than a true non-Hodgkin lymphoma (NHL). It is not possible to obtain a definite diagnosis without surgical intervention, because only three out of nine patients with orbital NHL had evidence of a monoclonal B cell population in peripheral blood on admission to the Orbital Centre. Furthermore it was confirmed that the identification of the various orbital lymphoid infiltrates becomes more distinct when immunological techniques are added to the clinical and histopathological methods of investigation. Multidisciplinary cooperation leads to further improvement of diagnosis and treatment of ocular adnexal lymphoproliferative disease.

Lymphoid proliferations of the ocular adnexa present a challenging problem for both the ophthalmologist and the pathologist. The lymphoid proliferations are reported to comprise 10 to 15% of all space filling lesions of the orbit.¹⁻³ Histologically these extranodal lymphoid proliferations can be classified into malignant non-Hodgkin lymphomas (NHL), benign pseudolymphomas, and chronic inflammatory lesions.^{4,5} Lesions which occupy either end of the histopathological spectrum, that is, either an obvious benign chronic inflammatory process or a malignant NHL, present few problems for the pathologist. However, not infrequently lymphoid proliferations lie between these two extremes morphologically, and the diagnosis is often a source of error.⁶⁻⁹ Not only is the histopathological diagnosis of such a lesion difficult, but the pathogenesis, the natural history, and the relationships with systemic lymphoprolifera-

tive diseases are poorly understood. Furthermore if classification of the tumour were possible without the use of surgical intervention to obtain biopsy tissue the patients with a pseudolymphoma or a chronic inflammatory lesion, amenable to drug treatment alone, would greatly benefit from prompt instigation of treatment. The wish to find a non-invasive method for the diagnosis of orbital space filling lesions prompted us to set up this multidisciplinary investigation of patients presenting at the Orbital Clinic over a period of three years. Cytological, histological, immunohistological, and electronmicroscopical studies of biopsy material were performed in conjunction with ophthalmological examination and immunological and haematological examination of blood and bone marrow.

Patients and methods

Twenty consecutive patients with an orbital tumour of lymphoid origin, referred to the Orbital Clinic

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over the last three years, were incorporated in this study. At presentation the age, sex, the affected eye, initial symptoms, visual acuity, motility, Hertel values, and the referral diagnosis, together with the general medical history were filed. CT scans were made on all patients in axial and/or coronal planes. In all patients an open biopsy was performed. In 18 patients with deep orbital lesions an orbitotomy was done. The lesions of 10 of these patients were approached via an anterior route. In the others a lateral orbitotomy was performed. The freshly obtained biopsy specimens were subdivided¹⁰ for routine histology, cytology, electron microscopy, immunohistochemistry, and immunological investigation of cells in suspension. Before surgery and treatment a heparinised venous blood sample was taken and a bone marrow aspirate was obtained.

HISTOLOGY AND CYTOLOGY

Histological diagnoses were made on tissue sections supplemented with imprint smears. Tissue specimens were fixed in buffered formalin and embedded in Paraplast and/or plastic when indicated. Routinely, haematoxylin and eosin, periodic acid-Schiff (PAS), methyl-green-pyronin, Giemsa, and reticulin stains were made. Imprints were stained with May-Grünwald-Giemsa. Imprints were of great use in determining the cell type of NHL especially when the biopsies were small and/or mechanically damaged.¹¹

ELECTRON MICROSCOPY

Small pieces of biopsy specimens were fixed in a solution of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, postfixed in 1% OsO₄ in the same buffer, stained en bloc with 1% aqueous uranyl acetate, dehydrated in ethanol, and embedded in a mixture of Araldite and Epon 812. Thin sections, stained with lead hydroxide, were examined with a Philips EM 301 electron microscope.

DETERMINATION OF CELL SURFACE AND CYTOPLASMIC MARKERS IN CRYOSTAT SECTIONS BY MEANS OF IMMUNOFLUORESCENCE

The tissue specimens were snap frozen and stored in liquid nitrogen. 4 µm Cryostat sections were air dried and fixed in acetone for 10 min at 20° C. The sections were incubated with labelled antisera directed against human lymphocytes,¹² against T lymphocytes,¹¹ against α γ δ ϵ μ heavy chains, and κ and λ light chains. Double staining was applied for μ and δ , and κ and λ , one antiserum being labelled with fluorescein isothiocyanate (FITC), the other with tetramethylrhodamine isothiocyanate (TRITC). Indirect immunofluorescence was done on the sections with monoclonal antibodies directed against HLA-DR (supernatant 0101-2007, Cappel, USA), transferrin

receptor (kindly donated by van Rijn *et al.*¹³ and against monocytes (MaHuman monocyte 1 33020 lot 30224, BRL, USA).

PREPARATION OF CELL SUSPENSIONS AND IMMUNOFLUORESCENCE TECHNIQUE

When enough biopsy material was available cells were teased from a portion. Dead cells, granulocytes, and erythrocytes were separated from the lymphoid cells by means of density gradient centrifugation on Ficoll-Paque (Pharmacia). Mononuclear cells from blood were isolated by means of density gradient centrifugation. All the cells for the immunofluorescence test were fixed with paraformaldehyde, to prevent aspecific binding of immunoglobulins via the Fc receptor,¹⁴ and washed twice prior to use. B cells were determined by a direct immunofluorescence test with FITC labelled antisera against human immunoglobulins and α , γ , δ , μ , heavy chains and κ and λ light chains.¹⁵ T cells were determined by either the E rosette test¹⁶ or OKT monoclonal antibodies.¹⁷

Statistical analysis was performed by Student's *t* test and the χ^2 test.

Case report

Patient No 4. This patient was chosen as a case history to illustrate how difficult and puzzling the diagnosis and management of orbital lymphoproliferative lesions can be. The patient first presented in 1975, aged 66, with a palpable mass in the right upper eyelid, a proptosis of 4 mm, and ptosis. Visual acuity, visual fields, skull x-rays, and blood tests were

Fig. 1 CT scan appearance of patient No 4 showing a mass in the superior eyelid, lacrimal gland, and extending posteriorly.

Fig. 2 CT scan image showing the mass on the orbital floor extending into the orbital apex.

normal. The CT scan (taken on 3 September 1975) (Fig. 1) showed a pathological structure in the superior eyelid extending into the right orbital apex. An anterior orbitotomy was performed, and the histopathological diagnosis was lymphocytic pseudotumour. No immunohistochemistry was done at that time. The patient was treated with a course of prednisolone and responded well. A year later the CT scan was normal. In 1978 the patient returned with a 3 mm proptosis in the left eye. The CT scan (Fig. 2) showed a pathological structure on the orbital floor, left, extending into the apex. After various conservative treatments without result the patient was put into this study. A biopsy was taken in 1980 and histopathology showed a diffuse centrocytic non-Hodgkin lymphoma.

Immunopathological examination of the biopsy

presented a diffuse pattern of rather small tumour cells with a ring type fluorescence, positive for μ , κ , and HLA-DR: monoclonal B cells.

The subpopulation distribution of cells obtained from the same biopsy, studied in suspension, was as follows: 15% T cells and 37% B cells, 26% bearing κ , 9% λ light chains, and 21% μ heavy chains.

General physical examination showed no evidence for systemic disease. No enlargement of the spleen or liver was found. Serum electrophoresis revealed no abnormalities. Both cytological and immunological examination of bone marrow cells were normal.

Left orbital irradiation (4000 rads) was performed with a good result, leaving the patient in a stable condition with a follow-up of two years (Hertel 19–17 mm).

The distribution of subpopulations of lymphoid cells in peripheral blood was determined at different points in time. The patient had a marked reduction of T cells except in July 1981 (Table 1). The percentage of B cells was increased at every determination, but the ratio of κ bearing to λ bearing cells remained within normal limits (smaller than 4 to 1) until September 1982, when an abnormal B cell population, bearing μ , δ , κ , was found.

Results

Twenty patients were included in this study. Table 2 summarises the clinical characteristics and the biopsy results. Eleven of these patients were female and nine male. The orbital lesions of the 20 patients were divided into three groups based on light microscopical examination of tissue sections and imprint smears. The space occupying lesion was an NHL in nine patients, characterised morphologically by a more or less monotonous cell proliferation of small and/or large lymphoid cells without normal follicular structures and with infiltrating capacity. The subdivision of the NHLs was based on the Kiel classifica-

Table 1 Lymphocyte subpopulations in peripheral blood of patient No 4

Month	Year	% Positive cells								
		Erfe*	OKT3	B cell	κ	λ	IgG	IgM	IgA	IgD
April	1980	35	ND	66	45	15	3	51	0	43
May	1981	40	ND	27	16	9	1	25	1	25
July	1981	55	ND	20	19	8	0	25	0	20
November	1981	35	ND	40	27	7	ND	ND	ND	ND
September	1982	ND	41	33	27	4	1	19	0	11
Normal values†										
	Mean	73.0	60.0	7.5	5.0	3.0	0.5	5.5	0.5	5.5
	SD	9.5	5.0	2.5	2.5	1.5	0.7	2.2	0.7	2.6

*Sheep erythrocyte rosette forming cell.

†Mean of 13 healthy blood donors.

ND = not done.

tion.¹⁸ Seven patients were found to have a pseudolymphoma and four a chronic inflammatory lesion. Morphologically these two groups are characterised respectively, by a comparatively monomorphous, hypercellular, reactive lymphoid hyperplasia, or by a heterogenous cell population with small and some large lymphocytes, plasma cells, eosinophils and histiocytes, by lymphfollicles with germinal centres, and by fibrosis.

The mean age of the patients in the different groups was: 66 years for patients with NHL (range 51–76), 62 years for patients with pseudolymphoma (range 51–73), and 40 years for patients with a chronic inflammatory process. The age difference between patients with NHL and with pseudolymphoma was not statistically significant ($p>0.2$), but the patients with a chronic inflammatory process were significantly younger than either the NHL group ($p<0.005$) or the pseudolymphoma group ($p<0.02$).

At presentation 16 patients had a unilateral and two a bilateral orbital process. Two had a bilateral conjunctival involvement. There was a marked preponderance for the right orbit in the patients with unilateral complaints: 14 right versus 2 left sided. The symptoms at presentation were as follows: proptosis (13 patients), pain (12 patients), diplopia (11 patients), chemosis (5 patients), decrease of visual acuity (4 patients), epiphora (4 patients), ptosis (4 patients), and lid oedema (2 patients). Several patients had more than one initial complaint.

In the patients with NHL the initial complaints such as pain, proptosis, and chemosis were less acute than in the other two groups, and the duration of the complaints was very much longer (months for NHL versus weeks for pseudolymphoma and chronic inflammation). The degree of proptosis was also less marked in the patients with NHL (2.4 mm) than in those with either pseudolymphoma or chronic inflammation (mean 4.6 mm). The CT scan showed that an NHL could be located anywhere within the orbit, whereas pseudolymphoma and chronic inflammation had a predilection for the lacrimal gland area.

All but one patient, who refused treatment, of the NHL group were treated with irradiation (4000 rads). Six patients with a pseudolymphoma were treated with a course of corticosteroids (60 mg/day for 14 days and then tapered off). The criteria used to define clinical improvement were established as follows: reduction of proptosis (mean improvement NHL 2 mm, the pseudolymphoma and chronic inflammation patients, 4 mm); improved ocular motility and increased visual acuity (unchanged or improved in all patients). The results of treatment were satisfactory for all the patients (Table 2).

Immunohistochemical analysis was performed on 19 out of 20 biopsies (Table 3). Six of the patients studied showed a definite monoclonal B cell population all characterised by IgM and HLA-DR. Three bore κ light chains, three others λ chains, and one lymphoma was also IgD positive. In two patients a discrepancy between the results of the histological and the immunohistochemical examination was present. Patient No 8 was diagnosed as having a diffuse centrocytic lymphoma, but no monoclonality of B cells was found. The biopsy taken from patient No 1 was negative for immunoglobulin chains but definitively positive for HLA-DR, and the monocyte antibody, and was therefore diagnosed immunohistochemically as a true histiocytic lymphoma but histologically as a diffuse centroblastic NHL. In these two cases the sections were reassessed, but both the light microscopical and immunohistochemical diagnosis remained unchanged, and consequently patient No 8 was treated for NHL. Patient No 1 refused treatment.

The density of the transferrin receptor on the cell membrane is a marker for proliferative activity of lymphoid cells.¹⁹ It has also been shown that there is a correlation between the density distribution of transferrin receptors on tumour cells and the degree of malignancy.²⁰ We looked for the presence or absence of the transferrin receptor on biopsy material from patients 1 to 7 using a monoclonal mouse antitransferrin receptor antibody (66 1 G10). The results show that out of the seven tumours investigated only two (Nos 1 and 2) had a moderately dense distribution of transferrin receptors. In the case of patient No 1 this might not have a predictive value, as monocytes always have transferrin receptors. But, on the other hand, if this patient had a diffuse centroblastic NHL as diagnosed morphologically, this tumour would be of high grade malignancy. Patient follow-up will have to show whether these findings in this patient and the other six patients have any significance regarding the prognostic value. Immunohistochemistry of the 11 other biopsies showed a T cell and polyclonal B cell infiltrate with a predominance of T cells over the B cells. In one of the pseudolymphoma patients a slight predominance of IgM/ κ plasma cells was observed. Follow-up of this patient is necessary in order to evaluate whether this pseudolymphoma will evolve into a monoclonal B cell lymphoma.

Cell suspensions teased from six biopsies were investigated for lymphocyte subpopulations (Table 3). In three biopsies, classified morphologically as NHL, the κ bearing cells outnumbered the λ bearing cells, but the ratio never exceeded 3.5 to 1. In patient No 8, described morphologically as having a diffuse centrocytic NHL but immunohistochemically as polyclonal, the number of T cells was greater than in the other

Table 2 Clinical, radiological, and histopathological findings. Treatment and course of disease

Case	Age/sex/date	Eye(s)	Initial complaints	Medical history	Location on CT-scan	Surgical approach
1	64/F 22 Dec 1981	OD	Pain, epiphora, double vision	None	Inferior/medial	Anterior orbitotomy
2	71/M 22 Dec 1981	OD	Double vision, pain, proptosis, chemosis	None	Retrobulbar	Lateral orbitotomy
3	51/M 13 Jan 1981	OD/OS	Ptosis, vision ↓	'79 Brill Symmers disease	Superior eyelid, first OD, later OS	Anterior orbitotomy
4	66/M 3 July 1980	OD/OS	Pain, proptosis, lid oedema, chemosis	Thrombosis leg, maturity onset diabetes	Superior eyelid, first OD, later OS and apex	Anterior orbitotomy
5	58/F 17 Sept 1981	OD	Proptosis, pain ptosis	None	Temporal	Lateral orbitotomy
6	76/F 27 Oct 1982	OD	Proptosis, double vision	None	Retrobulbar superior/medial	Anterior orbitotomy
7	72/M 19 May 1982	OD	Proptosis, double vision, epiphora	Stomach carcinoma 1980	Retrobulbar	Anterior orbitotomy
8	62/M 23 Oct 1980	OD	Proptosis, double vision	1975 kidney stone operation 1977 cholecystectomy	Lateral + retrobulbar	Anterior orbitotomy
9	71/F 12 Oct 1981	OD/OS	Chemosis	None	Conjunctival	Open biopsy anterior
10	57/F 26 Nov 1982	OD/OS	Pain + chemosis	None	Conjunctival	Open biopsy anterior
11	57/M 1 Oct 1981	OD	Proptosis, pain	Cardial disease	Retrobulbar	Lateral orbitotomy
12	67/F 13 April 1982	OD	Diplopia, pain, proptosis, ptosis	Hypertension	Retrobulbar medially	Transconjunctival orbitotomy Lateral orbitotomy
13	66/M 20 May 1980	OD	Diplopia, proptosis	None	Lateral/superior	Anterior orbitotomy
14	51/F 4 Nov 1980	OD	Pain	None	Lateral/superior	Lateral orbitotomy
15	60/F 19 August 1980	OD	Proptosis, diplopia epiphora	Diabetes	Lateral/superior	Anterior orbitotomy
16	73/M 25 March 1980	OS	Proptosis, pain, diplopia	Hyperthyroidism	Superior retrobulbar	Anterior orbitotomy
17	61/F 13 May 1981	OS	Pain, lid oedema diplopia	None	Lateral/superior	Anterior orbitotomy
18	45/F 1 June 1981	OD	Proptosis	None	Retrobulbar (enlarged muscle)	Open biopsy anterior
19	27/F 7 Sept 1981	OD	Ptosis, chemosis, double vision, pain	None	Lateral retrobulbar	Lateral orbitotomy
20	26/M 22 July 1980	OD	Pain, epiphora, proptosis	None	Lateral/superior	Lateral orbitotomy

two cases. In the three other biopsies, classified morphologically as pseudolymphoma and by immunohistochemistry as polyclonal, the number of T cells was greater or equal to 23%, and the B cells were polyclonal.

Three of the NHL and two of the pseudolymphoma biopsies were screened electron microscopically (Table 3). Of special interest is the result in patient No 1, in whom a discrepancy existed between the morphological and the immunohistochemical diag-

nosis. The tissue appeared very heterogeneous. In between a mixture of fibroblasts and small lymphocytes small groups of large, pale staining cells were observed. Ultrastructurally the cells had very few organelles and a regular or lobulated nucleus that frequently showed nuclear pockets. No cell contacts were observed between these cells. Based on their ultrastructure a distinction between a lymphocytic (most likely centroblastic) or a histiocytic character could not be made. The morphology of the two other

Biopsy results	VA		Motility		Exophthalmometer		Follow up (months)	Treatment
	Initial	Final	Initial	Final	Initial	Final		
Diffuse centroblastic NHL	20/20	20/20	5% Depression	5% Depression 20% Adduction 15% Elevation	13-11	14-13	26	Patient refused treatment
Diffuse lymphoplasmacytoid NHL	20/20	20/20	Strab conv + total limit of abduction	15% Abduction	18-17	14-15	8	4000 Rads
Follicular centroblastic/centrocytic NHL	20/40	20/20	90% depression + elevation	Full	18-18	15-17	24	4000 Rads later total body irradiation
1. Pseudotumour 2. Diffuse centrocytic NHL	20/20	20/20	Full	Full	21-17	19-17	90	1. Prednisolone 2. Endoxan 3. Immuran + pred 4. 4000 Rads
Diffuse centrocytic NHL	20/20	20/20	Full	Full	19-14	18-15	15	Prednisolone no effect, radiotherapy 4000 Rads
Diffuse centrocytic NHL	20/20	20/60	80% Elevation 85% Adduction 90% Depression	—	22-18	21-18	3	4000 Rads
Diffuse centrocytic NHL	20/20	20/30	20% Elevation 90% Abduction 80% Adduction	Ptosis	22-17	19-20	6	4000 Rads
Diffuse centrocytic NHL	20/20	20/20	90% Elevation 90% Depression	Full ptosis	22-18	12-13	28	4120 Rads
Diffuse centrocytic NHL	20/30	20/30	Full	Full	16-16	16-16	15	3171 Rads
Pseudolymphoma	20/20	20/20	Full	Full	16-16	16-16	4	Prednisone
Pseudolymphoma	20/20	20/20	Full	Full	28-24	20-20	9	Prednisone
Pseudolymphoma	20/30	20/30	Frozen globe	Frozen globe	17-12	12-14	8	Prednisone
Pseudolymphoma	20/30	20/30	5% Elevation 50% Abduction 80% Adduction	No follow up	23-18	18-18	21	Prednisone
Pseudolymphoma	20/20	20/20	Full	Full	22-17	15-17	28	None
Pseudolymphoma	20/40	Unknown	80% Elevation 80% Abduction	70% Elevation ptosis	18-12	No follow up	None	None
Pseudolymphoma	20/30	20/20	5% Elevation	Full	17-22	22-23	9	Prednisone
Chronic inflammation	20/20	20/20	90% Elevation	Full	21-21	20-20	18	None
Chronic inflammation	20/20	20/20	5% Elevation 20% abduction and adduction	No follow up	29-20	No follow up	None	None
Chronic inflammation	20/30	20/20	Full	Full	19-14	11-12	7	None (clamoxyl, vibramycine)
Chronic inflammation	20/30	20/20	Full	Full	19-17	20-19	30	Prednisone

NHLs (Nos 3 and 4) was very similar. The tissues consisted of small and somewhat enlarged lymphoid cells with a regular or slightly indented nucleus with moderately condensed chromatin, a distinct Golgi apparatus, few endoplasmic reticulum profiles, and free ribosomes. Very few granules were observed. The ultrastructure resembled that of centrocytes.²¹ The two biopsies of the patients with a pseudolymphoma were very different in appearance. The tissue of case 13 was very heterogeneous, consisting

of small lymphocytes (possibly B cells), somewhat larger lymphocytes with an indented nucleus (possibly activated T cells), immature plasma cells, histiocytes, and fibroblasts. As a whole this resembled the morphology of T cell areas within lymph nodes. The tissue blocks of case 14 were all derived from a follicular area with centroblasts surrounded by a network of cellular protrusions.

In order to understand the relationship of the orbital process with systemic disease and the wish to

Table 3 Immunohistopathological, electron microscopical, and immunological results

Patient No.	Age/sex	Clonality of lymphoid cells in the biopsy		Electron microscopy of the biopsy	Lymphocyte subpopulations in peripheral blood			
		Immuno-histochemistry	Cell suspension		% T cells	% B cells	% κ	% λ
1	64 F	Ia ⁺ , few T and B cells	ND	Mixed population + activated T cells	38	3	2	2
2	71 M	$\mu\lambda$ Ia ⁺	ND	ND	57	3	3	0
3*	51 M	$\mu\kappa$ Ia ⁺	9%T, 14%B, 10% κ , 5% λ	Homogenous centrocytic population	52	27	14	3
4	66 M	$\mu\kappa$ Ia ⁺	12%T, 36%B, 26% κ , 9% λ	CLL type lymphoid cells	See Table 1			
5	58 F	$\mu\lambda$ Ia ⁺	ND	ND	59	10	5	1
6	76 F	$\mu\sigma\kappa$ Ia ⁺	ND	ND	46	ND	45	1
7	72 M	$\mu\lambda$ Ia ⁺	ND	ND	54	9	7	5
8	62 M	Polyclonal	49%T, 20%B, 21% κ , 6% λ	ND	64	18	13	7
9	71 F	ND	ND	ND	46	5	2	1
10	57 F	Polyclonal	ND	ND	ND	ND	ND	ND
11	57 M	Lymphocytes polyclonal plasma cells $\mu\kappa$	ND	ND	58	3	3	1
12	67 F	Polyclonal	ND	ND	70	9	5	4
13	66 M	Polyclonal	23%T, 11%B, 6% κ , 7% λ	Mixed population lymphoid cells	56	24	13	6
14	51 M	Polyclonal	37%T, 36%B, 28% κ , 12% λ	Medium sized centroblasts	58	6	2	3
15	60 F	Polyclonal	52%T, 35%B, 20% κ , 13% λ	•ND	60	41	22	21
16	73 M	Polyclonal	ND	ND	36	21	10	7
17	61 F	Polyclonal	ND	ND	78	21	14	10
18	45 F	Polyclonal	ND	ND	77	5	2	2
19	27 F	Polyclonal	ND	ND	68	19	9	5
20	26 M	Polyclonal	ND	ND	76	12	3	4
Controls†					6.0±5.0‡	7.5±2.5	5.0±2.5	3.0±1.7

*Treated with Leukeran.

†Thirteen healthy blood donors.

‡Mean ± SD.

ND = not done.

find an ophthalmologically non-invasive method for diagnosis immunological studies were performed on bone marrow cells and peripheral blood lymphocytes. Immunocytological investigations of bone marrow cells were carried out in 11 patients (Nos 2, 4, 6, 7, 9, 10, 11, 12, 13, 17, and 18). In only one patient (No 6) could a monoclonal B cell lymphocytic population be detected. The distribution of T and B cells in peripheral blood was determined on the day after admission to the Orbital Centre (Table 3). Patient No 4 is reported separately as a case report. Of the other eight patients with NHL three had an abnormal distribution of κ versus λ bearing B cells, one had a polyclonal increase of B cells, and three had low T cell levels. In the patients with pseudolymphoma or chronic inflammation no abnormalities were detected in the κ : λ ratios. A polyclonal increase of B cells was seen in five patients, one patient had low T cell levels, and three patients raised T cell levels.

Discussion

Our results show that there was a difference in clinical

presentation between patients with NHL and patients with a pseudolymphoma or a chronic inflammatory lesion. This finding contrasts with that of some other investigators.^{6,9} Patients with a recent and acute history of clinical signs like pain, oedema, epiphora, double vision, and proptosis, and of fairly young age, were more liable to have a pseudolymphoma or chronic inflammation than patients of more advanced age with a longer history of complaints. These results concur with those of Garner *et al.*,⁹ when their final diagnosis table is integrated in the table of clinical and radiological findings. The localisation of the tumour inside the orbit²² on the CT scan²³ also gave a fairly accurate diagnosis as to its type. A marked preference for the lacrimal gland area was seen in patients with either a pseudolymphoma or chronic inflammation. This observation was clearly illustrated by our case report. This patient first presented with a pseudolymphoma in the lacrimal gland area of the right orbit and later with a retrobulbar NHL of the left orbit. There appeared to be little tendency to dissemination in the NHL patients. In one patient generalised disease was manifest before the orbital

complaints. In one other patient monoclonal B cells were found in the bone marrow aspirate. The distribution of κ versus λ bearing B cells in peripheral blood was abnormal in three out of nine NHL patients at presentation and became abnormal during follow-up in one case. Abnormal distribution of B cells in the blood is therefore found in only 33% of the patients with an orbital NHL at admittance. In one of the patients with a pseudolymphoma or chronic inflammation in the orbit was such a monoclonal B cell population found in the blood. These results suggest that no definite diagnosis can be made without a surgical intervention. In contrast to the recommendations advocating fine needle aspiration to obtain a biopsy of the tumour²⁴ we found that open surgery was the safest procedure to avoid sampling errors. In addition lateral orbitotomy was preferable to anterior orbitotomy for deep orbital lesions in order to avoid damage to delicate orbital structures ending up with complications such as ptosis, diplopia, orbital haemorrhage, or blindness.

Part of this study was devoted to the use of immunohistochemistry in addition to light microscopical examination for a more refined diagnosis of lymphoid proliferations. Analysis of cell surface and cytoplasmic markers with the immunofluorescence technique on tissue obtained during surgery clearly benefited the routine histopathological diagnosis, because cells which were morphologically identical could be classified as of monoclonal or polyclonal origin. On two occasions the immunopathological diagnosis differed from the light microscopical observations. An explanation for such discrepancies may lie in the subdivision of biopsied tumour, in which case one diagnosis was made on tumour tissue, whereas the other diagnosis was made on tissue with inflammatory infiltrate often accompanying neoplasms. Only serial sectioning of the whole biopsy would allow a definite conclusion to be drawn. In these doubtful cases treatment was based on the diagnosis which classified the process as malignant.

Additional diagnostic examinations included lymphoid cell subpopulation determination on tumour cells teased from the biopsy specimen and electron microscopic investigation. Studying cells in suspension had the advantage that numerical counts of different lymphocyte subsets could be done. Knowles and Jacobiec^{25, 26} reported that the percentage of T cells in a tumour cell suspension was indicative of malignancy if less than 20% of the cells were T cells, whereas a benign proliferation contained over 40% T cells. In the six cases where enough viable cells were teased from the tumour to perform a similar study the general trend of their investigation could be confirmed. Nevertheless there is a definite advantage in studying tissue sections¹¹ over cell suspensions,

because the structure of the tissue is maintained. Many different areas of one tumour can be screened, and no contamination with peripheral blood lymphocytes occurs. Moreover sclerotic tissue from which it is difficult to tease cells can also be investigated immunologically.

Although our series is small (three NHLs and two pseudolymphomas) the electron microscopic investigation did not contribute greatly to the light microscopical and immunohistochemical diagnosis. Material fixed for electron microscopy should therefore be stored and only processed for investigation if a definite diagnosis cannot be reached by clinical, pathological, and immunological investigations alone.

Accurate and prompt diagnosis of the lymphoid proliferation in the orbit resulted in better adjusted treatment for each group of patients. So far the results of treatment (mean follow-up period 15 months, range three to 90) consisting of no treatment or postoperative prednisolone in the case of pseudolymphoma and local radiation therapy (4000 rads) in the case of NHL were favourable and resulted in the decrease of oedema, epiphora, proptosis, and improvement of motility. Careful follow-up of the pseudolymphoma patients and the patients with a chronic inflammatory process is mandatory in order to study whether a neoplastic disorder can arise locally from a reactive process. To date we have not observed such an involvement in any of our patients.

This study therefore shows that it was not possible to obtain an accurate diagnosis of the space occupying orbital process by non-invasive means alone. But immunological and cytological investigation of bone marrow and peripheral blood, as part of the staging procedure, remain necessary to exclude generalised disease in the NHL patients. From this study we concluded that detailed ophthalmological examination appears to give a fairly accurate impression of the possible type of process one may encounter. Light microscopical examination should be supplemented when necessary with immunological examination of frozen tissue or tumour cells in suspension in order to discriminate NHL from pseudolymphomas. Good data, thorough integration of clinical symptoms, and light microscopical and immunohistochemical investigation have led to a clear discrimination between the three different kinds of lymphoid proliferations encountered in the orbit.

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