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Ocular Adnexal Lymphomas: Five Case Presentations and a Review of the Literature

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Abstract. The ocular adnexal lymphomas represent the malignant end of the spectrum of lymphoproliferative lesions that occur in these locations. The Revised European and American Lymphoma (REAL) Classification and the new World Health Organization Classification of Tumors of Hemopoietic and Lymphoid Tissues are the most suitable for subdividing the ocular adnexal lymphomas, whereby the extranodal marginal zone B-cell lymphoma represents the most common lymphoma subtype. This review is based on five cases subtyped according to the above classifications—three "typical" lymphomas (an extranodal marginal zone B-cell lymphoma, a diffuse large cell B-cell lymphoma arising from an extranodal marginal zone B-cell lymphoma, and a follicular lymphoma) and two "atypical" lymphomas (a non-endemic Burkitt lymphoma in an immune competent elderly patient, and a primary Hodgkin lymphoma of the eyelid) of the ocular adnexa. Management of patients with ocular adnexal lymphomas includes a thorough systemic medical examination to establish the clinical stage of the disease. The majority of patients with ocular adnexal lymphoma have stage IE disease. Current recommended therapy in stage IE tumors is radiotherapy, while disseminated disease is treated with chemotherapy. Despite usually demonstrating an indolent course, extranodal marginal zone B-cell lymphomas are renowned for recurrence in extranodal sites, including other ocular adnexal sites. Long-term follow-up with 6-month examinations are therefore recommended. Major prognostic criteria for the ocular adnexal lymphomas include anatomic location of the tumor; stage of disease at first presentation; lymphoma subtype as determined using the REAL classification; immunohistochemical markers determining factors such as tumor growth rate; and the serum lactate dehydrogenase level. (**Surv Ophthalmol 47:**470–490, 2002.

Key words. Burkitt lymphoma conjunctiva • extranodal marginal zone B-cell lymphoma • lymphoproliferative lesion • Hodgkin's disease • lymphoma • "mucosa-associated lymphatic tissue" lymphoma • ocular adnexa • orbit • REAL classification • WHO lymphoma classification

Ocular adnexal lymphomas represent malignant lymphoid neoplasms which develop as primary or secondary tumor manifestations in the orbit, the conjunctiva, and eyelid. The majority of lymphomas occurring in the ocular adnexa are B-cell non-Hodgkin lymphomas.[3,12,19,35,44,51,55,61,82] These are predominantly extranodal marginal zone B-cell lymphomas,[3,12,35,51,61,82] according to the Revised European American Lymphoma (REAL) Classification,[22] but they can also include diffuse large cell B-cell lymphomas and follicular lymphomas. Less common B-cell lymphoma subtypes include lymphoplasmocytic lymphoma/immunocytoma, mantle-cell lymphoma, plasmocytoma, and immunoblastic lymphoma in de-

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creasing frequency.[3,12,35,43] Both the endemic and non-endemic forms of Burkitt's lymphoma rarely involve the ocular adnexa in children;[14,81] the non-endemic form can also occur in adults, usually in association with acquired immunodeficiency syndrome and other forms of immunosuppression (e.g., post-transplant).[43,70]

Adnexal lymphomas of non-B-cell type are rare, representing approximately 1–3% of all lymphomas in these sites.[3,11,24,35] The majority of non–B-cell lymphomas represent secondary manifestations of a systemic T-cell lymphoma or an extension of the tumor stage of mycosis fungoides, usually involving the eyelid.[11,24,35,56,74] Only a very few cases of primary T-cell lymphomas of the ocular adnexa have been reported in the literature to date;[11,24,40,46,47] it remains a point of discussion, however, whether all these cases represent a clonal T-cell proliferation.[33] Further, recent ophthalmic literature has described the rare involvement of the adnexal tissues by angiocentric T-cell lymphoma,[35] anaplastic large cell lymphomas of T-phenotype, and by "T/natural killer-cell lymphoma of nasal type."[11] Lastly, although it accounts for approximately 30% of lymphomas in general,[30] Hodgkin's disease of the ocular adnexa is exceptionally rare, with only a few cases being reported in the literature.[18,31,64,66,67]

We present five cases of ocular adnexal lymphomas, classified according to the REAL and the new World Health Organization (WHO) Classification of Tumors of Hematopoeitic and Lymphoid Tissues:[30] three of these cases could be considered to be typical ocular adnexal B-cell lymphomas in Western Europe and North America—an extranodal marginal zone B-cell lymphoma, a diffuse large cell B-cell lymphoma, and a follicular lymphoma. The remaining two cases—a non-endemic Burkitt lymphoma in a non-immune deficient patient and a primary Hodgkin lymphoma of the eyelid—represent the atypical tumors which can occur very rarely at these sites. These five cases, taken from a pool of approximately 300 cases of ocular adnexal lymphoproliferative lesions, form the basis for a review of the literature on ocular adnexal lymphomas.

Case Histories

A summary of the relevant clinical information is presented in Table 1.

CASE 1

In 1996, an 84-year-old male with a tan-colored conjunctival swelling of the right eye, as well as with a swelling in the region of the right parotid gland, was referred for consultation. Significant to his medical history was a right orbital "pseudolymphoma," diagnosed in 1983, which was treated with localized radiotherapy (total dosage, 40 Gy). Furthermore, in 1995, the patient had undergone a right-sided parotidectomy and three cycles of chemotherapy consisting of cyclophosphamide, hydroxydaunorubicin (adriamycin), vincristine (Oncovin), and prednisone (CHOP) for an extranodal marginal zone B-cell lymphoma of the parotid gland. On initial ocular examination, a facial paresis was present on the right side; the best corrected visual acuity was 6/18 in the right eye and 6/6 in the left. Extraocular motility was full. Examination of the right upper and lower eyelids revealed a nodular subconjunctival mass, measuring 30 × 25 × 15 mm, involving the palpebral conjunctiva and inferior cul-de-sac were noted (Fig. 1A). The cornea, anterior chamber, iris, lens, and fundus of the right eye were normal. The left eye was completely unremarkable.

Conventional histological examination was performed on an incisional biopsy of the conjunctival tumor, demonstrating an extranodal marginal zone B-cell lymphoma according to the REAL/WHO lymphoma classification.[22,30] Furthermore, a biopsy of the swelling in the region of the remnants of the right parotid gland revealed a small cell B-cell lymphoma, demonstrating similar morphology and immunophenotype as the conjunctival tumor (see Results). Staging investigations, which included full blood count, chest ×-ray, computer tomography scans, and magnetic resonance imaging of the thorax, abdomen, and of the head and neck, as well as bone marrow puncture, confirmed the conjunctival swelling (Fig. 1B) and revealed right-sided cervical lymphadenopathy, as well as a swelling in the region of the parotid gland remnants (Fig. 1C) (stage II, according to the Ann Arbor clinical staging system[7]). Consequently, the patient was treated with radiotherapy to the right orbit, the right parotid gland remnants, and cervical lymph nodes with 36 Gy. This was followed by 50 Gy to the last two locations with ocular protection. A significant decrease in both the conjunctival and parotid tumors was achieved. At the last follow-up, 4 years after therapy, there was no sign of tumor recurrence, although the patient complained of dry eye symptoms, which had been treated with ocular lubricants.

CASE 2

In 1996, an 81-year-old Caucasian female with diabetes mellitus was referred for consultation with a 3-month history of painful swelling in the left orbit with diplopia. Other symptoms included nausea, general fatigue over the previous 6 weeks, and swelling in the area of the thyroid gland. Of significance in the patient history was the diagnosis of an "immunocytoma" of the left orbit in 1989 (stage IE). At this time, the patient had been treated successfully with

TABLE 1

Clinical Information of the Ocular Adnexal Lymphomas Presented

Case No.	Initials	Age	Sex	Presenting Symptoms	Diagnosis[#]	Stage at Diagnosis*	Treatment	Outcome
1	W.P.	84	M	Conjunctival swelling RE, swelling in region of right parotid gland	EMZL	II	Initial:Rx Secondary:Cx Tertiary:Rx	Alive, CR
2	E.R.	81	F	Painful swelling in the left orbit with diplopia, nausea, malais, thyroid swelling	DLBCL	II	Initial:Rx Secondary:CHOP	Alive, CR
3	E.A.	56	F	Painless right-sided orbital swelling	FL	IE	Initial:Excision Secondary:Rx	Alive, CR
4	H.R.	84	F	Tender nodule of the lid	BL	III	CHOP	Alive, PR
5	A.T.	29	F	Foreign body symptoms, small nodular conjuctival swelling	HL	IIE	CHOP	Alive, CR

[#]Diagnoses are given according to the R.E.A.L/WHO Classifications: EMZL = extranodal marginal zone B-cell lymphoma; DLBCL = diffuse large cell B-cell lymphoma; FL = follicular lymphoma; BL = Burkitt lymphoma; HL = Hodgkin's lymphoma.

*Stage according to the Ann Arbor clinical staging system in Cases 1, 2, 3, and 5; and according to Murphy and Magrath in Case 4.

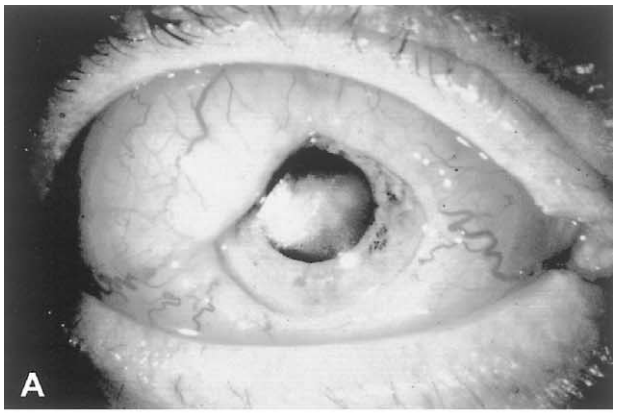
F = female; M = male; Rx = Radiotherapy; Cx = Chemotherapy; CHOP = cyclophosphamide, hydroxydaunorubicin (adriamycin), vincristine (Oncovin) and prednisone; CR = complete remission; PR = partial remission.

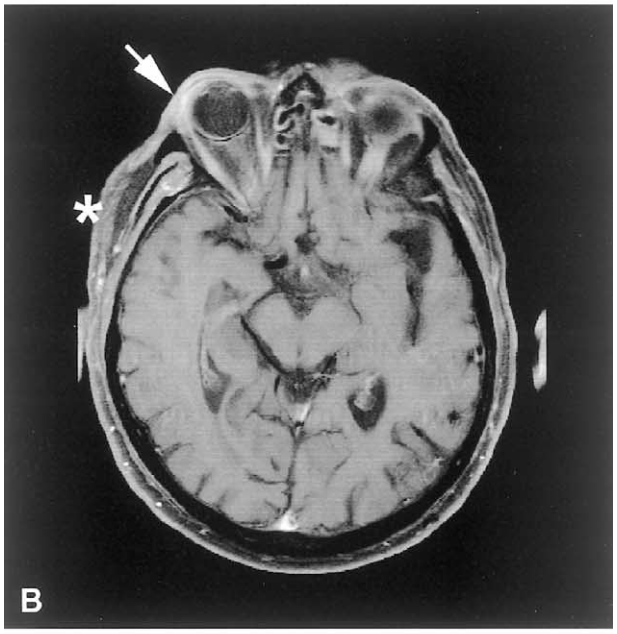
percutaneous radiotherapy with 30 Gy; she remained in remission until her re-presentation 7 years later. On examination, a mass of neoplastic tissue with an ulcerated surface protruded from the left orbit, producing a vertical squint in the left eye (Hertel 21 mm on the left side, 16 mm on the right) (Fig. 2A). There was massive lid edema and an ectropion of the left lower lid. Best corrected visual acuity in the left eye was 6/24 and in the right eye 6/18. Extraocular motility in the left eye was severely limited. The right eye was unremarkable with normal eyelids, conjunctiva, cornea, anterior chamber, lens, and fundus. General physical examination revealed an elderly woman of general good health, and it was otherwise unremarkable for lymphadenopathy, or for hepato- or splenomegaly. An incisional biopsy of the orbital tumor was performed, and histological and immunohistological examinations of this tissue disclosed the diagnosis of diffuse large cell B-cell lymphoma (see below). Magnetic resonance imaging of the left orbit demonstrated a 67 × 44 × 35-mm tumor with obvious ventral and lateral displacement of the globe (Fig. 2B). Staging investigations did not reveal any disseminated disease. The patient was treated with a total of 30 Gy to the left orbit, resulting in regression of the swelling.

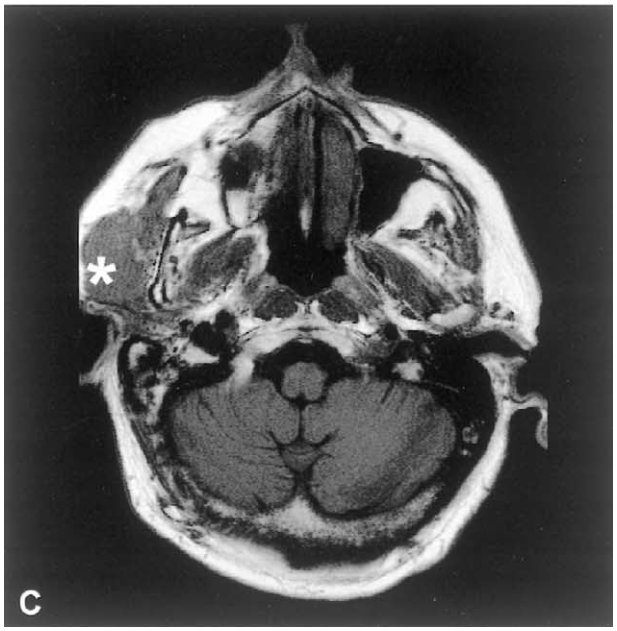
In 1997, the patient presented with an enlargement of the thyroid gland as well as with axillary and inguinal lymphadenopathy. A cytological smear of a thyroid aspirate revealed the diagnosis of an anaplastic B-cell lymphoma (see below). Blood investigations disclosed slightly elevated renal and liver function tests as well as a mild thrombocytopenia and anemia. Bone marrow puncture was negative for lymphoma infiltration. Thoracic and abdominal computer tomography scans revealed enlargement of the para-aortal lymph nodes in the region of the renal bifurcation. With stage III disease,[7] the patient was subsequently treated with a modified CHOP-Schema over 3 days. This lead to a successful regression of the thyroid tumor as well as the lymphadenopathy. At the last medical examination in March 2000, the patient was alive and in complete remission.

CASE 3

A 56-year-old Caucasian female presented to her general practitioner with a painless swelling of the right orbit with increasing diplopia. Past medical history was significant for hypertension and rheumatoid arthritis. She was referred for ophthalmic consultation. On initial examination, best corrected visual acuity was 6/18 in the right eye and 6/6 in the left. Extraocular motility was limited in the right eye on lateral gaze. Examination of the anterior and posterior segments of both eyes was unremarkable. A computed tomography scan of the right orbit demonstrated a poorly defined 15 × 13 × 10-mm tumor in the region of the lateral rectus muscle in the right orbit. Histological and immunohistological examination of the incisional biopsy revealed the diagnosis of a follicular lymphoma, according to the REAL/WHO lymphoma classifications.[22,30] Subsequent staging investigations did not reveal any lymph node or bone marrow involvement, that is, the orbital tumor represented a primary ocular adnexal tumor, stage IE.[7] The patient was subsequently treated with localized percutaneous radiotherapy with a total dose of 40 Gy. This treatment resulted in







total regression of the lesion. Complications from the radiotherapy included sicca syndrome, which was treated with ocular lubricants. Five years after diagnosis, there has been no local recurrence or evidence of systemic disease.

CASE 4

An 84-year-old female was referred by her ophthalmologist to the Ophthalmology and Head and Neck Surgical Departments for evaluation of double vision. She first complained of this symptom in September 1999, when a swelling in the left orbit was observed; she was treated with steroids for 14 days on the basis of a superior orbital biopsy which established the diagnosis of myositis. The symptoms initially improved but returned 2 months later. Past medical history was significant for diabetes mellitus and reflux gastritis due to a hiatus hernia. Further, in 1994 the patient suffered a pulmonary embolus following deep vein thrombosis, which was treated with a vena cava umbrella and warfarin anticoagulation therapy. On initial ocular examination, there was an obvious swelling in the mediosuperior region of the left orbit, causing a mild proptosis of the left eye of 2.5 mm (Fig. 3A). The best corrected visual acuity was 20/30 in the right eye and 20/30 in the left. The extraocular motility was limited on gaze to the left, resulting in double vision. The right eye was unremarkable. The physical examination was negative for lymph node swelling or enlargement of the liver or spleen. Histopathological examination including immunohistochemistry was performed on a left orbital biopsy revealing Burkitt lymphoma. The patient was referred to the Hematological Department for consultation, staging examinations, and further treatment. X-ray and magnetic resonance imaging of the head and neck disclosed an oval-shaped mass approximately 22 × 17 × 10 mm (Fig. 3B). Computed tomography of the thorax revealed enlarged paravertebral and hilar lymph nodes, with thickening of the visceral pleura laterobasal on the left side, of the diaphragm, and of the pericardium. Furthermore, the left lower lobe of the lung was atelectatic and a pleura effusion could be seen. A computed tomography scan of the abdomen was unremarkable. Blood investigations revealed a mild leucocytosis, and bone marrow biopsy, lymphomatous infiltration (stage IV).[50,60] Investigations for HIV-infection were nega-

Fig. 1. A: Conjunctival swelling of the right eye of Case 1 with dilated overlying vessels. *B:* Thickening of the bulbal conjunctiva on magnetic resonance imaging (arrow), with associated enlargement of the parotid gland remnants(*). *C:* Magnetic resonance imaging of the right parotid gland swelling (*) taken in 1995, prior to parotidectomy.

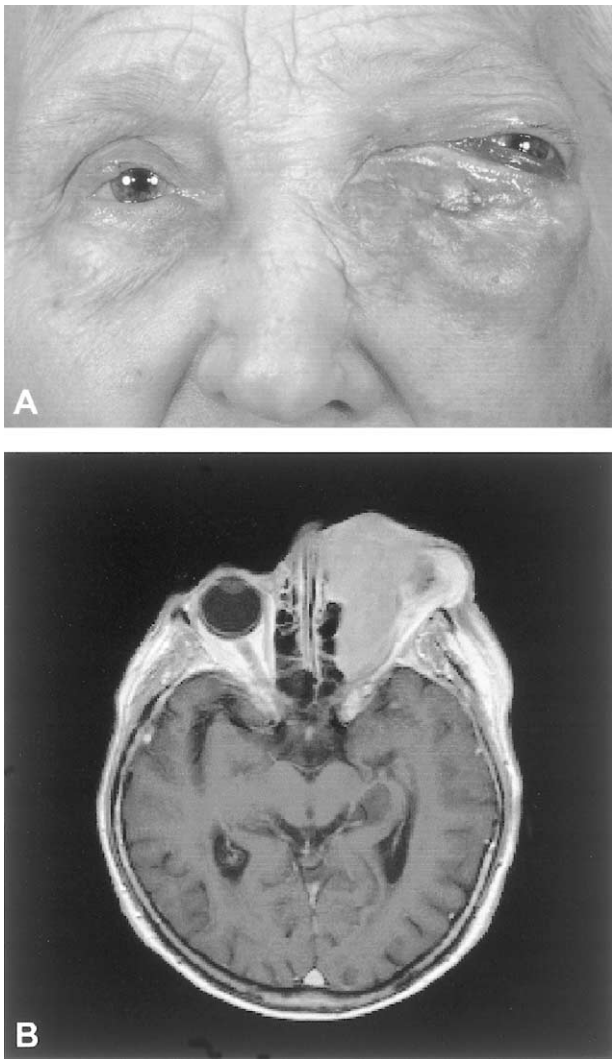


Fig. 2. A: Gross anterior and lateral displacement of the left eye of Case 2. *B:* Demonstration of the extent of tumor infiltration in the orbit with compression of the nasal cavities on MRI.

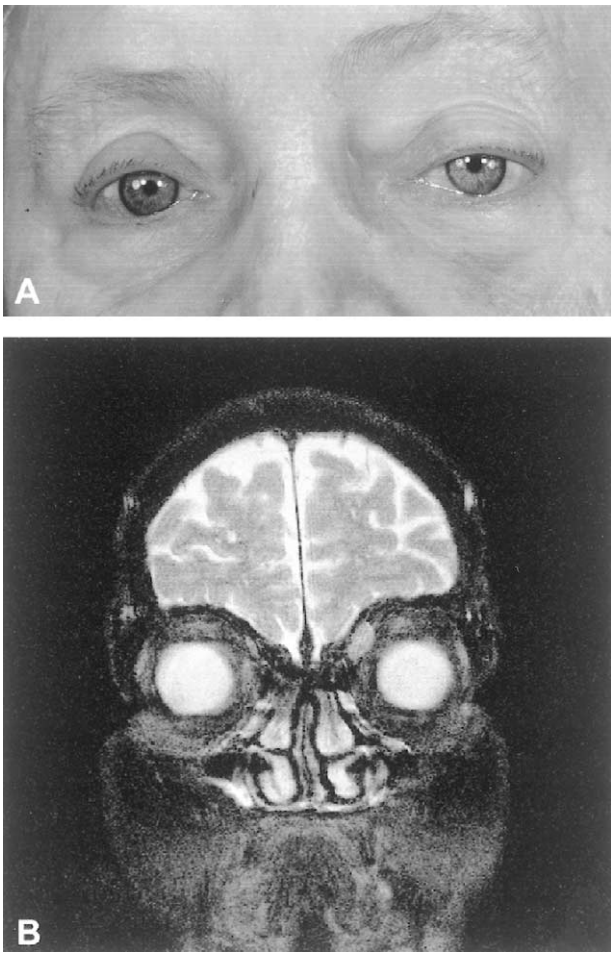


Fig. 3. A: Swelling in the mediosuperior corner of the left orbit in Case 4. *B:* Tumor demonstration on MRI scan with enhancement.

tive. Chemotherapy, consisting of cycle modified CHOP (four cycles) and cyclophosphamide, vincristine, methotrexate, and prednisone (COMP) (two cycles), was given between December 1999 and April 2000, and was well tolerated by the patient. The last clinical investigations revealed complete regression of the orbital tumor, an obvious decrease in the bone marrow and pulmonary infiltrates with improvement in cardiopulmonary function, but persistence of the mediastinal lymphadenopathy.

CASE 5

A 29-year-old Caucasian female presented to her ophthalmologist with a foreign body feeling and a small tender nodule approximately 8 mm in diameter on her right upper eyelid. Of clinical relevance in her medical history was the diagnosis of a high-grade malignant B-cell lymphoma of the pharynx at the age of 14. This had been treated with polychemotherapy and the patient was considered to be in remission at the time of presentation. The initial diagnosis was a chalazion and the patient was given antibiotic gel. As the patient returned 2 weeks later with a double-sized, tender, and ulcerated lesion, she was referred for ophthalmologic consultation. On examination, an ulcerated scaling lesion, measuring $28 \times 12 \times 4$ mm, surrounded by erythema, and edema was observed on the tarsus of the right eye. Levator function was normal, as were ocular motility and confrontation visual fields. Anterior and posterior segment examinations were unremarkable. Best corrected visual acuity was 6/6 both sides. Systemic examination did not reveal any abnormalities. Histological examination of an incisional biopsy of the tarsus revealed the diagnosis of classical Hodgkin's lymphoma in conjunctival tissue. The patient was subsequently referred for hematological consultation for staging procedures, disclosing swelling of the mediastinal

lymph nodes (stage IIE disease). She was treated with CHOP chemotherapy, and was in remission at the last follow-up examination.

Materials and Methods

TISSUE SAMPLES

The five cases were collected from the consultation files of the Reference Center for Hematopathology, Pathology Department of University Hospital Benjamin Franklin, Berlin, Germany. All tissue biopsies had been fixed in 4% buffered formalin and embedded in paraffin. Conventional histological stains included hematoxylin and eosin, Giemsa, and periodic-acid Schiff (PAS).

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Additional slides were stained with several monoclonal and polyclonal antibodies that are reactive in paraffin sections for immunohistochemical studies. An antigen-retrieval method using a pressure cooker was performed before immunohistochemical staining.[63] The staining consisted of a first-stage incubation with the following primary monoclonal antibodies: CD79a (clone JCB117); CD20 (L26); CD10; CD5 (54-F6); CD4 (1F6); CD8 (C8-144); CD21 (1F8); BCL-2 (124); CD30 (BerH2); CD15 (C3D1); cyclin D1 (P2D11F11); CD23 (1B12); CD43 (DFT1); terminal desoxynucleotidyl transferase (TdT); CD34 (BIRMK3); LMP (Cs1-4); and MIB-1 (Ki-67 antigen). Polyclonal antibodies were used to test the expression of CD3 antigen and of the Ig chains κ, λ, μ, γ, δ, and α.

The antibodies were made visible with an indirect immunoperoxidase method for the antibodies for the heavy and light chains of the immunoglobulin molecule, whereas the alkaline phosphatase anti-alkaline phosphatase (APAAP) method was used to demonstrate the binding of the remaining antibodies.[8] Antibodies were obtained from either DAKO (Glostrup, Denmark), Novocastra (Newcastle-upon-Tyne, England), or Monosan (Uden, Netherlands), except for MIB-1 and JCB-117, which were kindly provided by Dr. J. Gerdes (Borstel, Germany) and Dr. D. Mason (Oxford, UK), respectively. The number of MIB-1-positive cells was determined by counting the number of cells with clear nuclear positivity for these markers per 100 cells in five high-power fields (40 × objective, BH2-Olympus, Germany).

IN-SITU HYBRIDIZATION FOR EPSTEIN-BARR VIRUS ENCODED RNA

Epstein-Barr virus EBV RNA in-situ hybridization studies were performed as previously described,[11] using a 30-base oligonucleotide probe complementary to a portion of the Epstein-Barr virus encoded RNA (EBER1) gene. Five-micron thick paraffin sections were deparaffinized, rehydrated, digested with proteinase K, and hybridized overnight with a concentration of 0.25 ng/ml of biotinylated probe. Detection was accomplished using avidin-alkaline phosphatase conjugate. A known Epstein-Barr virus-positive neoplasm served as a positive control and Epstein-Barr virus-negative lymphoid tissue served as a negative control for each assay. All cases showed adequate reactivity with an oligo-T probe, indicating the integrity of the mRNA in the tissue samples.

POLYMERASE CHAIN REACTION (PCR)

DNA was extracted after de-waxing from 20-μm thick paraffin sections of all specimens employing QIAEX (Qiagen, Germany), according to the manufacturers' recommendations. A nested PCR for rearrangements of the heavy chain of the immunoglobulin gene (IgH) was performed as described previously.[12] The first round of DNA amplification was performed with six family-specific FW1 primers in conjunction with a JH consensus primer. The re-amplification was carried out with an aliquot (1%) of the first PCR employing six family-specific FW2 primers in conjunction with a consensus J4 primer. Reactive tonsils were used as polyclonal controls whereas B-cell line DNA served as monoclonal controls. Sufficient numbers of PCRs without DNA were included as negative controls. Products were analyzed on 6% polyacrylamide gel stained with ethidium bromide and viewed under ultraviolet light. A discrete band (230–280 base pairs long) after electrophoresis indicated monoclonality.

GENE SCAN

For precise size determination, the amplificates were separated on an automated DNA-sequencer (GeneScan analysis). For this purpose the re-amplification was performed as described above with the exception being a fluorescence (FAM)-labeled VLJH primer and separation on a DNA sequencing gel. Fluorescence-labeled PCR products were detected by a laser scanner and their size was automatically calculated according to a differently labeled size standard separated in the same lane.

DNA SEQUENCE AND SOMATIC MUTATION ANALYSIS

The DNA sequence analysis was performed using an automated DNA sequencer (Applied Biosystems 377A) by using the DyeDeoxy Terminator Method for usage solely with this system. For direct sequencing, the amplified products were separated by PAGE and appropriate bands were isolated by excising them under ultraviolet light. The excised bands were placed in separate Eppendorf tubes with 20 μl of distilled water for 24 hours and 5 μl of this were

used for sequencing. The isolated products were sequenced in both directions by using the re-amplification primers, FR2A and VLJH, respectively, in two separate sequencing reactions. A comparison was performed with published VH germline sequences (VBASE; German Cancer Research Center, Heidelberg, Germany).[79]

Results

The results of the immunohistochemical, in-situ hybridization and molecular biological findings of the cases examined are summarized in Table 2.

CASE 1

Microscopically, the tan-pink conjunctival specimen consisted of a diffuse dense lymphocytic infiltrates which displayed an expansive growth pattern within the marginal zone, surrounding reactive follicles. The neoplastic cells were cytologically heterogeneous, consisting of small lymphocytes, centrocyte-like cells, a variable number of blasts, monocytoid cells, as well as plasmacytoid cells (Fig. 4A). Occasional Dutcher bodies were present (Fig. 4B). The tumor cells infiltrated the conjunctival epithelium with the formation of lymphoepithelial lesions (not shown). Immunohistochemically, they were characterized by positivity for CD79a, CD20 (Fig. 4C), BCL-2, and CD43 with absence of staining for CD5, CD10, CD23, and cyclin D1 (Table 2). A monotypical expression of light (κ) (Figs. 4D and 4E) and heavy (IgM) immunoglobulin chains could be demonstrated. The plasmacellular differentiation of some tumor cells could be highlighted with plasma cell-related markers, namely Vs38c, CD38 (Fig. 4F), and CD138. Residual germinal centers, which were negative for BCL-2 and for BCL-6, were demonstrated with the antibody CD21, directed against the meshwork of follicular dendritic cells. The growth fraction of the tumor cells in the marginal zone was 15% (not shown). On the basis of morphology and immunohistology, a malignant small cell B-cell non-Hodgkin lymphoma could be diagnosed and sub-typed as extranodal marginal zone B-cell lymphoma. The biopsy from the parotid gland remnants demonstrated a lymphocytic infiltrate with similar morphological features and with a similar immunophenotype. Furthermore, review of histological slides of the orbital biopsy taken in 1983, which was diagnosed as "pseudolymphoma," combined with new immunohistochemical stainings, lead to a re-interpretation of the original diagnosis as an extranodal marginal zone B-cell lymphoma. The diagnosis of a B-cell lymphoma in the conjunctiva and parotid biopsies was confirmed by IgH-PCR and gene scan analysis demonstrating a clonal B-cell population of the same size (248 base pairs) (Figs. 5 and 6).

CASE 2

Conventional histology of the orbital tumor biopsy demonstrated an extensive, diffuse infiltrate of B-lymphocytes of medium to large size with conspicuous nucleoli and basophilic cytoplasm as well as numerous mitoses including atypical mitotic figures (Fig. 7A). The bony walls of the lateral orbital cavity were also infiltrated by tumor (not shown). The immunophenotype of the neoplastic cells was CD79a+, CD20+ (Fig. 7B), BCL-2+/−, BCL-6+/− and the growth rate was large, approximately 80% (Table 2). On the edge of the large cell tumor component, reactive germinal centers surrounded by expanded marginal zones with the cytomorphology and immunophenotype for extranodal marginal zone B-cell lymphoma, as described above, were observed. An EBV-infection could not be demonstrated either immunohistochemically or using in-situ hybridization. The diagnosis of diffuse large cell B-cell lymphoma arising from an extranodal marginal zone B-cell lymphoma was made. Despite repetition with various primer sets, a clonal population could not be demonstrated on IgH-PCR. Conventional stains of the thyroid puncture demonstrated numerous lymphocytic blasts which were positive for B-cell antigens and negative for T-cell markers (Fig. 7C).

TABLE 2

Results of the Immunohistochemical Examinations, In-Situ Hybridization and of the IgH-PCR of the Presented Ocular Adnexal Lymphomas

Case No.	CD79a	CD20	CD3	CD5	CD43	CD23	CD10	BCL-2	BCL-6	Cyclin D1	CD30	CD15	LMP	EBER	Ki-67%	IgH
1	+	+	−	−	+	−	−	+	−	−	−	−	−	−	15	Mono
2	+	+	−	−	−	−	−	±	±	−	−	−	−	−	80	n.p.
3	+	+	−	−	−	−	+	+	+	−	−	−	−	−	20	Mono
4	+	+	−	−	+	−	+	−	−	−	−	−	−	−	100	Mono
5	+	+	−	−	−	−	−	−	−	−	+	+	+	−	90	Mono

LMP = latent membrane protein of the Epstein-Barr virus; EBER = Epstein = Barr virus-encoded early nuclear RNA; + = positive; − = negative; ± = mostly positive; mono = monoclonal amplification product; poly = polyclonal amplification product; n.p = no amplification product.

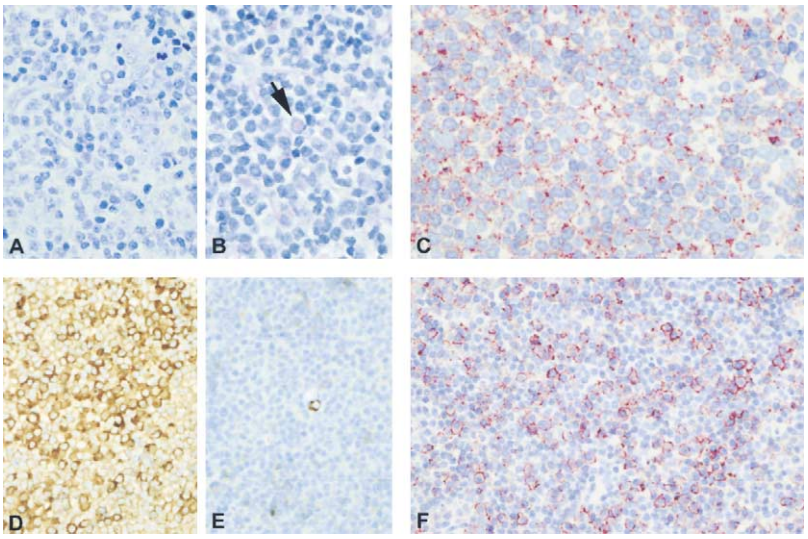

Fig. 4. *A:* Diffuse and dense lymphocytic infiltrates consisting of a heterogeneous population, with small lymphocytes, centrocyte-like cells, a variable number of blasts, monocytoid cells, as well as plasmacytoid cells (Giemsa, original magnification × 200). *B:* Within the infiltrates, some Dutcher bodies were present (arrow) (PAS stain, original magnification × 200). *C:* Tumor cell positivity for CD20 (APAAP, original magnification × 400). *D:* Monotypical expression of the tumor cells for the immunogloblin light chain Kappa (PAP, original magnification × 200). *E:* For comparison, only occasional reactive plasma cells were positive for the immunoglobin light chain lamda (PAP, original magnification × 200). *F:* The extent of the plasmacellular differentiation of the tumor cells could be highlighted using CD38 antibody (APAAP, original magnification × 200).

CASE 3

Microscopically, the orbital specimen displayed a follicular arrangement of tumor cells, with the neoplastic follicles being bland without the typical arrangement of zones seen in reactive follicles. They extended between the acini of the lacrimal gland and consisted of both centrocytes and centroblasts, with a dominance of centrocytes (Fig. 8A). An interfollicular infiltration of the tumor cells, as well as some areas of sclerosis were also observed. The immunohistochemical profile of the neoplastic germinal center cells included positivity for CD79a, CD20 (Fig. 8B), BCL-2 (Fig. 8C), CD10, and BCL-6 (Table 2). The growth fraction of the tumor cells was 20% (Fig. 8D), considerably reduced when compared to reactive germinal centers. On the basis of the morphology and immunophenotype, the diagnosis of FL (grade II) was made. A monoclonal amplification product of size 247 base pairs was obtained on IgH-PCR (Fig. 5).

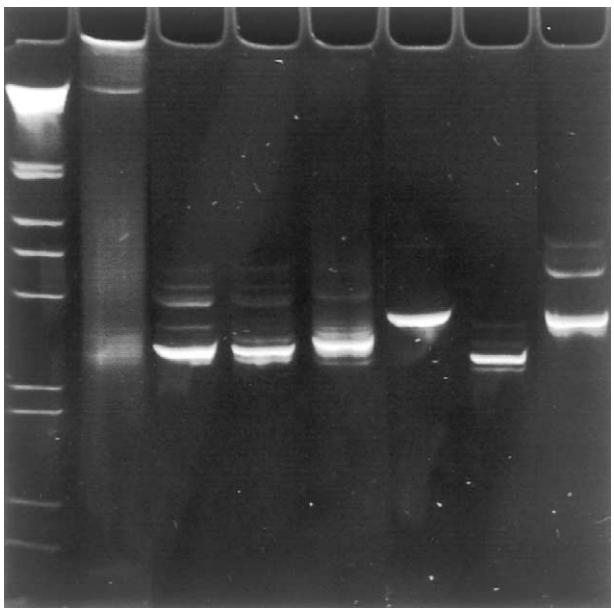

Fig. 5. Electrophoresed PCR gel demonstrating the DNA amplicates obtained for the various cases: Lane 1: standard; Lane 2: tonsil as a polyclonal control demonstrating a smear of DNA products of differing sizes; Lanes 3 and 4: DNA amplificates of identical size (248 base pairs), obtained from the conjunctival and parotid tumors of case 1, respectively; Lane 5: DNA amplificate of size 247 base pairs, obtained from the tumor of Case 3; Lane 6: DNA amplificate of size 276 base pairs, obtained from the tumor of case 4; Lane 7: DNA amplificate of size 236, obtained from the tumor of case 5; Lane 8: Raji B-cell clone used as a positive control (263 base pairs).

CASE 4

Conventional histology demonstrated a dense infiltration of mainly medium-sized closely packed lymphocytes interspersed with phagocytic histiocytes, giving a "starry sky" appearance when observed at lower magnification (Fig. 9A). The tumor cells were characterized by oval or round nuclei, sur-

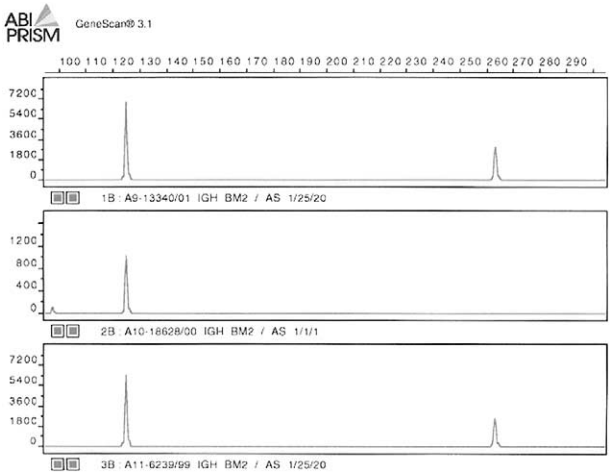

Fig. 6. Gene scan of Case 1 demonstrating the monoclonal peaks obtained in the parotid (*top*) and conjunctival (*bottom*) tumors, measuring 248 base pairs. An amplificate could not be obtained from the parotid recurrent tumor (*middle*).

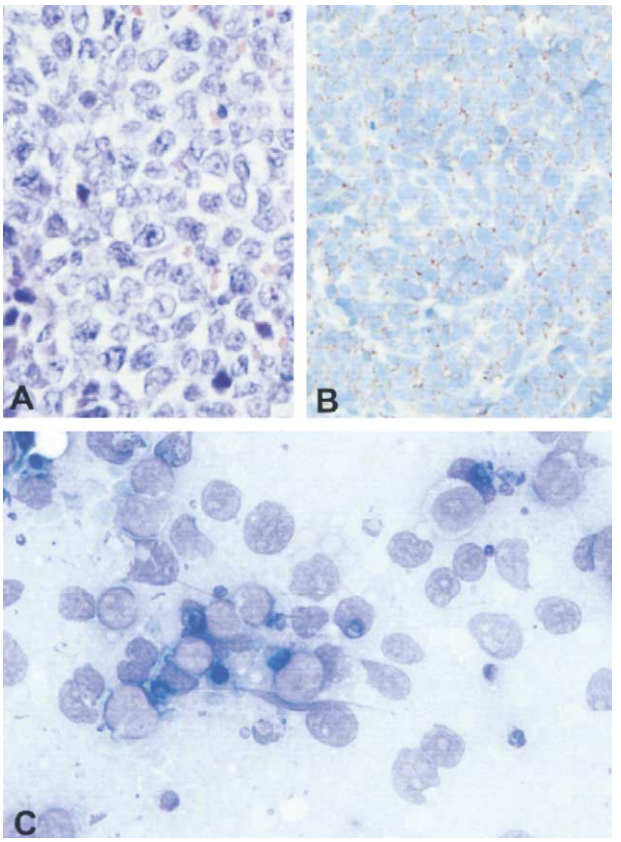

Fig. 7. A: Extensive, diffuse infiltrate of lymphocytes of medium to large size with conspicuous nucleoli and basophilic cytoplasm in the orbit (PAS stain, originial magnification 200 x). *B:* Positivity of the tumor cells for CD20 (APAAP, original magnification 200 x). *C:* Cytological smear of the thyroid aspirate, demonstrating extensive infiltration by large polymorphic tumor cells as well as apoptotic debris (May-Grünewald-Giemsa stain, original magnification 400×).

rounded by eccentric basophilic cytoplasm, and with one to several distinct basophilic nucleoli. Mitotic figures were frequent. Similarly, numerous apoptotic cells could be observed. Immunophenotypically, the tumor cells were positive for CD79a, CD20 (Fig. 9B), CD10, CD43, and BCL-6 (Table 2) with absence of expression of Tdt, CD34, CD3 (Fig. 7C), and BCL-2 (Fig. 7D). Furthermore, the tumor cells demonstrated moderate expression for the plasma cell marker Vs38c and monotypic intracytoplasmic immunoglobulin (not shown). The growth fraction of the tumor cells was 100% (Fig. 7D). On the basis of the morphology and the immunophenotype, the diagnosis of a plasmacytoid variant of Burkitt lymphoma was made. On IgH-PCR and gene scan analysis, a monoclonal population of tumor cells with a PCR amplificate of 276 bp could be demonstrated (Fig. 5).

CASE 5

Histopathological examination of the conjunctival specimen demonstrated an atypical heterogeneous infiltrate consisting of a mixture of small lymphocytes, eosinophils, occasional plasma cells and atypical blasts (Fig. 10A). The latter demonstrated the morphology of Hodgkin cells and Reed-Sternberg cells, with several nuclei and distinct nucleoli. The tumor cells were positive for CD20 (Fig. 10B), and were surrounded by rosettes of T-lymphocytes (Fig. 10C). Furthermore, CD30 and CD15 expression by most tumor cells was observed (Figs. 10D and 10E). An Epstein-Barr infection could be demonstrated using the antibody Cs1-4, directed against the latent membrane protein of the virus (not shown). In-situ hybridization for Epstein-Barr–encoded early nuclear RNA was negative. The growth fraction of the tumor cells was 90% (Fig. 10F) (Table 2). On IgH-PCR and gene scan analysis, a monoclonal population of tumor cells with a PCR amplificate of 236 bp could be demonstrated (Fig. 5).

Discussion

The lymphomas of the ocular adnexa are almost exclusively extranodal B-cell non-Hodgkin lymphoma.[3,12,35,44,55,61,82] They delineate one end of the spectrum of lymphoproliferative lesions which occur in these tissues; the other pole being represented by reactive lymphoid hyperplasia. A group of tumors, termed atypical lymphoid hyperplasia, exists between the two ends of this spectrum: these are lymphoproliferative lesions, which cannot be placed unequivocally in either of the two major groups.[12,35,44,55,61]

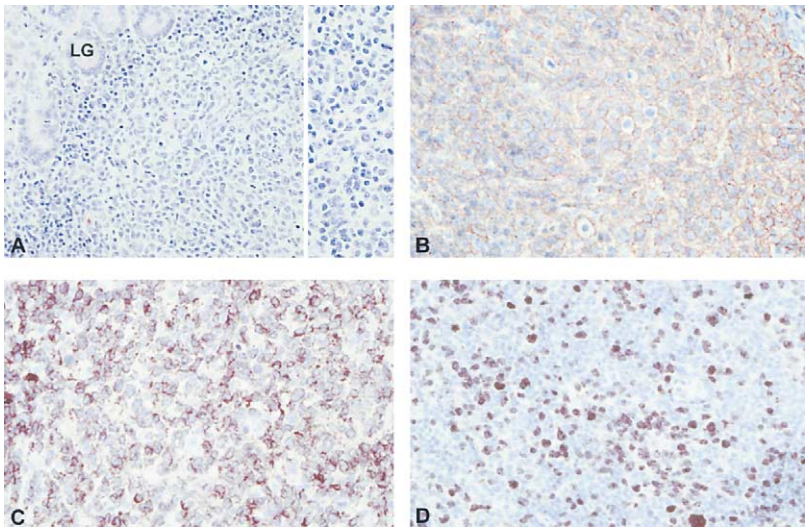

Fig. 8. A: Follicular arrangement of the tumor cells with the monomorphic neoplastic follicles consisting of centrocytes and centroblasts (insert). The tumor cells extend between the acini of the lacrimal gland (lg) (Giemsa, original magnifications, 200 and 400 x). *B:* Positivity of the tumor cells for CD20 (APAAP, original magnification, 200 x). *C:* BCL-2 expression by the neoplastic cells (APAAP, original magnification, 400 x). *D:* Reduced growth fraction of the germinal center cells, compared to reactive follicles (Ki-67, APAAP, original magnification, 400 x).

CLINICAL CHARACTERISTICS

Regardless of the histopathological entity (reactive lymphoid hyperplasia, atypical lymphoid hyperplasia, or malignant lymphoma), patients with ocular adnexal lymphoid proliferations do not differ significantly with respect to age, sex, presenting signs and symptoms, duration of symptoms, or ophthalmic findings.[12,44,51] With the exception of pain with associated bone erosion on radiological examination (most often observed in so-called high-grade lymphomas[12,82]) there are few symptoms or signs that aid the clinical distinction between reactive lymphoid hyperplasia and malignant lymphoma.

The ocular adnexal lymphoid proliferations can occur at all ages, but they are most commonly seen in patients in the fifth to seventh decades of life.[3,12,35,44,51,61,75,82] They tend to affect women more often than men (female:male = 1.5–2:1), and occur most frequently in the orbit (particularly, in the superior anterior orbit), followed by the conjunctiva, and the eyelids.[3,12,44,82] Approximately 10–17% of cases demonstrate bilateral involvement,[12,44,52,61,82] with the bilaterality occurring either simultaneously (approximately 80%) or subsequently (approximately 20%).[52]

Typical presenting signs and symptoms included an orbital mass with or without proptosis, diplopia, conjunctival ("salmon-pink") swelling, conjunctival redness and irritation, and ptosis, when involving the dermis of the orbicularis muscle of the superior eyelid.[12,44,51,75,82] The duration of symptoms before presentation varies considerably between patients, with the average duration being approximately 6 months.[12,44,82] The non-ophthalmic findings discovered on general physical examination depend on the presence or absence of systemic malignant lymphoma. The percentage of patients with a history or

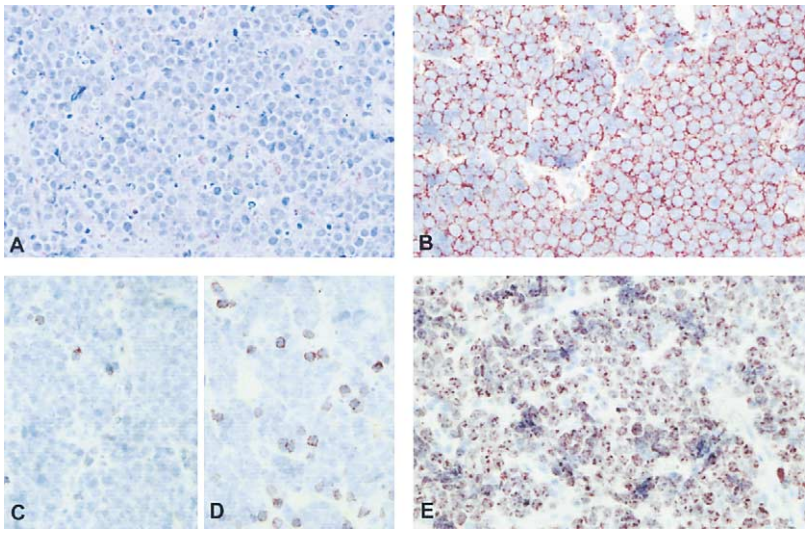

Fig. 9. A: Low-grade magnification of the orbital tumor of Case 4, demonstrating an infiltration of medium-sized tumor cells with minimal cytoplasm, prominent nuclei and nucleoli. The apoptotic bodies and interspersed macrophages lead to the so-called "starry-sky" appearance (Giemsa, original magnification x 200). *B:* Tumor cell positivity for CD20 (APAAP, original magnification 400 x). *C:* Occassional CD3-positive lymphocytes are present between the neoplastic cells (APAAP, original magnification 200 x). *D:* The admixed T-cells are positive for BCL-2, for which the tumor cells are negative (APAAP, orignial magnification, 200 x). *E:* The growth fraction of the tumor cells is 100% (APAAP, Ki-67 antigen, original magnification 200 x).

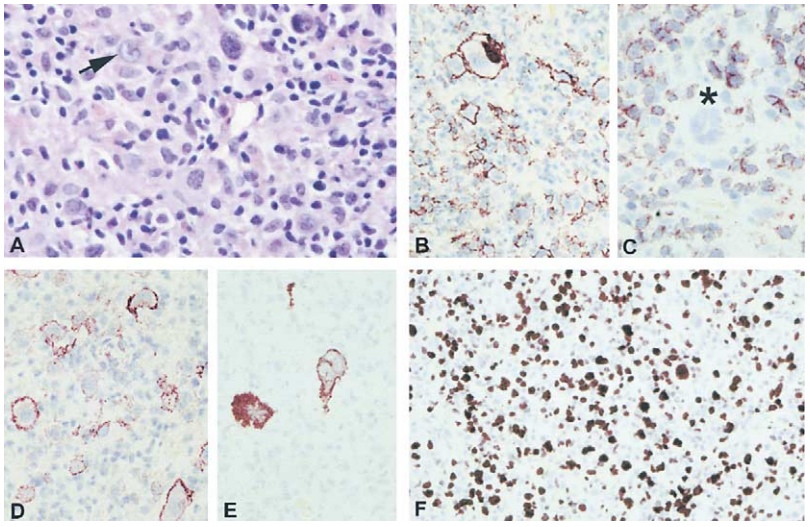

Fig. 10. A: A heterogeneous infiltrate consisting of eosinophilic granulocytes, macrophages, small lymphocytes as well as blasts with prominent multiple nuclei (arrow) (HE, original magnification 200 x). *B:* The latter cells demonstrated positivity for CD20 (APAAP, original magnification, 400 x). *C:* The neoplastic blasts (star) were also surrounded by T-cell rosettes (APAAP, CD3, original magnification, 200 x). *D:* Expression of CD30 and *E:* CD15 by the blasts confirmed the diagnosis of Hodgkin's disease (APAAP, original magnification, 400 x). The growth fraction of the tumor cells was large, approximately 90% (APAAP, Ki-67 antigen, original magnification, 200 x).

concurrent evidence of extraocular lymphoma at diagnosis varies considerably between investigations, with 20–40% of patients being reported.[12,37,44,61,75,82]

DIAGNOSTIC TECHNIQUES

Although ultrasonography has proven useful in the detection of anterior and intraconal neoplasms, computed tomography scans or magnetic resonance imaging with enhancement are usually the primary diagnostic imaging investigation in the evaluation of ocular adnexal lymphoid proliferations.[17,68,87] Both procedures are of use to determine the size, the number, and the degree of infiltration of these lesions. Most orbital ocular adnexal lymphoid lesions are unifocal homogeneous masses of relatively high density that demonstrate mild enhancement with contrast media.[17] They are usually located within the orbital adipose tissue, although very rare cases have been reported within an extraocular muscle.[25,87] They tend to mold themselves along the orbital structures, with bony destruction or infiltration only being observed in high-grade lymphomas. When lacrimal gland involvement is present, a diffuse wedge-shaped expansion may be observed. At the present time, neither computed tomography scans nor magnetic resonance imaging can reliably differentiate reactive lymphoid hyperplasia from atypical lymphoid hyperplasia or from malignant lymphoma.[17,68]

The ultimate diagnosis is usually established through histopathological examinations of a tissue biopsy of a suitable size. As a result of the development of antigen-demasking and DNA-extraction techniques, the majority of the above-mentioned immunohistochemical and molecular biological examinations can be performed on formalin-fixed and paraffin-embedded tissue. Consequently, it is not necessary for tissue probes to be frozen; instead, most biopsies can be placed in containers with 4% buffered formalin, which enables better transport of specimens to diagnostic centers. The role of fine-needle aspiration biopsy in diagnosing ocular adnexal lesions is controversial. Some centers with experience in aspiration cytology use this diagnostic tool successfully.[39] It should be mentioned, however, that mechanical artifacts, insufficient material, as well as sampling errors can limit the interpretation of these specimens, particularly in the differentiation between reactive lymphoid hyperplasia and malignant lymphoma as well as in the subtyping of a malignant lymphoma.

HISTOPATHOLOGY

Terminology and Classifications

In the past, the malignant lymphomas of the ocular adnexa represented a considerable diagnostic challenge for the pathologist. This was possibly due to a number of factors, including the usual low-grade nature of these neoplasias; their extranodal location together with their composition predominantly of small relatively bland lymphocytes; and the fact that the criteria previously used to distinguish between small cell lymphoma and reactive lymphoid hyperplasia were unclear, and were based mainly on cellular morphology as well as on the dogma of older lymphoma classifications.[65,72] The rate of accuracy in prediction of the behavior of the ocular adnexal lymphoproliferative lesions was poor on the basis of the histological findings.[42,58] These difficulties and resulting confusion (remnants of which are still present today) were compounded by the variety of terms attributed to the ocular adnexal lymphoma, including benign lymphoma, inflammatory pseudotumors, or orbital pseudotumor. It was only with the advent of immunohistochemistry and molecular biological studies, such as Southern blot hybridization

and PCR, together with the recognition of lymphoma entities, in particular, the mucosa-associated-lymphatic-tissue lymphoma,[27] that the distinction between malignant lymphoma and reactive lymphoid hyperplasia became clearer. Furthermore, the number of borderline lesions or atypical lymphoid hyperplasia diagnosed could be reduced.

In the past, the larger studies[15,44,53,55] investigating ocular adnexal lymphomas have classified them using the "Working Formulation."[69] More recent analyses[3,12,35,37,51,61,82] have applied the REAL Classification,[22] which is probably more appropriate due to its inclusion of both nodal and extranodal lymphomas. The five cases of ocular adnexal lymphomas reported in this article have been sub-typed according to the new WHO Classification of Tumors of Hemopoietic and Lymphoid Tissues,[30] which was based on the REAL Classification.[22] The five lymphoma subtypes will be briefly summarized below.

Extranodal Marginal Zone B-Cell Lymphoma

The small cell B-cell lymphoma diagnosed in Case 1 represents the most common subtype of B-cell lymphoma in the ocular adnexa—the extranodal marginal zone B-cell lymphoma.[3,12,19,35,61,82,83] This term was proposed by the International Lymphoma Study Group to include "mucosa-associated lymphatic tissue lymphomas" with and without an association with mucosa.[22] Extranodal marginal zone B-cell lymphomas develop in a variety of locations normally possessing minimal lymphoid tissue, the most common site being the stomach; others include the salivary glands, the thyroid, and the ocular adnexa.[28] Regardless of the location of origin, extranodal marginal zone B-cell lymphoma have similar clinical, pathological, and molecular features[28] (Table 3).

Extranodal marginal zone B-cell lymphomas usually demonstrate an indolent clinical course, often remaining localized to their sites of origin for many years prior to dissemination.[20] Bone marrow or peripheral blood involvement is very unusual. A typical clinical feature of extranodal marginal zone B-cell lymphoma is the concurrent or subsequent involvement of ocular adnexal tissues and/or other extranodal sites, such as the lung or salivary glands.[55] This was exemplified by Case 1 with an initial orbital tumor followed by ipsilateral parotid and conjunctival tumor 12 and 13 years, respectively, after the initial presentation.

Morphologically, extranodal marginal zone B-cell lymphomas are characterized by an expansion of a heterogeneous cell population consisting of centrocyte-like, monocytoid, and plasmacytoid tumor cells with occasional blasts in the marginal zone surrounding reactive follicles (Table 3). The degree of monocytoid or plasmacellular differentiated tumor cells varies considerably between cases: it is possible that this cellular heterogeneity of extranodal marginal zone B-cell lymphomas, together with the presence of reactive germinal centers in most tumors, resulted in some of them being misinterpreted as reactive lymphoid hyperplasia (as in Case 1, initial orbital tumor) or grouped with other low-grade B-cell lymphomas (e.g. immunocytomas, as in Case 2, initial orbital tumor) in the past.[21,35]

Another characteristic feature of extranodal marginal zone B-cell lymphomas is "follicular colonization," a secondary infiltration of the germinal centers by the neoplastic marginal zone B-cells.[27] When arising in tissues associated with epithelium, such as the conjunctiva, lacrimal gland, or retinal pigment epithelium,[9] nests of extranodal marginal zone B-cell lymphoma cells may infiltrate the neighboring structures, forming lymphoepithelial lesions; these are further evidence of the neoplastic nature of these lesions and aid in the differentiation between extranodal marginal zone B-cell lymphoma and reactive lymphoid hyperplasia.

Immunophenotypically, extranodal marginal zone B-cell lymphomas have a characteristic profile (Tables 2 and 3), which aids their distinction from other small cell B-cell lymphomas. In particular, they are usually negative for CD5, which helps differentiate them from mantle cell lymphoma and chronic lymphocytic leukemia of B-cell type. Furthermore, they are negative for CD10 and BCL-6, for which FL are usually positive (see Case 3). Monotypic expression for immunoglobulin light and heavy chains (typically IgM) can usually be demonstrated. Light chain immunohistochemistry and its interpretation is, however, known for being problematic, and it may often require repetition using different methods. In such cases, additional staining with plasma cell-related antigens, with demonstration of an aberrant expression compared to reactive plasma cells, may be of help in establishing the diagnosis of malignancy.[9]

The neoplastic nature of the infiltrating lymphocytes in extranodal marginal zone B-cell lymphoma can be further supported (if necessary) through the demonstration of rearrangements of the immunoglobulin heavy chain gene with either Southern blot hybridization[34,37,62] or PCR.[10,12] It is important to note, however, that although these investigations are helpful in establishing a diagnosis, they do not have any value in terms of predicting the prognosis of patients with ocular adnexal lymphoproliferative lesions.[37,44] Furthermore, the success of these procedures depends on a number of factors, including the size and fixation solution of the biopsy as well as the size of the tumor population within the infiltrating cells. Results vary between studies with some authors

TABLE 3

Morphological, Immunophenotypic, Molecular-Biological and Clinical Characteristics of the 5 Lymphoma Subtypes Presented

Lymphoma Subtype	Morphology	Tumor Cell Immune Profile	Molecular Biological Changes*	Cell of Origin	Clinical Characteristics
EMZL	• Expansive growth in the marginal zone between reactive secondary follicles • Heterogeneous cell population: centrocyte-like cells, monocytoid B-cells, plasmacytoid cells, occasional blasts • Possibly "Follicular Colonization" • Possibly "Lymphoepithelial lesions" • Often multifocal growth	• CD79a+, CD20+, CD43+ (usually), BCL−2± • IGM+, IgD− • CD10−, CD23−, CD5−, cyclin D1− • Presence of FDC's in reactive secondary follicles • Monotypic cytoplasmic Ig in 10%	• Clonal Ig-H and Ig-L rearrangements • Mutations in V-region of IgH-gene • t(11;18)(q21;q21)in ca. 50% • Trisomy 3 in ca. 50%	• "Memory" B-cell	• 8% of all NHL • Peak age, 65 years • Females > Males • Rare involvement of BM or spleen at time of diagnosis • Possible concurrent or subsequent involvement of other extranodal sites • Tendency to recur
DLBCL	• Diffuse growth pattern • Centroblastic: large centroblast-like tumor cells with variable content of immunoblasts • Immunoblastic: >90% immunoblastic tumor cells • Anaplastic: polymorphic often bizarre tumor cells • T-cell rich: only 10% tumor cells with 90% T-cell infiltrate and macrophages	• CD79a+, CD20+, • BCL-6+ (ca. 70% of cases) • CD10+ (ca. 25–50%) • IgM> IgG> IgA in 50%–75% of cases • CD30+ in lymphoma with anaplastic morphology • Rarely CD5+ or CD23+ • No FDC-MW • Ki-67 nearly always >40%	• Clonal Ig-H and Ig-L rearrangements** • Numerous mutations in V-Region of IgH-Gene • Bcl-6 gene rearrangements in up to 40% of cases • Bcl-2 gene rearrangements in 20–30% of cases • C-myc gene rearrangements extremely rare • REL gene amplification in 20% mainly extranodal lymphoma • P53-Gene mutations only in secondary lymphoma arising from a FL	• Mature germinal center-B-cell or post-germinal center-B-cell (memory) B-cell	• 40% extranodal (gastrointestinal tract > skin > soft tissue > central nervous system) • 60% nodal • Average age: 60–70 years • Rapidly growing solitary nodal or extranodal tumor • Aggressive clinical course
FL	• Usually follicular growth pattern with occasional diffuse areas; rarely purely diffuse • Mixture of centrocytes and centroblasts with dominance of former • Monomorphic GCs with loss of zonation • Minimal or no apoptosis in GC • Usually no macrophages with tingible bodies • Thin or even absence of the follicle mantle	• CD20+, CD10+, BCL-2+ (90%), BCL-6+, IgM+ (50%), IgG (50%) • CD43- (95%), CD23-, CD5- • Dense follicular FDC MW • Obvious reduction in growth fraction in neoplastic GCs versus reactive GCs, particularly in BCL-2+ cases • Often CD10+ and BCL-6+ B-cells in the interfollicular region	• Clonal Ig-H and Ig-L rearrangements** • Numerous mutations in V-region of IgH-Gene with "ongoing" mutations (intraclonal diversity) • t(14;18) in 90%, resulting in the expression of BCL-2 in neoplastic germinal centers • Mutations of p 53 gene and c-myc-rearrangement in high-grade transformed cases	• Germinal center-B-cell	• 40% of all NHL in the USA, 20–30% in Europe • Fifth and sixth decades of life (mean age, 59 years), unusual before 20 years of age • M:F = 1:1 • Lymph nodes mainly infiltrated, but also spleen, bone marrow and skin

(*continued*)

TABLE 3

Continued

Lymphoma Subtype	Morphology	Tumor Cell Immune Profile	Molecular Biological Changes*	Cell of Origin	Clinical Characteristics
FL	• Rarely pure diffuse growth pattern	• Dense well-defined FDC meshworks in neoplastic germinal centers (demonstrated with CD21)			• Often advanced disease (Stage III/IV) at the time of diagnosis • 5-year survival rate: 75% Transformation to DLBCL in 30% of cases
Classical BL	• Diffuse monotonous infiltration pattern • Medium-sized tumor cells, round nuclei, clumped chromatin, basophilic cytoplasm • Extremely high proliferation rate with numerous mitoses and apoptotic bodies • Starry sky pattern due to admixed histiocytes	• CD79a+, CD20+, CD10+, BCL-6+, IgM+ • CD21+ (endemic form) • CD5−, CD23−, TdT−, BCL-2- • Ki-67 = 100%	• Clonal IgH-rearrangements with somatic mutations • Translocation of MYC: t(8;14), t(2;8) or t(8;22) • Inactivation of TP53 due to mutations (30%) • EBV genomes can be demonstrated in tumor cells in nearly all endemic cases, 25–40% immunodeficient cases and <30% in sporadic cases	• Germinal center-B-cell	• Endemic form: children > adults (ages 4–7 years), M:F = 2:1, mandible, maxilla and orbital bones • Sporadic form: children > adults, 1–2% of all NHL in USA, M:F = 2 or 3:1, distal ileum, cecum and mesenteric lymph nodes • Immunodeficiency associated BL: adults > children, HIV-infection, predominantly lymph nodes • Often bulky tumor disease due to rapid proliferation rate of tumors • Prognosis dependant on Stage, particularly bone marrow involvement

(*continued*)

TABLE 3

Continued

Lymphoma Subtype	Morphology	Tumor Cell Immune Profile	Molecular Biological Changes*	Cell of Origin	Clinical Characteristics
Classical HL	• Tumor cells: typical HRS-cells • Architecture: mainly diffuse or an interfollicular infiltrate composed of esoinophils, neutorphils, lymphocytes, plasmacells and macrophages	• CD30+, CD15+ • EBV+ (40–50%) • EMA 5%+ • CD20−/+, CD79−/+ • CD45−, J-chain-	• Clonal IgH-rearrangements with numerous somatic mutations without "ongoing" mutations	• Germinal center-B-cell	• Mainly 30–40 years • M:F − 3:1 • Lymph node enlargement, particularly cervical, axillary and inguinal • Extranodal involvement mainly in mediastinum, spleen, less often lung, liver and bone marrow • B-symptoms in 35% of cases • Prognosis dependant on Stage of disease at diagnosis • 5 year survival: 85–90%

*These results arise from investigations of Non-Hodgkin lymphomas in other locations.

**Rearrangements demonstrable only in 50–70% of cases due to presence of somatic mutations.

EMZL = Extranodal marginal zone B-cell lymphoma; DLBCL = Diffuse large cell B-cell lymphoma+; FL = Follicular lymphoma; BL = Burkitt lymphoma; HL = Hodgkin's lymphoma; NHL = Non-Hodgkin's lymphoma; FDC-MW = follicular dendritic cell meshworks; Ig-L = Immunoglobulin Light chain; Ig-H = Immunoglobin Heavy chain; EBV = Epstein-Barr Virus.

demonstrating either monoclonality or oligoclonality in up to 90% of ocular adnexal lymphomas with Southern blot hybridization[37] and up to 78% with PCR.[12] Some authors have been able to demonstrate that bilateral as well as concurrent ocular adnexal and extraocular lymphomas have arisen from the same B-cell clone.[52,62] We could demonstrate here that the parotid and conjunctival extranodal marginal zone B-cell lymphomas in Case 1 arose from the same neoplastic B-cell clone (Fig. 5 and 6).

Although the pathogenesis of extranodal marginal zone B-cell lymphoma remains unclear, recent cytogenetic studies have demonstrated the translocation t(11;18), resulting in a fusion of the apoptosis inhibitor-2 (API2) gene on chromosome 11 and the "mucosa-associated-lymphatic tissue" lymphoma-associated translocation (MLT) gene on chromosome 18 (Table 3).[25,59] This transcription appears to have clinical significance, possibly resulting in therapy resistance when present in a extranodal marginal zone B-cell lymphoma.[49] Furthermore, trisomy 3 has been demonstrated in a significant proportion of extranodal marginal zone B-cell lymphoma in different locations. Finally, the development of extranodal marginal zone B-cell lymphoma in the stomach, salivary gland, and thyroid gland has been associated with autoimmune disease or bacterial infection prior to tumor development (Table 3). Indirect evidence suggests that the development and progression of extranodal marginal zone B-cell lymphoma in these locations is dependent on antigen stimulation provided by the associated immune reactions.[20,84] This evidence includes not only clinical studies[84,85] but the analysis of somatic mutations in the variable region of the immunoglobulin heavy chain gene of these tumors as well. We have recently demonstrated that antigen selection may have had a role in the development of ocular adnexal extranodal marginal zone B-cell lymphomas.[10] These data have been supported by recent studies of other investigators.[51]

Diffuse Large Cell B-Cell Lymphoma

A diffuse large cell B-cell lymphoma was diagnosed in the recurrent orbital tumor of the patient in Case 2. According to our most recent analysis of 212 cases of ocular adnexal lymphoid tumors[13] and others,[3,61] diffuse large cell B-cell lymphoma represents the second most common lymphoma subtype of B-NHL occurring in these locations.

The morphological, immunohistological, and molecular biological characteristics of diffuse large cell B-cell lymphoma are summarized in Table 3. Briefly, these tumors show a broad morphological spectrum but essentially are characterized by a diffuse proliferation of medium to large-sized atypical lymphoid cells with large nuclei, prominent sometimes multiple nucleoli and numerous atypical mitotic figures. Diffuse large cell B-cell lymphomas may evolve either de novo or secondarily during the course of a less aggressive lymphoma. Most commonly a secondary diffuse large cell B-cell lymphoma develops from follicular lymphoma;[30] however, they may also arise from B-cell chronic lymphocytic leukemia (Richter syndrome) and from extranodal marginal zone B-cell lymphomas, as demonstrated in Case 2. Such a high-grade malignant transformation of an extranodal marginal zone B-cell lymphoma to a diffuse large cell B-cell lymphoma has been described in extranodal marginal zone B-cell lymphoma in several locations,[71] including in the ocular adnexa in the recent literature.[4,35,51] Supporting the presumption of high-grade transformation in our case is the history of a stage I orbital extranodal marginal zone B-cell lymphoma (initially misinterpreted as immunocytoma), together with the presence of remnants of extranodal marginal zone B-cell lymphoma in the immediate vicinity of the diffuse large cell B-cell lymphoma. That we could not demonstrate a clonal amplificate in this case does not at all detract from the diagnosis of a malignant lymphoma. Both diffuse large cell B-cell lymphoma[45] and follicular lymphoma[86] are well known for their large number of somatic mutations in the variable region genes of the immunoglobulin molecule, which can prevent the docking of primer sets in PCR.

Follicular Lymphoma

The patient in Case 3 was diagnosed with follicular lymphoma, the third most common B-cell lymphoma occurring in the ocular adnexa.[3,13] It is interesting to note that in the studies investigating ocular adnexal lymphomas in Japan, only a few follicular lymphoma were described.[73] This discrepancy possibly reflects the low incidence of this tumor in Asia.[2,76]

The morphological, immunohistological, and molecular biological characteristics of follicular lymphoma are summarized in Table 3. Typically, these tumors consist predominantly of centrocytes with admixed centroblasts arranged in neoplastic follicles, which have lost the typical zonal arrangement of reactive B-cell follicles. There is an obvious decrease in apoptosis in the neoplastic germinal centers compared to reactive secondary follicles. An interfollicular growth is also observed almost in all tumors, but is seen particularly in grade 3 tumors, which consists of diffuse sheets of centroblasts. In addition to B-cell antigens, the tumor cells are usually positive for CD10, BCL-2, and BCL-6.[22]

Burkitt Lymphoma

Burkitt lymphoma is a high-grade B-cell lymphoma more commonly seen in children than in

adults; when occurring in adults, it is usually associated with immunodeficiency.[30] Three different clinical presentations can be distinguished:[30] 1) the endemic form of Burkitt lymphoma is seen in central Africa and New Guinea, and typically involves the mandible, maxilla, and orbital bones of children; 2) the non-endemic (sporadic) form of Burkitt lymphoma is present world wide and most often involves the abdomen, particularly the distal ileum, cecum as well as mesenteric lymph nodes. In contrast to the endemic form, it very rarely involves ocular tissues; and 3) the non-endemic form of Burkitt lymphoma associated with immunodeficiency most often affects adults who are HIV-positive, involving predominantly lymph nodes, but it can occur in extranodal sites such as the orbit[6] (Table 3).

Case 4 is very unusual in that it represents a non-endemic (sporadic) Burkitt lymphoma occurring in an immune-competent elderly woman, with the first clinical manifestation being diplopia due to orbital disease. The majority of sporadic Burkitt lymphoma involving ocular tissues have occurred in children;[14,81] extremely rare cases have been reported in immune competent adults.[41,80]

The morphological, immunohistological, molecular biological, and clinical characteristics of Burkitt lymphoma are summarized in Table 3. Morphologically, Burkitt lymphoma is composed of monomorphic medium-sized cells with basophilic cytoplasm and numerous mitotic figures. There are, however, morphological variants which deviate slightly from this classical form—a Burkitt lymphoma with plasmacytoid differentiation, and another with centroblasts and/or immunoblasts.[30] The plasmacytoid variant of Burkitt lymphoma is most often seen in non-endemic Burkitt lymphoma, as seen in our Case 4, whereas the latter variant is frequently seen in the non-endemic Burkitt lymphoma associated with immunodeficiency.

Genetically, most cases have a translocation of *c-myc* from chromosome 8 to the Ig heavy chain region on chromosome 14 (t(8;14)) or, less commonly, to light chain loci on 2 (t(2;8)) or 22 (t(8;22))[30] (Table 3). In endemic cases, the breakpoint on chromosome 14 involves the heavy chain joining region (early B-cell) whereas in non-endemic cases, the translocation involves the Ig switch region (later stage of B-cell). Epstein-Barr virus genomes can be demonstrated in the tumor cells in most African cases, and in 25–40% of the cases associated with HIV infection, but less frequently in non-endemic, non-immune deficient cases (Table 3). Epstein-Barr virus-encoded nuclear RNA was absent in the tumor cells of our patient.

Hodgkin's Lymphoma

Case 5 is a very rare case representing a primary manifestation of Hodgkin's lymphoma in the ocular adnexa. Hodgkin's lymphoma occurs extremely rarely in the ocular adnexa and is usually a secondary manifestation of advanced systemic disease.[31]

Our understanding of Hodgkin's lymphoma has progressed enormously in the last decade, with molecular biological examinations demonstrating that almost all cases of this disease are clonal B-cell neoplasms. Hodgkin's lymphoma can essentially be divided into four subtypes: nodular sclerosis, mixed cellularity, lymphocyte depletion, and lymphocyte predominant Hodgkin's lymphoma.[22] The first three forms display a mostly identical antigen profile and similar clinical characteristics; they are, therefore, grouped together in the WHO classifications under the heading of "classical Hodgkin's lymphoma."[30] Lymphocyte predominant Hodgkin's lymphoma differs in respect to morphology, immunophenotype, and clinical features from the other forms of Hodgkin's lymphoma and represents its own distinct entity. The morphological, immunohistological, and molecular biological characteristics of classical Hodgkin's lymphoma are summarized in Table 3. As seen in our case, classical Hodgkin's lymphoma is characterized by 1) the occurrence of Hodgkin- and Sternberg-Reed cells; 2) a dominant reactive cellular background consisting of small lymphocytes, eosinophils, neutrophils, histiocytes, plasma cells, fibroblasts, blood vessels, and collagen fibers in varying proportions; and 3) a particular immunophenotype of the tumor cells, namely CD30+, CD15+ (Figs. 8D and 8E) (Table 3).

TREATMENT OF OCULAR ADNEXAL LYMPHOMA

The initial step in the treatment of a patient, in whom the diagnosis of ocular adnexal lymphoma has been established, is referral to a hematologist/oncologist for "staging investigations" to determine the extent of the disease at the time of presentation or diagnosis. These investigations, which usually include full blood count, chest x-ray, thorax and abdominal computed tomography scans or magnetic resonance imaging, as well as bone marrow examination, determine the extent of disease infiltration. This is given in terms of a clinical stage usually according to the Ann-Arbor Classification,[7] or, in the case of Burkitt lymphoma, according to Murphy[60] and Magrath.[50] Approximately 60–80% of ocular adnexal B-cell lymphomas are limited in their extension at the time of diagnosis and represent stage IE—or primary ocular adnexal—disease.[3,12,75] Previous studies suggest that the development of systemic disease occurs usually within the first 6 months after presentation.[12,29,35,44,75] It may occur, however, many years after primary treatment and, therefore, it is recommended that systemic evaluation be repeated every 6 months at least for the first 5 years.[35,44]

In patients with stage IE ocular adnexal lymphomas, localized radiotherapy is the therapy of choice, [4,5,38,39,48,57,78] as can be seen in Cases 1–3. All tumors in these patients demonstrated an initial, and in Case 3, a lasting response. Pure surgical therapy is usually not recommended in stage IE ocular adnexal lymphomas, as tumor microinfiltration into surrounding tissue is usually present.[4] Adjuvant cryotherapy to the conjunctiva is performed to circumvent this problem in some centers.[75] In others, a wait-and-see policy for stage IE extranodal marginal zone lymphoma following excisional biopsy has been adopted.[61] Such a policy is, however, to be discouraged due to risks of developing either local recurrence or systemic disease, with the ever present risk of high-grade transformation of the tumor with more aggressive behavior (see Case 2).[4,35,51] Finally, systemic corticosteroids, which may produce an initial decrease in tumor size, have been proven to be ineffective for long-term control of ocular adnexal lymphomas.

The dosage of localized radiotherapy for ocular adnexal lymphomas remains controversial and varies between a total dose of 28 and 36 Gy for low-grade lymphomas (e.g., extranodal marginal zone B-cell lymphoma) and 30–40 Gy for medium to high-grade lymphomas (e.g., mantle cell lymphoma, diffuse large cell B-cell lymphoma)[4,13]. A total dosage less than 28 Gy has been reported to result in an increase in tumor recurrence rates,[57] whereas those greater than 36 Gy result only in an increase in side effects. These include xerophthalmia, corneal ulceration, cataract formation, and vasculopathy of the retina and the optic nerve.[16,23,36] Recent large studies analyzing the results of treatment with radiotherapy in patients with ocular adnexal lymphoid neoplasms report local control rates varying between 75–100%.[5,38,39,48,57,78] Some patients may require repeat radiation therapy to achieve total tumor eradication.[12] In the follow-up investigations, the contralateral ocular adnexa should be examined as this is a potential site of relapse in approximately 20% of patients.[52]

Should disseminated disease be demonstrated in ocular adnexal lymphoma, such as in Cases 2, 4, and 5, systemic chemotherapy is required. Most regimens are based upon the CHOP scheme, although as seen in Case 4, COMP was additionally administered. Combined radiotherapy and chemotherapy is used in rare cases in which the tumors are bulky or vision-threatening. In general, however, the combination is avoided due to the potentiation of radiotoxicity.

PROGNOSIS

Various parameters have been described to have an influence on the clinical course of the patients with ocular adnexal lymphoma (Table 4). In such analyses, three endpoints are of clinical relevance: the development of local tumor recurrence; the development of systemic disease, and death related to lymphoma. The first two endpoints pertain to patients with primary ocular adnexal disease, whereas the latter takes all patients with ocular adnexal lymphomas (i.e., also including those with secondary manifestations of a systemic lymphoma) into account.

TABLE 4

Major documented Prognostic Factors in Ocular Adnexal Lymphoma

Factor
Anatomic location
Stage at diagnosis
B-cell subtype (REAL Classification)
Immunophenotype: Ki-67 growth fraction; p53*
Serum lactate dehydrogenase

*Weaker association.

The majority of published studies have concentrated on the second endpoint in the establishment of prognostic parameters. Confusingly, in some investigations both patient groups (i.e., those with primary and secondary ocular adnexal disease) have been included in the evaluation. Consequently, the percentage of patients with ocular adnexal lymphoma who develop disseminated disease varies considerably in the literature, ranging from 17–68%.[12,37,44,61,75,82] When considering primary ocular adnexal disease only, however, the dissemination rate is approximately 30%.[44]

The anatomic location of an adnexal lymphoma correlated with the risk of disseminated disease in some investigations.[32,44] Knowles and Jakobiec found approximately 35%, 20%, and 67% of patients with ocular adnexal lymphoid tumors of the orbit, conjunctiva, and eyelids, respectively, had prior, concurrent, or subsequent extraocular lymphoma.[32,44] These authors, therefore, concluded that conjunctival ocular adnexal lymphomas had the better prognosis in this respect. This was supported by some investigators,[3,37,77,82] but not by others.[12,15,55] Shields et al have recently demonstrated in an analysis of 117 conjunctival lymphoid tumors that disseminated disease occurred more frequently in patients with lymphomas located at an extralimbal site (fornix or midbulbar conjunctiva), and in those with multiple tumors.[75]

Prognosis also relates to the clinical stage at the time of presentation and diagnosis. The majority of patients with ocular adnexal lymphoma with unilateral or bilateral clinical stage I disease respond well to therapy, demonstrate a benign indolent clinical

course, and fail to develop extraocular adnexal lymphoma.[3,12,44] Approximately one-quarter of patients with ocular adnexal lymphomas, however, have prior or concurrent extraocular lymphoma, that is, stage II–IV disease. These patients display a worse survival rate than stage I ocular adnexal lymphoma patients.[3,12,44]

The histopathological subdivision of the ocular adnexal lymphomas using the REAL Classification[22] appears useful in predicting stage of disease at diagnosis, persistence of disease at final examination as well as lymphoma-related death.[12,61] Most (but not all) patients with extranodal marginal zone B-cell lymphoma had stage I disease, which usually demonstrated a less aggressive clinical course compared to the high-grade malignant B-cell lymphomas, such as mantle cell lymphoma or diffuse large cell B-cell lymphoma.[12,35,61] The risk of the development of systemic disease is less (but certainly not absent) in ocular adnexal extranodal marginal zone B-cell lymphoma than in non-extranodal marginal zone B-cell lymphoma tumors, varying between 32% and 54% for extranodal marginal zone B-cell lymphoma[3,12,35,37] and 66–68% for non-extranodal marginal zone B-cell lymphoma[3,35] at a follow-up longer than 3 years. Furthermore, the estimated hazard ratios for lymphoma related death increased significantly in diffuse large cell B-cell lymphoma when compared to extranodal marginal zone B-cell lymphoma.[12,35]

Immunohistological characteristics of the ocular adnexal lymphomas of prognostic value include the MIB-1 growth fraction and, less convincingly, tumor cell positivity for p53.[3,12] In an analysis of 112 ocular adnexal lymphoid tumors with 99 malignant lymphomas, tumors with large growth fractions (>20%) correlated significantly with stage of disease at presentation, stage of disease at final follow-up, and the occurrence of lymphoma-related death ($p < 0.001$).[12] The majority of the extranodal marginal zone B-cell lymphoma had growth fractions less than 20% with a corresponding low disease stage at presentation. Forty-one of the 99 lymphoma cases, however, had a growth fraction greater than 20%, and 31 of these 41 patients (75%) had disseminated disease at the time of diagnosis.[12] Most of these cases were represented by diffuse large cell B-cell lymphoma and plasmocytoma; however, 15 cases were diagnosed as extranodal marginal zone lymphoma.[12] These results would support the suggestion of Medeiros and Harris[54] that growth fractions greater than 20% in usually low-grade malignant tumors may be predictors of disseminated disease.

Recently, Nakata et al analyzed the survival data of 57 patients with ocular adnexal lymphomas with a follow-up period of 5 years.[61] They found an association between abnormal serum lactate dehydrogenase values and poor survival in ocular adnexal lymphoma on both univariate and multivariate analysis.[61] Raised serum lactate dehydrogenase values, which are included in the International Prognostic Index used to predict the outcome of patients with aggressive lymphomas,[1] would tend to suggest systemic disease, which is only seen in the minority of patients with ocular adnexal lymphomas at first diagnosis.

Summary

Lymphoproliferative disease of the ocular adnexa includes a spectrum of lesions that can present with similar clinical and radiological features. They represented considerable diagnostic dilemmas for both the clinician and pathologist in the past; however, by employing morphological, immunohistochemical, and molecular biological criteria, the ocular adnexal lymphoid lesions can be better distinguished today into reactive lymphoid hyperplasia, atypical lymphoid hyperplasia, and malignant lymphoma. The term "pseudolymphoma" for such lesions should be avoided. The REAL and WHO Lymphoma Classifications are the most suitable for subdividing the ocular adnexal lymphomas, whereby the extranodal marginal zone B-cell lymphoma represents the most common lymphoma subtype in these locations. Essential for the management of patients with ocular adnexal lymphomas is a thorough systemic medical examination for systemic lymphoma to establish the clinical stage of the disease. The therapy of choice in Stage I tumors is radiotherapy, while disseminated disease is treated with chemotherapy. Despite the indolent course of extranodal marginal zone B-cell lymphoma, they are renowned for recurrence in extranodal sites, including the lung, salivary glands, as well as other ocular adnexal sites. Therefore, long-term follow-up with 6-month examinations is recommended. Major prognostic criteria for the ocular adnexal lymphomas include anatomic location of the tumor; stage of disease at first presentation; lymphoma subtype as determined using the REAL classification; immunohistochemical markers determining factors such as tumor growth rate; and the serum lactate dehydrogenase level.

Method of Literature Search

Literature selection for this article was based on a Medline search spanning the period 1966 to September 2001 for all articles using the following keywords, singularly and in various combinations: *ocular adnexa, orbit, conjunctiva, lymphoma, lymphoproliferative lesion, eye, extranodal marginal zone B-cell lymphoma, "mucosa-associated lymphatic tissue" lymphoma, Burkitt lymphoma, Hodgkin's disease, treatment, prognosis, REAL Classification.* The German, French, and Japanese ar-

ticles were translated. English abstracts were used for other non-English articles.

References

1. ___: A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. N Engl J Med 329:987–94, 1993
2. ___: Statistical analyses of clinico-pathological, virological and epidemiological data on lymphoid malignancies with special reference to adult T-cell leukemia/lymphoma: a report of the second nationwide study of Japan. The T- and B-Cell Malignancy Study Group. Jpn J Clin Oncol 15:517–35, 1985
3. Auw-Haedrich C, Coupland SE, Kapp A, et al: Long term outcome of ocular adnexal lymphoma subtyped according to the REAL classification. Revised European and American Lymphoma. Br J Ophthalmol 85:63–9, 2001
4. Baldini L, Blini M, Guffanti A, et al: Treatment and prognosis in a series of primary extranodal lymphomas of the ocular adnexa. Ann Oncol 9:779–81, 1998
5. Bessell EM, Henk JM, Wright JE, Whitelocke RA: Orbital and conjunctival lymphoma treatment and prognosis. Radiother Oncol 13:237–44, 1988
6. Brooks HL Jr, Downing J, McClure JA, Engel HM: Orbital Burkitt's lymphoma in a homosexual man with acquired immune deficiency. Arch Ophthalmol 102:1533–7, 1984
7. Carbone PP, Kaplan HS, Musshoff K, et al: Report of the Committee on Hodgkin's Disease Staging Classification. Cancer Res 31:1860–1, 1971
8. Cordell JL, Falini B, Erber WN, et al: Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). J Histochem Cytochem 32:219–29, 1984
9. Coupland SE, Foss HD, Hidayat AA, et al: Extranodal marginal zone B-cell lymphomas of the uvea: an analysis of 13 cases. J Pathol 197:333–40, 2002
10. Coupland SE, Foss HD, Anagnostopoulos I, et al: Immunoglobulin VH gene expression among extranodal marginal zone B-cell lymphomas of the ocular adnexa. Invest Ophthalmol Vis Sci 40:555–62, 1999
11. Coupland SE, Foss HD, Assaf C, et al: T-cell and T/natural killer-cell lymphomas involving ocular and ocular adnexal tissues: a clinicopathologic, immunohistochemical, and molecular study of seven cases. Ophthalmology 106:2109–20, 1999
12. Coupland SE, Krause L, Delecluse HJ, et al: Lymphoproliferative lesions of the ocular adnexa. Analysis of 112 cases. Ophthalmology 105:1430–41, 1998
13. Coupland SE, Wiegel T, Auw-Haedrich C, et al: Lymphoproliferative Läsionen der okulären Adnexe und intraokuläre Lymphome. Der Onkologe 5:792ã804, 1999
14. Edelstein C, Shields JA, Shields CL, et al: Non-African Burkitt lymphoma presenting with oral thrush and an orbital mass in a child. Am J Ophthalmol 124:859–61, 1997
15. Ellis JH, Banks PM, Campbell RJ, Liesegang TJ: Lymphoid tumors of the ocular adnexa. Clinical correlation with the working formulation classification and immunoperoxidase staining of paraffin sections. Ophthalmology 92:1311–24, 1985
16. Esik O, Ikeda H, Mukai K, Kaneko A: A retrospective analysis of different modalities for treatment of primary orbital non-Hodgkin's lymphomas. Radiother Oncol 38:13–8, 1996
17. Flanders AE, Espinosa GA, Markiewicz DA, Howell DD: Orbital lymphoma. Role of CT and MRI. Radiol Clin North Am 25:601–13, 1987
18. Fratkin JD, Shammas HF, Miller SD: Disseminated Hodgkin's disease with bilateral orbital involvement. Arch Ophthalmol 96:102–4, 1978
19. Hardman Lea S, Kerr Muir M, Wotherspoon AC, et al: Mucosal-associated lymphoid tissue lymphoma of the conjunctiva. Arch Ophthalmol 112:1207–12, 1994
20. Harris NL: Extranodal lymphoid infiltrates and mucosa-associated lymphoid tissue (MALT): a unifying concept. Am J Surg Pathol 15:879–84, 1991
21. Harris NL: Low-grade B-cell lymphoma of mucosa-associated lymphoid tissue and monocytoid B-cell lymphoma. Related entities that are distinct from other low-grade B-cell lymphomas. Arch Pathol Lab Med 117:771–5, 1993
22. Harris NL, Jaffe ES, Stein H, et al: A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group [see comments]. Blood 84:1361–92, 1994
23. Hempel M, Hinkelbein W: Eye sequelae following external irradiation. Recent Results Cancer Res 130:231–6, 1993
24. Henderson JW, Banks PM, Yeatts RP: T-cell lymphoma of the orbit. Mayo Clin Proc 64:940–4, 1989
25. Hornblass A, Jakobiec FA, Reifler DM, Mines J: Orbital lymphoid tumors located predominantly within extraocular muscles. Ophthalmology 94:688–97, 1987
26. Inagaki H, Okabe M, Seto M, et al: API2-MALT1 fusion transcripts involved in mucosa-associated lymphoid tissue lymphoma: multiplex RT-PCR detection using formalin-fixed paraffin-embedded specimens. Am J Pathol 158:699–706, 2001
27. Isaacson P, Wright DH: Malignant lymphoma of mucosa-associated lymphoid tissue: a distinctive type of B-cell lymphoma. Cancer 52:1410–6, 1983
28. Isaacson PG: Mucosa-associated lymphoid tissue lymphoma. Semin Hematol 36:139–47, 1999
29. Isaacson PG, Norton AJ: Extranodal Lymphomas. Edinburgh: Churchill Livingstone, 1994
30. Jaffe ES, Harris NL, Stein H, Vardiman JW: World Health Organization Classification of Tumours. Tumours of Haematopoietic and Lymphoid Tissues. Pathology and Genetics. Lyon: IARC Press, 2001
31. Jakobiec FA: Orbital Hodgkin's disease: clinicopathologic conference. N Eng J Med 320:447–57, 1989
32. Jakobiec FA, Knowles DM: An overview of ocular adnexal lymphoid tumors. Trans Am Ophthalmol Soc 87:420–42; discussion 442–4, 1989
33. Jakobiec FA, Lefkowitch J, Knowles DM: B- and T-lymphocytes in ocular disease. Ophthalmology 91:635–54, 1984
34. Jakobiec FA, Neri A, Knowles DM: Genotypic monoclonality in immunophenotypically polyclonal orbital lymphoid tumors. A model of tumor progression in the lymphoid system. The 1986 Wendell Hughes lecture.Ophthalmology 94:980–94, 1987
35. Jenkins C, Rose GE, Bunce C, et al: Histological features of ocular adnexal lymphoma (REAL classification) and their association with patient morbidity and survival. Br J Ophthalmol 84:907–13, 2000
36. Jereb B, Lee H, Jakobiec FA, Kutcher J: Radiation therapy of conjunctival and orbital lymphoid tumors. Int J Radiat Oncol Biol Phys 10:1013–9, 1984
37. Johnson TE, Tse DT, Byrne GE Jr, et al: Ocular-adnexal lymphoid tumors: a clinicopathologic and molecular genetic study of 77 patients. Ophthal Plast Reconstr Surg 15:171–9, 1999
38. Keleti D, Flickinger JC, Hobson SR, Mittal BB: Radiotherapy of lymphoproliferative diseases of the orbit. Surveillance of 65 cases. Am J Clin Oncol 15:422–7, 1992
39. Kennerdell JS, Flores NE, Hartsock RJ: Low-dose radiotherapy for lymphoid lesions of the orbit and ocular adnexa. Ophthal Plast Reconstr Surg 15:129–33, 1999
40. Kirsch LS, Brownstein S, Codere F: Immunoblastic T-cell lymphoma presenting as an eyelid tumor. Ophthalmology 97:1352–7, 1990
41. Knieper P: Lymphoblastic lymphoma of the orbit. Orbit 1: 235–40, 1982
42. Knowles DM: The extranodal lymphoid infiltrate: a diagnostic dilemma. Semin Diagn Pathol 2:147–51, 1985
43. Knowles DM, Chamulak G, Subar M, et al: Clinicopathologic, immunophenotypic, and molecular genetic analysis of AIDS-associated lymphoid neoplasia. Clinical and biologic implications. Pathol Annu 23(Pt 2):33–67, 1988
44. Knowles DM, Jakobiec FA, McNally L, Burke JS: Lymphoid hyperplasia and malignant lymphoma occurring in the ocular adnexa (orbit, conjunctiva, and eyelids): a prospective multiparametric analysis of 108 cases during 1977 to 1987. Hum Pathol 21:959–73, 1990
45. Kuppers R, Rajewsky K, Hansmann ML: Diffuse large cell lymphomas are derived from mature B cells carrying V region genes with a high load of somatic mutation and evi-

dence of selection for antibody expression. Eur J Immunol 27:1398–405, 1997
46. Laroche L, Laroche L, Pavlakis E, Saraux H: Immunological characterization of an ocular adnexal lymphoid T tumor by monoclonal antibodies. Ophthalmologica 187:43–9, 1983
47. Leidenix MJ, Mamalis N, Olson RJ, et al: Primary T-cell immunoblastic lymphoma of the orbit in a pediatric patient. Ophthalmology 100:998–1002, 1993
48. Letschert JG, Gonzalez D, Oskam J, et al: Results of radiotherapy in patients with stage I orbital non-Hodgkin's lymphoma. Radiother Oncol 22:36–44, 1991
49. Liu H, Ruskon-Fourmestraux A, Lavergne-Slove A, et al: Resistance of t(11;18) positive gastric mucosa-associated lymphoid tissue lymphoma to Helicobacter pylori eradication therapy. Lancet 357:39–40, 2001
50. Magrath IT, Janus C, Edwards BK, et al: An effective therapy for both undifferentiated (including Burkitt's) lymphomas and lymphoblastic lymphomas in children and young adults. Blood 63:1102–11, 1984
51. Mannami T, Yoshino T, Oshima K, et al: Clinical, histopathological, and immunogenetic analysis of ocular adnexal lymphoproliferative disorders: characterization of malt lymphoma and reactive lymphoid hyperplasia. Mod Pathol 14:641–9, 2001
52. McNally L, Jakobiec FA, Knowles DMD: Clinical, morphologic, immunophenotypic, and molecular genetic analysis of bilateral ocular adnexal lymphoid neoplasms in 17 patients. Am J Ophthalmol 103:555–68, 1987
53. Medeiros LJ, Harmon DC, Linggood RM, Harris NL: Immunohistologic features predict clinical behavior of orbital and conjunctival lymphoid infiltrates. Blood 74:2121–9, 1989
54. Medeiros LJ, Harris NL: Immunohistologic analysis of small lymphocytic infiltrates of the orbit and conjunctiva. Hum Pathol 21:1126–31, 1990
55. Medeiros LJ, Harris NL: Lymphoid infiltrates of the orbit and conjunctiva. A morphologic and immunophenotypic study of 99 cases. Am J Surg Pathol 13:459–71, 1989
56. Meekins B, Proia AD, Klintworth GK: Cutaneous T-cell lymphoma presenting as a rapidly enlarging ocular adnexal tumor. Ophthalmology 92:1288–93, 1985
57. Minehan KJ, Martenson JA Jr, Garrity JA, et al: Local control and complications after radiation therapy for primary orbital lymphoma: a case for low-dose treatment. Int J Radiat Oncol Biol Phys 20:791–6, 1991
58. Morgan G: Lymphocytic tumors of the orbit. Modern Problems in Ophthalmology, Vol 14. Basel: Karger, 1975, pp 35–8
59. Motegi M, Yonezumi M, Suzuki H, et al: API2-MALT1 chimeric transcripts involved in mucosa-associated lymphoid tissue type lymphoma predict heterogeneous products. Am J Pathol 156:807–12, 2000
60. Murphy SB, Hustu HO: A randomized trial of combined modality therapy of childhood non-Hodgkin's lymphoma. Cancer 45:630–7, 1980
61. Nakata M, Matsuno Y, Katsumata N, et al: Histology according to the Revised European-American Lymphoma Classification significantly predicts the prognosis of ocular adnexal lymphoma. Leuk Lymphoma 32:533–43, 1999
62. Neri A, Jakobiec FA, Pelicci PG, et al: Immunoglobulin and T cell receptor beta chain gene rearrangement analysis of ocular adnexal lymphoid neoplasms: clinical and biologic implications. Blood 70:1519–29, 1987
63. Norton AJ, Jordan S, Yeomans P: Brief, high-temperature heat denaturation (pressure cooking): a simple and effective method of antigen retrieval for routinely processed tissues. J Pathol 173:371–9, 1994
64. Okuda K, Matsuo N, Matsuo H, Nakano K: [A case of orbital lymphoma (Hodgkin's paragranuloma)]. Nippon Ganka Kiyo 18:504–10, 1967
65. Pangalis GA, Nathwani BN, Rappaport H: Malignant lymphoma, well differentiated lymphocytic: its relationship with chronic lymphocytic leukemia and macroglobulinemia of Waldenstrom. Cancer 39:999–1010, 1977
66. Park KL, Goins KM: Hodgkin's lymphoma of the orbit associated with acquired immunodeficiency syndrome [letter]. Am J Ophthalmol 116:111–2, 1993
67. Patel S, Rootman J: Nodular sclerosing Hodgkin's disease of the orbit. Ophthalmology 90:1433–6, 1983.
68. Polito E, Leccisotti A, Galieni P: Clinical and radiological presentation of 95 orbital lymphoid tumors. Graefe Arch Clin Exp Ophthalmol 234:504–9, 1996
69. Project N-HsLPC: National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas: summary and description of a working formulation for clinical usage. Cancer 49:2112–35, 1982
70. Reifler DM, Warzynski MJ, Blount WR, et al: Orbital lymphoma associated with acquired immune deficiency syndrome (AIDS). Surv Ophthalmol 38:371–80, 1994
71. Salhany KE, Pietra GG: Extranodal lymphoid disorders. Am J Clin Pathol 99:472–85, 1993
72. Saltzstein S: Extranodal malignant lymphomas and pseudolymphomas. Path Ann 4:159–84, 1969
73. Sasai K, Yamabe H, Dodo Y, et al: Non-Hodgkin's lymphoma of the ocular adnexa. Acta Oncol 40:485–90, 2001
74. Sherman MD, Van Dalen JT, Conrad K: Bilateral orbital infiltration as the initial sign of a peripheral T-cell lymphoma presenting in a leukemic phase. Ann Ophthalmol 22:93–5, 1990
75. Shields CL, Shields JA, Carvalho C, et al: Conjunctival lymphoid tumors: clinical analysis of 117 cases and relationship to systemic lymphoma. Ophthalmology 108:979–84, 2001
76. Shih LY, Liang DC: Non-Hodgkin's lymphomas in Asia. Hematol Oncol Clin North Am 5:983–1001, 1991
77. Sigelman J, Jakobiec FA: Lymphoid lesions of the conjunctiva: relation of histopathology to clinical outcome. Ophthalmology 85:818–43, 1978
78. Smitt MC, Donaldson SS: Radiotherapy is successful treatment for orbital lymphoma. Int J Radiat Oncol Biol Phys 26:59–66, 1993
79. Tomlinson IM, Cook GP, Walter G, et al: A complete map of the human immunoglobulin VH locus. Ann NY Acad Sci 764:43–6, 1995
80. Wegner A, Schmidt T, Fellbaum C: [Primary manifestation of Burkitt's lymphoma of the non-African type in the orbits]. Klin Monatsbl Augenheilkd 203:128–31, 1993
81. Weisenthal RW, Streeten BW, Dubansky AS, et al: Burkitt lymphoma presenting as a conjunctival mass. Ophthalmology 102:129–34, 1995
82. White WA, Ferry JA, Harris NL, Grove AS: Ocular adnexal lymphoma. A clinicopathologic study with identification of lymphomas of mucosa-associated lymphoid tissue type. Ophthalmology 102:1994–2006, 1995
83. Wotherspoon AC, Diss TC, Pan LX, et al: Primary low-grade B-cell lymphoma of the conjunctiva: a mucosa-associated lymphoid tissue type lymphoma. Histopathology 23:417–24, 1993
84. Wotherspoon AC, Doglioni C, Diss TC, et al: Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of Helicobacter pylori. Lancet 342:575–7, 1993
85. Wotherspoon AC, Ortiz Hidalgo C, Falzon MR, Isaacson PG: Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. Lancet 338:1175–6, 1991
86. Wu Hy, Kaartinen M: The somatic hypermutation activity of a follicular lymphoma links to large insertions and deletions of immunoglobulin genes. Scand J Immunol 42:52–9, 1995
87. Yeo JH, Jakobiec FA, Abbott GF, Trokel SL: Combined clinical and computed tomographic diagnosis of orbital lymphoid tumors. Am J Ophthalmol 94:235–45, 1982.

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