Lymphoproliferative Lesions of the Ocular Adnexa

Analysis of 112 Cases

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Objective: Lymphoproliferative lesions of the ocular adnexa were analyzed to examine (1) the suitability of the Revised European–American Lymphoma (REAL) classification for the subtyping of the lymphomas in these sites; (2) the predictive value of the REAL classification for the evolution of these tumors; and (3) the frequency and prognostic impact of tumor type, location, proliferation rate (Ki-67 index), p53, and CD5 positivity and the presence of monoclonality within these tumors.

Design: Retrospective review.

Methods: The clinical, histomorphologic, immunohistochemical, and molecular biologic (polymerase chain reaction [PCR]) features of lymphoid proliferations of the ocular adnexa were studied.

Study Materials: The ocular adnexal lymphoproliferative lesions were located as follows: orbit in 52 patients (46%), conjunctiva in 32 patients (29%), eyelid in 23 patients (21%), and caruncle in 5 patients (4%).

Results: Reactive lymphoid hyperplasia was diagnosed in 12 cases and lymphoma in 99 cases; 1 case remained indeterminate. The five main subtypes of lymphoma according to the REAL classification were extranodal marginal-zone B-cell lymphoma (64%), follicle center lymphoma (10%), diffuse large cell B-cell lymphoma (9%), plasmacytoma (6%), and lymphoplasmocytic lymphoma (5%). Age, gender, and anatomic localization of the lymphomas did not have prognostic significance during a follow-up period of 6 months to 16.5 years (mean, 3.3 years). Extent of disease at time of presentation was the most important clinical prognostic factor: advanced disease correlated with increased risk ratios of having persistent disease at the final follow-up and with lymphoma-related death ($P < 0.001$). Histomorphologic features and immunohistochemical markers positively correlating with disseminated disease at presentation, stage at final follow-up, and occurrence of lymphoma-related death included cytologic atypia ($P < 0.001$), MIB-1 proliferation rate ($P < 0.001$), and tumor cell p53 positivity ($P < 0.001$). The MIB-1 proliferation rates greater than 20% in extranodal marginal-zone B-cell lymphoma corresponded to at least stage II lymphoma ($P < 0.05$).

Conclusion: The REAL classification is suitable for the subdivision of the ocular adnexal lymphomas. The MIB-1 proliferation rate and p53 positivity may aid the prediction of disease stage and disease progression, whereas PCR can support the diagnosis and reduce the number of histologically indeterminate lesions.
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Lymphomas of the ocular adnexa (i.e., the conjunctiva, orbit, and lacrimal gland) represent approximately 8% of all extranodal lymphomas.[1] They form one end of the spectrum of lymphoproliferative lesions that are seen at these sites; the other end of the spectrum is represented by reactive lymphoid hyperplasia (RLH), also previously termed benign lymphoma,[2] pseudolymphoma,[3–5] and inflammatory pseudotumor.[6] The majority of lymphomas of the ocular adnexa are composed of small lymphocytic cells with a relatively bland cytologic appearance and are, therefore, not easily distinguished from their benign counterparts on conventional histology. To further compound diagnostic difficulties, a "gray zone" lies between the two poles of the spectrum and consists of a group of borderline cases in which the diagnosis of the lesion cannot be determined with any certainty using conventional histologic techniques. Lesions allocated to this group consist of diffuse or follicular lymphoid proliferating cells that either manifest borderline maturity or contain a subpopulation of atypical cells with large hy-

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perchromatic nuclei. On immunohistochemical staining, these cells are polyclonal for the immunoglobulin (Ig) light or heavy chains.[7] Such specimens have been termed atypical lymphoid hyperplasia,[3,7,8] lymphocytic infiltrates of indeterminate nature,[2,9] or histologically indeterminate[10–14] and have been reported to represent between 3% and 12% of the lymphoproliferative lesions in the ocular adnexa.[8,11] A variable proportion of these lymphoproliferative lesions develop ultimately into systemic lymphoma.[3,15,16]

The advent of immunohistochemistry and of molecular biologic techniques, such as the polymerase chain reaction (PCR), has allowed a better understanding and definition of lymphoproliferative lesions in these sites. Despite this, a number of contentious issues remain. One of these is the classification of the lymphomas of the ocular adnexa, subdivided into eight to ten different subtypes, with up to five different types of borderline lesions.[11,17] The prognostic value of subdividing the lymphoproliferative lesions of the ocular adnexa into benign and malignant and polyclonal and monoclonal also has been questioned by some authors.[8,18] Similarly, the relationship between prognosis and anatomic localization of the lymphomas has been controversial. Some groups suggested that lymphomas of the orbit and lid have worse prognoses than those of the conjunctiva,[8,19–22] but this could not be verified by other researchers.[11,12,23] Finally, reports on the frequency of the expression of CD5 in lymphomas of the ocular adnexa have varied considerably.[8,11,18,22,24] In light of recent results,[25] this may be of use in predicting disease progression.

A well-characterized and large series of patients was included in the current study to: (1) summarize the immunohistochemical profiles of the lymphomas of the ocular adnexa to aid their recognition from their benign counterparts; (2) examine the suitability of the Revised European–American Lymphoma (REAL) classification[26] for the subtyping of the lymphomas of the ocular adnexa; (3) test the REAL classification in predicting the evolution of these tumors; and (4) determine the frequency and prognostic impact of tumor type, location, proliferation rate (Ki-67 index), p53, CD5 positivity, as well as of monoclonality within these tumors.

Methods

Tissue Samples

One hundred twelve cases of lymphoid proliferation of the ocular adnexa in 112 patients were collected from the consultation files of the Pathology Departments of Universitätsklinikum Benjamin Franklin, Berlin, Germany, and of Western Infirmary, Glasgow, Scotland. Patients with inflammatory pseudotumor or lymphoid proliferations of the choroid, vitreous, or retina were excluded. The majority of the tissue biopsy specimens had been fixed in 10% formaldehyde solution and embedded in paraffin; some biopsy specimens had been fixed in 2.5% buffered glutaraldehyde. Conventional histologic stains included hematoxylin and eosin, Giemsa, and periodic acid-Schiff. The histologic and immunohistochemical pattern of each lesion was reviewed by a panel of three observers (SEC, HJD, and HS) without knowledge of the previous diagnosis made nor of the clinical outcome. The cases were subdivided into RLH, lymphoma, and histologically indeterminate. The lymphomas were further classified on the bases of morphologic features and immunophenotype according to the REAL classification. In all cases, the presence of germinal centers, cytologic atypia, Dutcher bodies, and tumor infiltration of extraocular muscle, the lacrimal gland, or blood vessels was assessed systematically.

Immunohistology

Additional slides of all cases were stained for immunohistochemical studies using several monoclonal and polyclonal antibodies that are reactive in paraffin sections. An antigen-retrieval method using a pressure cooker was performed before immunohistochemical staining.[27] The staining consisted of a first-stage incubation with the following primary monoclonal antibodies: LC (CD45); L26 (CD20); 54-F6 (CD5); 1F6 (CD4); C8-144 (CD8); 1F8 (CD21); MIB-1 (antigen Ki-67); BCL-2 (clone 124); VS38c, Cs1-4 (LMP-1); JCB117 (CD79a); BerH2 (CD30); cyclin D1 (clone P2D11F11); Bu38 (CD23); DFT1 (CD43); p53 (clone DO7); and the pan-cytokeratin marker MNF-116. Polyclonal antibodies were used to test the expression of CD3 antigen and of the immunoglobulin chains κ, λ, μ, γ, δ, and α.

The antibodies were made visible with an indirect immunoperoxidase method for the antibodies to the heavy and light chains as well as for p53, whereas the alkaline phosphatase antialkaline phosphatase method was used to show the binding of the remaining antibodies.[28] All antibodies were obtained from DAKO (Glostrup, Denmark) or Novocastra (Newcastle-upon-Tyne, England), except for MIB-1, JCB 117, and CD5, which were kindly provided by Dr. J. Gerdes (Borstel, Germany), Dr. D. Mason (Oxford, England), and Dr. K. Gatter (Oxford, England), respectively. In those cases in which frozen material also was available, staining for CD10 was performed. The number of MIB-1 and p53-positive cells was determined by counting the number of cells with clear nuclear positivity for these markers per 5×100 tumor cells using the $40\times$ objective.

Polymerase Chain Reaction Amplification Method

The DNA was extracted from 20-μm-thick paraffin sections of all cases with an automated DNA extractor (Applied Biosystems 341A, Welterstadt, Germany) according to manufacturer recommendations. A nested PCR was performed to determine the clonality of the cases. Five hundred nanograms of genomic DNA was amplified using high-performance liquid chromatography-purified consensus oligonucleotides specific to the variable (FW1; set of 6 family-specific FW1 primers; 50 ng/reaction) and joining (LJH; 100 ng/reaction) Ig heavy chain segments[29] in 100 ml of reaction mixture (62.5 mmol/l potassium chloride, 12.5 mmol/l TRIS–hydrochloric acid, 2 mmol/l magnesium chloride, 0.8 mmol deoxynucleotriphosphate, 2 U of Amplitaq polymerase; Perkin–Elmer Cetus, Norwalk, CT). The PCR conditions included five initial stringent cycles (96° C for 15 seconds, 63° C for 30 seconds with a ramping time of 45 seconds, and 72° C for 30 seconds) followed by 40 cycles with a lower annealing temperature (96° C for 15 seconds, 57° C for 30 seconds with a ramping time of 45 seconds, and 72° C for 30 seconds) in a GeneAmp PCR system 9600A (Perkin–Elmer Cetus). One microliter of the amplified product was reamplified using a set of six family-specific FW2 primers (50 ng each per reaction) and an oligonucleotide internal to LJH (VLJH; 200 ng per reaction) in the same buffer conditions, with the exception of the magnesium chloride concentration (1.5

mmol/l). For the reamplification, 25 cycles of PCR (96° C for 15 seconds, 63° C for 30 seconds with a ramping time of 45 seconds, and 72° C for 30 seconds) were performed. Controls used were polyclonal (reactive tonsil), monoclonal (Raji B-cell line), and a negative control (no-template DNA). Products were analyzed on 6% polyacrylamide gel stained with ethidium bromide and viewed under ultraviolet light. A discrete band (220–270 base-pairs long) in the electrophoresed PCR-amplified material indicated monoclonality. Monoclonality showed either immunohistochemically for surface or cytoplasmic immunoglobulin light and/or heavy chains or, using PCR, showing immunoglobulin gene rearrangement was considered to define the lesion as a lymphoma when consistent with the histomorphologic findings.

Medical History

The clinical information gathered included patient age, gender, medical history, presenting symptoms, principal clinical findings, precise anatomic location of the lesion, results of laboratory and radiologic studies, stage of disease at diagnosis (Ann Arbor), type and extent of therapy, course of disease, disease-free period, duration of survival, and stage of disease at final follow-up. Patients who had only bilateral ocular adnexal disease were considered to have stage IE disease. The anatomic localization of the lesions was defined as proposed by Knowles et al.[8]

Statistical Analysis

Actuarial survival curves were plotted using the Kaplan–Meier method.[30] Statistical significance was calculated on the 111 defined cases using the log–rank test[31] for invariate analysis. The Cox regression model was used for the calculation of the hazards ratio and its confidence interval.[32] No evaluation was performed solely on the individual lymphoma subgroups because each series was too small. Consequently, the lymphomas were grouped into (1) high- and low-grade malignancy[26] and (2) extranodal marginal-zone B-cell lymphoma and other lymphomas for the evaluation of the significance of MIB-1 proliferation rate and positivity for p53.

Results

Clinical Features and Anatomic Distribution

The patient group consisted of 79 females and 32 males in total with an age range of 14 to 91 years; median, 61 years (Fig 1). There was no significant age difference between the patients diagnosed with RLH and those with lymphoma (median age, 59 and 61 years, respectively) (Fig 1). None of the patients were suffering from Sjögren's syndrome, Wegener's granulomatosis, or Mikulicz syndrome.

The ocular adnexal lymphoid proliferations were distributed as follows: orbit in 52 (46%) with involvement of the lacrimal gland in 9% of these cases; conjunctiva in 32 (29%); eyelid in 23 (21%); and caruncle in 5 (4%). The chief presenting signs and symptoms included a palpable mass or swelling (65%), proptosis (34%), conjunctival redness and irritation (42%), diplopia (26%), upper lid ptosis (8%), pain (5%), and epiphora (3%). There were few symptoms, clinical or radiologic signs that distinguished RLH from lymphoma; bilateral lesions as well as pain with associated bone erosion on radiologic examination were observed only in patients with lymphoma.

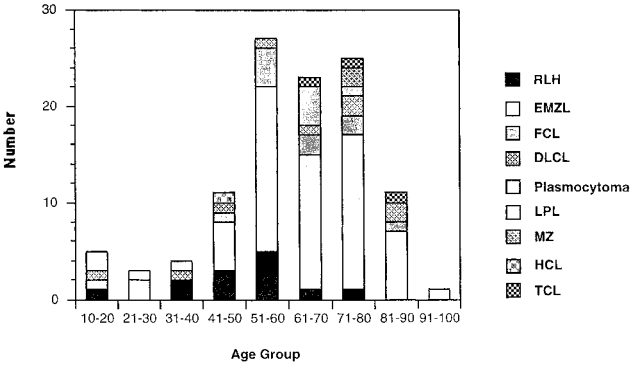

Figure 1. Age distribution of patients with lymphoproliferative lesions of the ocular adnexa. RLH = reactive lymphoid hyperplasia; EMZL = extranodal marginal-zone B-cell lymphoma; FCL = follicle center lymphoma; DLCL = diffuse large cell B-cell lymphoma; LPL = lymphoplasmocytic lymphoma; MZ = mantle zone lymphoma; HCL = hairy cell leukemia; TCL = T-cell lymphoma.

After extensive histomorphologic and immunohistochemical analysis, RLH was diagnosed in 10 cases and lymphoma in 97 cases; 5 specimens remained histologically indeterminate. After further analysis using PCR, it was possible to classify four of the five indeterminate lesions. Two lesions showed monoclonality and two, polyclonal bands. An amplificate could not be obtained in the fifth case because of insufficient biopsy material. The histology and the immunohistochemistry of these four lesions were reassessed on the basis of PCR investigations and the diagnosis correspondingly modified. Consequently, final analyses showed 12 cases of RLH (10.7% of cases), 99 cases of lymphoma (88.3%), and 1 indeterminate lesion. The RLH lesions were located as follows: two in eyelid, three in conjunctiva, and seven in orbit, with four of the latter involving the lacrimal gland. The 99 ocular adnexal lymphoid proliferations diagnosed as lymphoma were distributed as follows: 44 in orbit, 29 in conjunctiva (Fig 2A), 21 in eyelid, and 5 in caruncle (Table 1).

Histology and Immunohistology

Reactive Lymphoid Hyperplasia. On conventional staining, RLH was characterized by a dense infiltration of small histologically bland lymphocytes with the formation of reactive lymphoid follicles of varying size reminiscent of a normal lymph node architecture. Mitoses were restricted to the germinal centers where macrophages containing scattered debris (tingible bodies) also were observed. Dutcher bodies were absent. Immunohistochemically, the tumors consisted of mixed T- and B-lymphocyte infiltrates with the percentage T-cell infiltrate varying between 42% and 65% (mean, 57%). The B-cell component present varied between 15% and 47%, and these showed polyclonality for κ and λ. Proliferation of cells, determined with the MIB-1 antibody, was largely restricted to the germinal centers; BCL-2 positivity was observed, in contrast, in the interfollicular zones only. Occasional cells in the germinal center were positive for p53. Lymphoepithelial lesions were not observed.

Malignant Lymphoma. Of the 99 lymphoma cases, 63 were diagnosed as extranodal marginal-zone B-cell lymphoma (Table 2). These tumors consisted of small cells and occasional large blasts surrounding reactive B-cell follicles (Figs 2C, D; 3A), the presence of which was reflected by staining for the follicle dendritic cells (FDCs) using CD21. The cytology of the tumor cells varied, resembling centrocytes, monocytoid B-cells, or small lym-

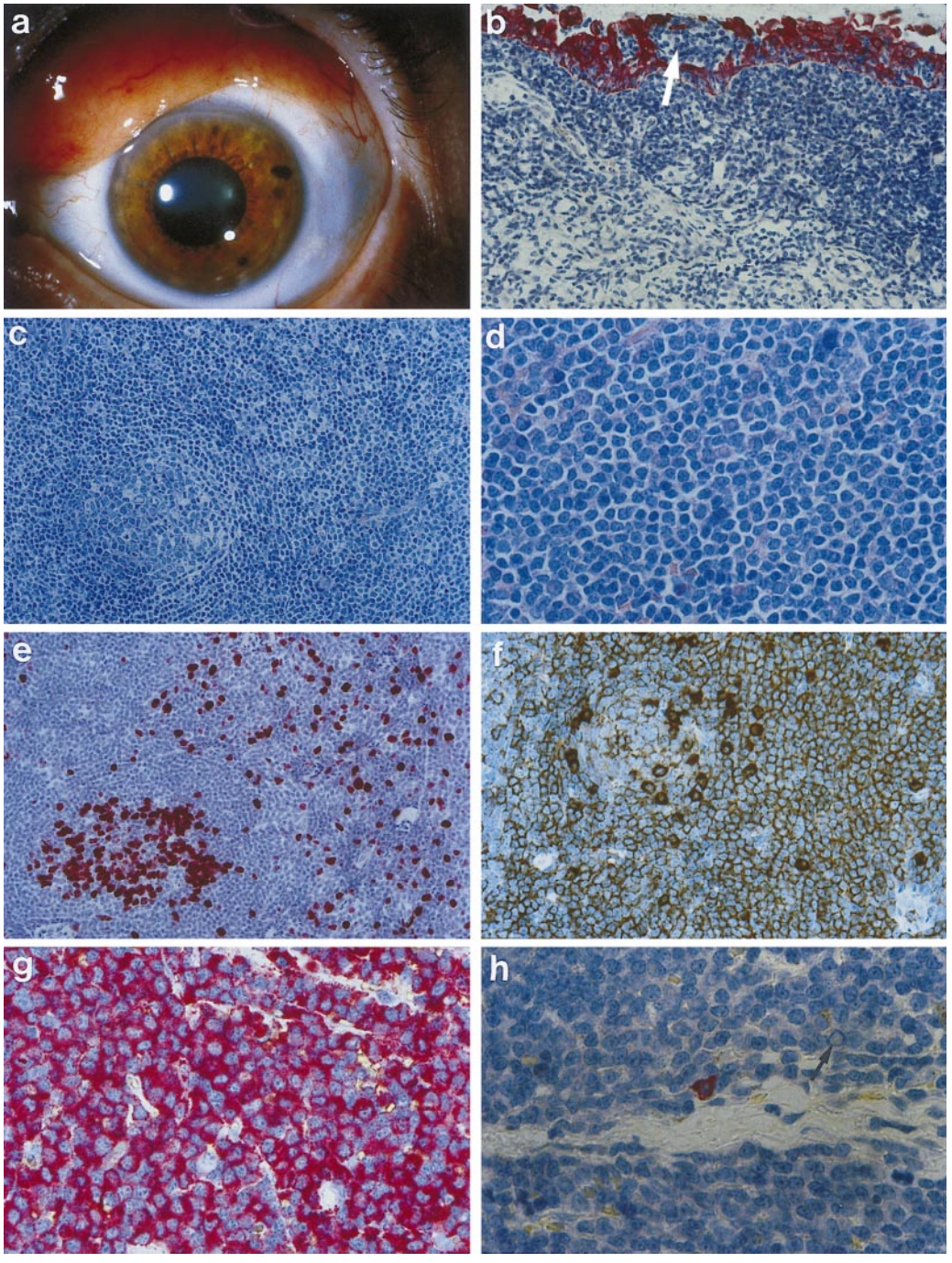

Figure 2. A, clinical photograph of the classical "salmon-patch" conjunctival lymphoma. **B,** lymphoepithelial lesion of a conjunctival extranodal marginal-zone B-cell lymphoma (arrow) illustrated using pan-cytokeratin marker, MNF-116. **C,** histology of an extranodal marginal-zone B-cell lymphoma consisting of small lymphocytes. **D,** occasional blasts. **E,** MIB-1 staining showing proliferation of the cells in the follicle center and those of the tumor cells in the wider-than-usual marginal zone. **F,** immunoglobulin M monoclonality. **G,** monoclonality for kappa. **H,** in contrast, staining for the immunoglobulin light chain lambda. The arrow shows a Dutcher body.

phocytes. Varying degrees of plasmacellular differentiation were apparent (Fig 2D) but was particularly pronounced in orbital lesions. Fourteen (22%) of the extranodal marginal-zone B-cell lymphomas were characterized by the presence of Dutcher bodies (Fig 2H). The tumor cells were positive in 58 (92%) of the cases for BCL-2: this staining was heterogenous in 31 tumors (53%); 5 cases only were BCL-2 negative. The proliferation rate of the tumor cells, as determined with the antibody MIB-1, varied between 10% and 70% (mean, 15%) (Figs 2E, 3B). In only seven cases (11%) of extranodal marginal-zone B-cell lymphoma were occasional blasts positive for p53. Some reactive follicles were partially infiltrated and even replaced by the tumor cells (follicular colonization).[33] Infiltration of the conjunctival or lacrimal gland epithelium by the tumor cells gave rise to lymphoepithelial lesions

Table 1. Anatomic Localization of Ocular Adnexal Lymphomas in Relation to Disease Stage

Anatomic Localization	Total	Stage I (n = 58)	Stage II (n = 21)	Stage III (n = 10)	Stage IV (n = 9)
Eyelid	21	13	2	5	1
Conjunctiva	29	19	5	2	3
Orbit (with or without lacrimal gland involvement)	44	24	9	4	5
Caruncle	5	2	2	1	—

in 26 (70%) of 37 cases, highlighted by the pancytokeratin marker, MNF-116 (Fig 2B). Monoclonality could be shown for Ig light chains (κ, 64.5%; λ, 35.5%) (Figs 2G, H) and for Ig heavy chains (μ, 92%; combined μ and δ, 8%) (Fig 2F). Although the tumor cells were negative for CD5 in the majority of specimens, positivity for this antigen was shown in two tumors.

Follicle center lymphoma was diagnosed in ten patients. These tumors displayed a follicular arrangement of cells, with the tumor cells consisting of varying proportions of centrocytes and centroblasts (Figs 4A, B). One patient was diagnosed as grade I (predominantly small cell) and nine as grade II (mixed small and large cells). The immunohistochemical profile of the cells included positivity for CD45, CD20, BCL-2, cytoplasmic Ig (mainly κ), and Ig heavy chains ($\mu > \delta > \gamma$). The proliferation rate of the tumor cells varied between 20% and 50% (grade I, 25%; grade II, mean, 32%) (Fig 4C); their positivity for p53 was between 10% and 40% (grade I, 7.5%; grade II, mean, 19.5%).

Diffuse large cell B-cell lymphoma was diagnosed in nine patients. These tumors were characterized by diffuse infiltrates of B-lymphocytes of medium to large size with conspicuous nucleoli and basophilic cytoplasm as well as numerous mitoses, including atypical mitotic figures (Fig 5A). The aggressiveness of these tumors was reflected by their infiltration and destruction of surrounding tissues such as the bony walls of the orbital cavity. The immunophenotype of these tumor cells was CD45+, CD20+, and the proliferation rates were high, varying between 50% and 80% (mean, 66.5%). This corresponded to an increased percentage of cells positive for p53 (range, 5%–80%; mean, 35%) (Fig 5B). One case of diffuse large cell B-cell lymphoma occurred in a patient who was positive for human immunodeficiency virus. In this specimen, infection with Epstein–Barr virus was shown immunohistochemically with Cs1-4 for the latent membrane protein of Epstein–Barr virus and was confirmed with in situ hybridization using the Epstein–Barr virus-encoded small RNAs 1 and 2 probes that are specific to the viral nontranslated small RNAs.

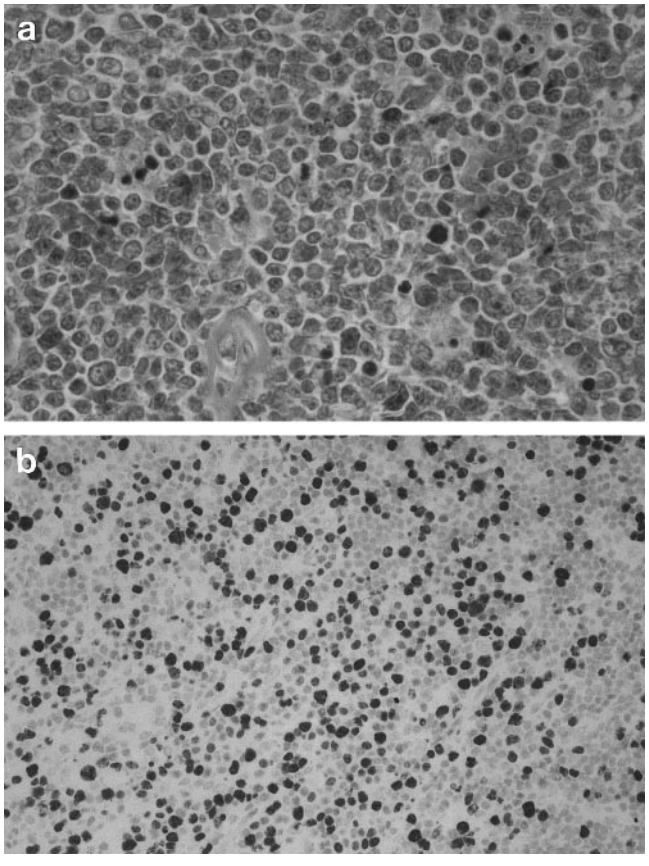

Figure 3. A, extranodal marginal-zone B-cell lymphoma with higher percentage of blasts, more frequent mitotic figures. **B,** a higher MIB-1 proliferation rate in a patient with stage III disease.

Plasmacytomas were diagnosed in six patients and were characterized by a mixture of mature and immature plasma cells. The cells were CD20 negative, surface Ig negative, cytoplasmic Ig positive, VS38c, and CD79a positive; only occasional cells were p53 positive (<5%). Five cases represented extramedullary plasmacytomas: two patients had disease localized to the ocular adnexa; one patient, 19 years of age, had additional extensive involvement of the mucosa of the upper respiratory tract; the remaining two patients had both nodal and gastrointestinal involvement. The sixth patient had bone marrow involvement with the production of paraprotein (myeloma) (Table 2).

Lymphoplasmocytic lymphoma–immunocytoma was diagnosed in five cases. These lesions consisted of diffuse proliferation of small lymphocytes, plasmacytoid lymphocytes, and plasma cells without reactive follicles. The tumor cells were positive for surface and cytoplasmic Ig (predominantly μ) and expressed B-cell surface antigens. They were negative for CD5, CD10, and p53. Some patients had systemic manifestation of the disease elsewhere (1 patient, stage II; 1 patient, stage III), as well as serologic evidence of serum paraprotein of IgM type.

Mantle cell lymphoma was diagnosed in two patients only and consisted of diffusely arranged small- to medium-sized cells with pale cytoplasm, inconspicuous nucleoli, and irregular or "cleaved" nuclei (Fig 6A). The meshwork of FDCs was prominent and disorganized as shown by the CD21 antibody. The tumor cells were characterized by positivity for CD45, CD20, CD5, and cyclin D1 protein, together with monoclonality for IgD (Fig 6B) and IgM. Occasional tumor cells were positive for p53.

One patient was diagnosed with orbital manifestation of hairy cell leukemia, with positivity of the infiltrating cells for CD103 on frozen sections. In three other patients suffering from systemic T-cell lymphoma, the initial presentation took the form of an orbital tumor. The T-cell tumors were characterized by diffuse mixed infiltrates of medium and large cells with positivity for the T-cell-associated antigens (CD3, CD5), with a CD4 predominance. The tumor cells were negative for B-cell-associated antigens. The proliferation rates were high (range, 60%–70%) and, similarly, the proportion of cells positive for p53 (range, 45%–60%).

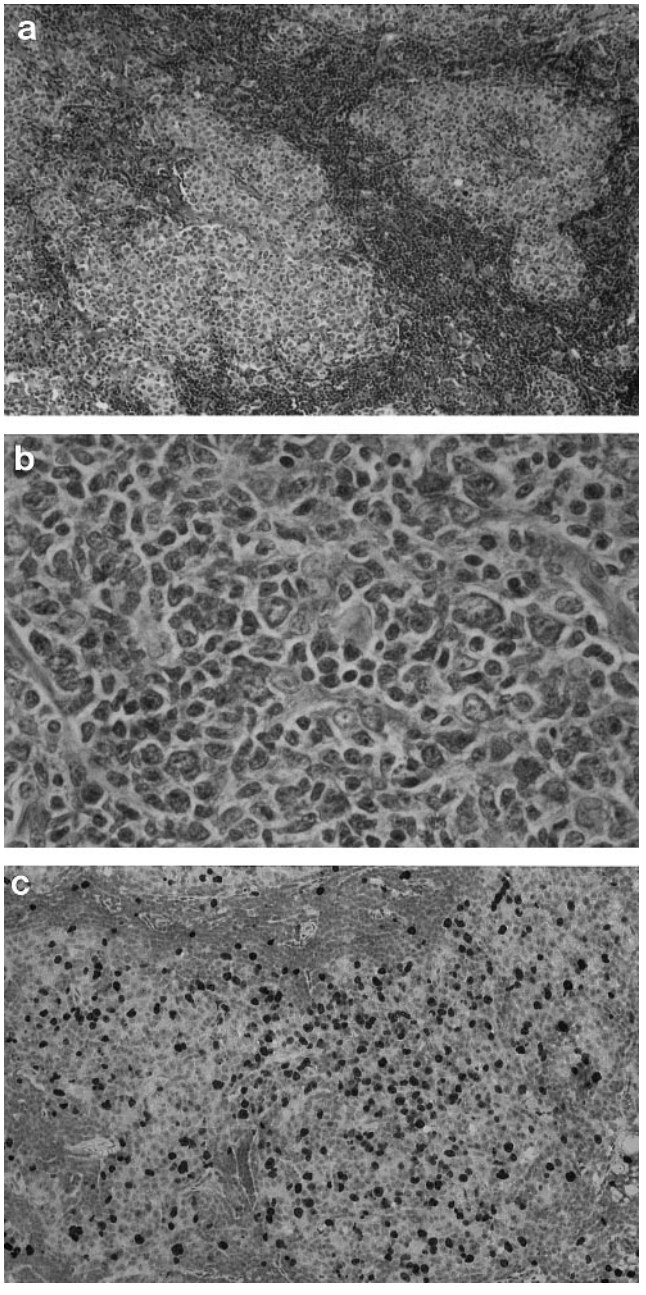

Figure 4. A, follicle center lymphoma (stain, periodic acid-Schiff). **B,** follicle center lymphoma, grade II (stain, Giemsa). **C,** MIB-1 proliferation rate of the neoplastic follicle center cells (grade II).

Polymerase Chain Reaction Amplification

A single amplification product was obtained after PCR with Ig heavy chain-specific primers in 79 of the 99 diagnosed lymphomas, confirming their B-cell origin and their monoclonality. In five cases, an oligoclonal picture was obtained. Examination of most RLH lesions with PCR showed a polyclonal picture; a monoclonal band was not seen in these cases. An amplificate could not be shown in 16 cases; this could be explained by either an inadequate quantity of material or by glutaraldehyde fixation of some specimens.

Clinical Follow-up

The patients with RLH were treated either with steroids (n = 5) or localized radiation therapy (n = 7). Ten patients responded well to therapy; in one, recurrence of the reactive lesion occurred within 5 years after steroid treatment. Although the follow-up in some cases is short (range, 6.4–125.5 months; median, 31.3 months), none of the patients with RLH have developed localized or systemic manifestations of lymphoma. In the patient with the histologically indeterminate lesion, there has not been a recurrence after steroid therapy (follow-up, 72 months).

Of the patients with lymphoma (n = 99), 58 had stage IE, 18 had stage II, 13 had stage III, and 10 had stage IV disease at diagnosis (Tables 1–3). Stage IE lymphoma, representing primary disease of the ocular adnexa, was distributed in the following locations: 13 in eyelid, 18 in conjunctiva, 24 in orbit (with or without lacrimal gland involvement), and 3 in caruncle (Table 1). Forty-five (77.5%) of the stage IE patients were diagnosed with extranodal marginal-zone B-cell lymphoma. Five of these patients had bilateral disease; the bilateral tumors were immunophenotypically and genotypically identical. The next major lymphoma subtype of stage IE disease was follicle center lymphoma (1 in grade I, 6 in grade II). Diffuse large cell B-cell lymphoma was never observed as stage I disease.

Stage II lymphoma was represented by nine extranodal marginal-zone B-cell lymphomas, two follicle center lymphomas (grade II only), three plasmacytomas, two diffuse large cell B-cell lymphomas, one lymphoplasmocytic lymphoma, and one mantle cell lymphoma (Table 2). Fifteen (71%) of these cases represented primary ocular adnexal disease with secondary systemic involve-

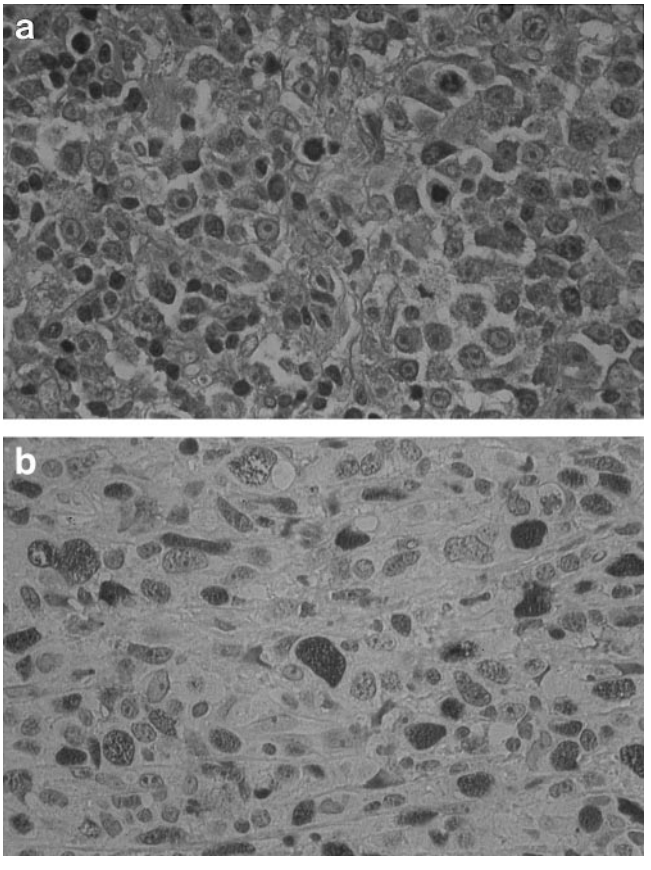

Figure 5. A, diffuse cell large B-cell lymphoma (stain, Giemsa). **B,** diffuse cell large B-cell lymphoma, p53 (DO7) staining.

Table 2. Lymphoma Subtypes and Stage at Diagnosis

Subtype	Total	Stage I (n = 58)	Stage II (n = 18)	Stage III (n = 13)	Stage IV (n = 10)
Extranodal marginal zone lymphoma	63	45	9	7	2
Follicle center lymphoma	10	7	2	1	—
Diffuse large cell B-cell lymphoma	9	—	2	4	3
Plasmacytoma	6	2	3	—	1
Lymphoplasmocytic lymphoma	5	3	1	1	—
Mantle cell lymphoma	2	1	1	—	—
Hairy cell leukemia	1	—	—	—	1
T-cell lymphoma	3	—	—	—	3

ment. The lymphoma subtypes observed in patients with stage III or IV disease were extranodal marginal-zone B-cell lymphoma (n = 9), diffuse large cell B-cell lymphoma (n = 7), systemic T-cell lymphoma (3), follicle center lymphoma (grade II) (1), plasmocytoma–myeloma (1), and hairy cell leukemia (1) (Table 2). In most patients with stages III and IV disease, a primary lymphoma of extraocular adnexal origin could be established (i.e., the lymphomatous manifestations in the ocular adnexa tended to represent secondary disease).

The methods applied for the treatment of the patients with lymphoma are summarized in Table 3. The follow-up period of the patients with lymphoma varied between 3 and 197.8 months (16.5 years; mean, 39.8 months; 3.3 years). Two patients with lymphoma with stage IE disease were treated with surgical excision only: in one of these patients, disease recurrence has not occurred; the other patient has been lost to follow-up.

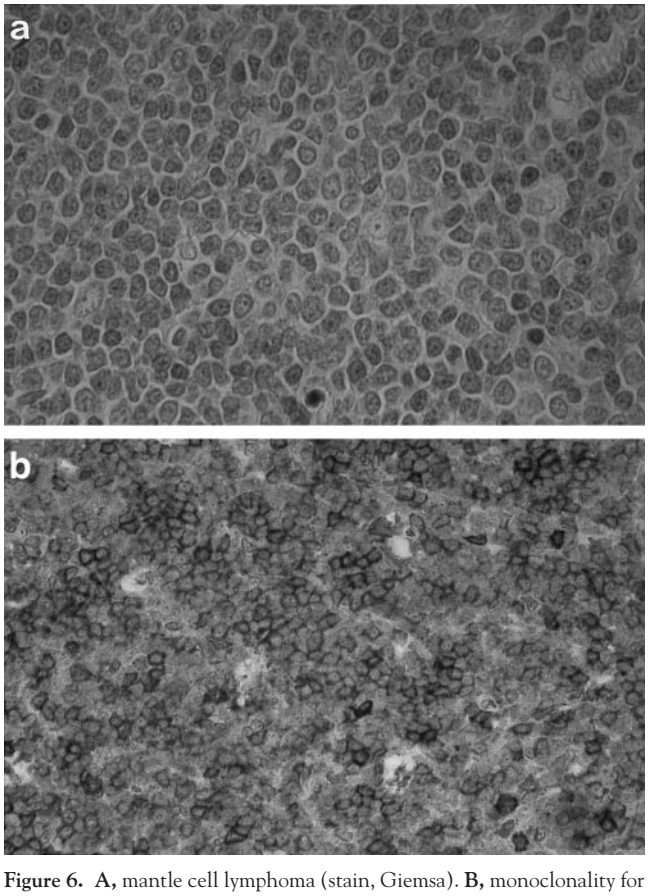

Figure 6. A, mantle cell lymphoma (stain, Giemsa). **B,** monoclonality for immunoglobulin D.

Fifty-four patients with lymphoma (all stage IE) were treated with localized radiation therapy alone (range, 20–40 Gy). Of these, 44 patients (80%) had complete remission; in 8 patients, repeated radiation therapy was required for complete tumor eradication. In two patients, manifestation of disseminated disease became apparent at 2 and 5 years after initial diagnosis and disease staging. These patients, diagnosed with extranodal marginal-zone lymphoma and follicle center lymphoma (grade II), respectively, required additional systemic chemotherapy.

Thirty-four patients (2 patients, stage IE; 16, stage II; 12, stage III, and 4, stage IV) underwent combined localized radiation therapy and systemic chemotherapy (Table 3). The chemotherapy regimens for this and the following group (treated only with chemotherapy) varied considerably; the regimen used most often was cyclophosphamide, doxorubicin, vincristine, and prednisone. Complete remission was obtained in seven patients (2 patients, stage IE; 4, stage II; 1, stage III). In 13 patients (6 patients stage II; 5, stage III; 2, stage IV), complete eradication of tumor was only achieved after repetition of either chemotherapy or radiation therapy. At final examination, persistent tumor was observed in six patients who had stage III disease or worse and an average follow-up period of 8 months. Eight patients had died of their disease.

In nine patients (2, stage II; 1, stage III; and 6, stage IV), the treatment was confined to chemotherapy only (Table 3). Five patients (2, stage II; 3, stage IV) had persistent tumor at the last examination (follow-up period between 7–89 months), whereas four had died of their disease (1, stage III; 3, stage IV).

Statistical Analysis

Prognosis was not influenced by patient age, gender, or tumor localization ($P > 0.05$ for all factors). Bilateral involvement and disease dissemination at the time of diagnosis occurred only in lymphomas ($P < 0.05$ and $P < 0.001$, respectively). The extent of disease at time of presentation was the most important clinical prognostic factor: patients presenting with stage III or IV lymphoma had risk ratios of 3.7 and 4.1 for persistent disease at the final follow-up ($P < 0.001$ and $P < 0.001$, respectively). Furthermore, the risk ratios of lymphoma-related deaths with stages III and IV disease were 2.6 and 3.2, respectively, both at the $P < 0.001$ level (Fig 7).

The presence of cytologic atypia ($P < 0.05$) and Dutcher bodies ($P < 0.05$) correlated with disseminated disease at presentation, stage at final follow-up, and the occurrence of lymphoma-related death. Immunohistochemical markers that were of prognostic significance included the MIB-1 proliferation rate and tumor cell positivity for p53. High proliferation rates within tumors corresponded significantly with tumor subdivision into high- and low-grade malignancy according to the REAL classification ($P < 0.001$) as well as into extranodal marginal-zone lymphoma and other lymphomas ($P < 0.05$). Further, high proliferation rates of

Table 3. Treatment Modalities Applied

Treatment Modality	Stage I (n = 58)	Stage II (n = 18)	Stage III (n = 13)	Stage IV (n = 10)
Surgical excision only	2	—	—	—
Localized radiotherapy only	54	—	—	—
Localized radiotherapy and systemic chemotherapy	2	16	12	4
Chemotherapy only	—	2	1	6

tumor cells positively corresponded with the stage of disease at presentation ($P < 0.001$) (Fig 8A), the stage at final follow-up ($P < 0.001$), and the occurrence of lymphoma-related death ($P < 0.001$) (Fig 9). The average MIB-1 proliferation rate of the extranodal marginal-zone B-cell lymphoma was 15%, which corresponded to stage I disease. However, 15 cases had a proliferation rate greater than 20%, and 14 of these cases had at least stage II disease. Similar correlations with respect to tumor subdivision (high- and low-grade malignancy) ($P < 0.001$), stage of disease at presentation ($P < 0.05$) (Fig 8B), stage at final follow-up ($P < 0.001$), and occurrence of lymphoma-related death ($P < 0.001$) were observed with tumor cell p53 positivity. Multivariate analysis showed that the MIB-1 proliferation rate had the highest risk ratio for predicting persistence of disease at follow-up (1.32) as well as lymphoma-related deaths (4.10).

Monoclonality (proved either immunohistochemically or after PCR analysis) correlated significantly with dissemination of disease at diagnosis ($P < 0.05$), persistence of lymphoma at follow-up ($P < 0.05$), as well as lymphoma-related death ($P < 0.05$).

Discussion

Benign and malignant lymphoid proliferations of the ocular adnexa cannot be diagnosed accurately on the basis of clinical or radiologic criteria[3,22,34,35]: the distinction often requires extensive histomorphologic, immunophenotypical, and molecular biologic analyses. In the current study, the majority of the lymphoproliferative lesions of the ocular adnexa could be divided into two groups—RLH or lymphoma—using conventional histology and immunohistochemistry. A gray zone of so-called histologically indeterminate tumors was observed in this investigation, as also reported by others.[3,8–10,13,14,18,22] The number of these cases, however, was small (n = 5) and could be reduced further (n = 1) with the aid of PCR analysis.

Reactive lymphoid hyperplasia was diagnosed in 10% of the patients with lymphoid proliferations of the ocular adnexa in the current study, which represented 16% of all lymphoproliferative lesions in the orbit and 10% of those located in the conjunctiva. The distribution of reactive lesions in the ocular adnexa varies in the literature, with some authors suggesting an RLH dominance in the conjunctiva compared to the orbit,[36] whereas other authors state the opposite.[12] These differences may be explained to some extent by differing selection criteria for biopsy between clinicians with a resulting bias in the tissues sent for examination.

In the current study, a discrepancy between the diagnosis of RLH and clinical course (i.e., the development of local or systemic manifestations of lymphoma) was not observed in the cases thus diagnosed during a median follow-up period of 31.3 months. It has been suggested by some authors that 27% to 29% of patients with RLH[3,8] and up to 45% with histologically indeterminate lesions[34] of the ocular adnexa ultimately develop disseminated disease. This led to a questioning of the value of dividing lymphoid proliferations in the ocular adnexa into polyclonal and monoclonal tumors.[8] The results of the current study and those of previous investigators[11,37] showed, however, that dissemination of disease only occurred in lesions with monoclonal immunoglobulin light or heavy chain expression ($P < 0.05$). Differences in methodology have been proposed to explain the higher incidence of dissemination in previously reported cases of RLH.[11] The possibility of tissue sampling error as well as of the reactive hyperplastic lymphoid populations harboring or ultimately giving rise to small monoclonal populations cannot, of course, be excluded completely.[20] Consequently, clinical follow-up still is to be recommended in all cases of RLH.

Application of the REAL classification, the only standard lymphoma classification that takes both nodal and extranodal lymphomas into account, proved suitable for the subtyping of the ocular adnexal lymphomas, dividing these extranodal lymphomas in the current study into seven groups on the bases of morphology and immunophenotype. The five main subtypes of lymphoma were extranodal marginal-zone B-cell lymphoma (63.6%), follicle center lymphoma (10%), diffuse large cell lymphoma (9%), plasmacytoma (6%), and lymphoplasmocytic lymphoma (5%) (Table 2).

Extranodal marginal-zone B-cell lymphoma corresponds to the small lymphocytic lymphoma of the Working For-

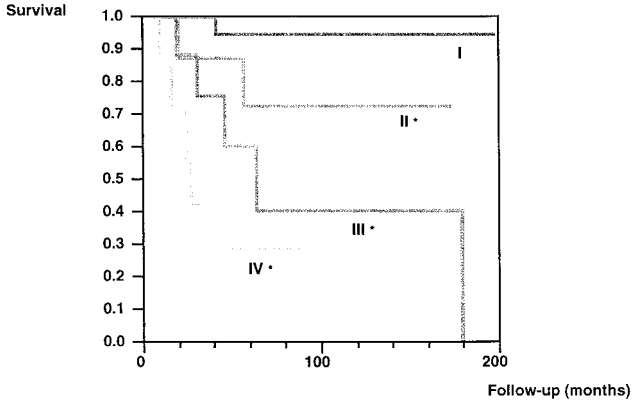

Figure 7. Kaplan–Meier curve. Stage of disease at the time of diagnosis related to survival (key: I = stage I; II = stage II; III = stage III; IV = stage IV; * = statistically significant, $P < 0.001$).

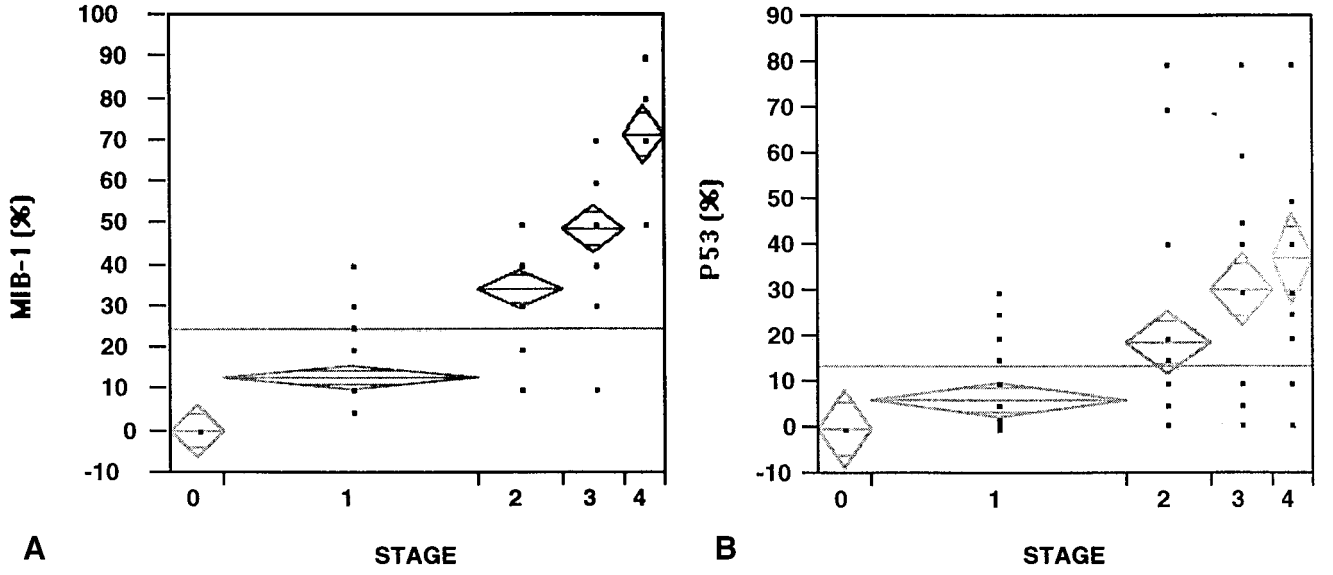

Figure 8. Relationship of MIB-1 proliferation rate (**A**) and p53 (**B**) to stage of disease at presentation (key: 0 = reactive lymphoid hyperplasia; 1 = stage I; 2 = stage II; 3 = stage III; 4 = stage IV).

mulation, described by previous authors as the most common lymphoma of the ocular adnexa.[4,5,8,10,11] Extranodal marginal-zone lymphomas of the ocular adnexa have very similar features to the so-called mucosa-associated lymphoid tissue (MALT) lymphoma, a specific form of tumor that has distinctive characteristics that distinguish it from other forms of primary non-Hodgkin extranodal lymphoma.[38] This morphologic similarity has been increasingly noted during the past decade[11,14,24,39–41] and facilitated the recognition of extranodal marginal-zone lymphoma in the current study. There was little variation in their immunohistomorphologic pattern between the different sites of the ocular adnexa, although some difficulties did arise in the orbital lesions due to the lack of architectural features—including the absence of epithelium—together with commonly occurring distortion artifacts. The similarity of the extranodal marginal-zone lymphomas of each site within the ocular adnexa, as well as with MALT lymphoma in other localizations,[42] was reflected further in the similar indolent clinical course of these tumors. In contrast to some previous reports[8,19,21] but in agreement with others,[12,23] there was no difference in the clinical course of an extranodal marginal-zone B-cell lymphoma located in the conjunctiva as compared to those in the lid or orbit.

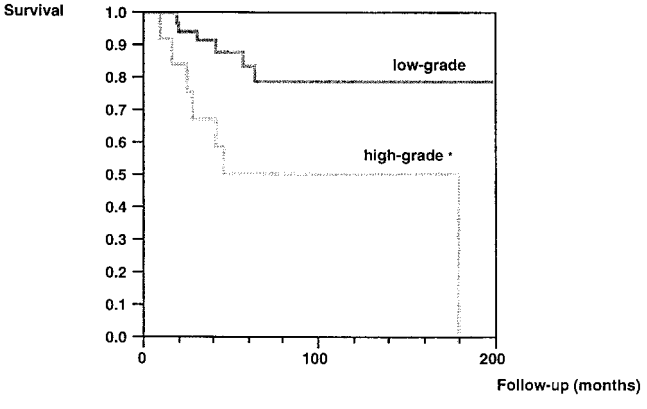

Figure 9. Kaplan–Meier curve. Lymphomas of high- and low-grade malignancy related to survival (*, statistically significant; $P = 0.01$).

In the current study, subdivision of the ocular adnexal lymphomas using the REAL classification was useful in predicting the stage of disease at diagnosis, the persistence of disease at final examination, and the progression to lymphoma-related death. The majority of the low-grade malignant lymphomas of the REAL classification (extranodal marginal-zone lymphomas, follicle center lymphoma, mantle cell lymphoma, and lymphoplasmocytic lymphoma)[26] were classified clinically as stage IE disease. Of these, extranodal marginal-zone lymphomas, followed by follicle center lymphoma, represented the most common primary lymphoma subtypes in the ocular adnexa: 45 (78%) of the 58 patients diagnosed with stage IE lymphoma were diagnosed with an extranodal marginal-zone B-cell lymphoma and 7 (12%) with follicle center lymphoma. Ninety-three percent of patients with unilateral or bilateral clinical stage IE, regardless of histologic subdivision, had a benign indolent clinical course and failed to develop extraocular adnexal lymphoma during a median follow-up of 44.3 months. This percentage value is comparable with the findings of Knowles et al,[8] who reported the absence of dissemination in 86% of stage IE patients after 51 months' follow-up. In contrast, advanced disease at the time of diagnosis (stage III or IV) with secondary manifestation of lymphoma in the orbit or ocular adnexa—represented particularly by diffuse large cell B-cell lymphoma and T-cell lymphoma—correlated significantly with increased risk ratios for persistent disease at final follow-up and lymphoma-related death.

Important histomorphologic features of ocular adnexal lymphomas consistent with monoclonality were the presence of

cytologic atypia and Dutcher bodies. These findings are in agreement with those of previous investigations.[11] Those immunohistologic characteristics of the ocular adnexal lymphomas of prognostic value, which had not been addressed in detail in previous investigations, included the MIB-1 proliferation rate and the tumor cell positivity for p53. Rapidly proliferating tumors correlated significantly with the stage of disease at presentation, the stage of disease at final follow-up, and the occurrence of lymphoma-related death to a significant statistical level (Fig 8A). The majority of the patients with low-grade malignant lymphoma of the REAL classification had proliferation rates less than 20% with a corresponding low disease stage at presentation. Forty-one patients with lymphoma in total had a proliferation rate greater than 20%, and 31 (75%) of these patients had disseminated disease at the time of diagnosis. The percentage of disseminated disease increased to 90% in lymphomas with a proliferation rate greater than 30%. Most lymphomas possessing greater proliferation rates were formed by diffuse large cell B-cell lymphomas, T-cell lymphomas, and plasmacytomas. However, 15 patients with proliferation rates greater than 20% were diagnosed as extranodal marginal-zone lymphoma, and 14 of these patients had stage II or more disease at diagnosis. These results would support the suggestion of Medeiros and Harris[43] that proliferation rates greater than 20% in ostensibly low-grade malignant tumors may be predictors of disseminated disease.

After subdivision of the lymphomas of the ocular adnexa into low grade and high grade, the marker for the tumor suppressor gene, p53, also proved to be of prognostic significance with regard to the stage of disease at presentation (Fig 8B), the disease course, and the risk of lymphoma-related death. These findings are consistent with the recognition that p53 is overexpressed more commonly in high-grade than low-grade lymphomas of other sites[44] and is a poor prognostic indicator in patients with high-grade lymphomas.[45] Recent investigations have also shown that p53 mutation may be involved in high-grade lymphoma transformation.[46,47]

An antigen that may have significance in the prognosis of the patients with extranodal marginal-zone B-cell lymphoma of the ocular adnexa is CD5. Anomalous T-cell antigen expression by non-Hodgkin lymphoma B-cell lymphomas of the ocular adnexa has been reported in varying percentages in the literature: 40%,[22,24] 22%,[8] 15.6%,[43] 11%,[18] and 3%.[14] Where Jakobiec et al[24] did not find any correlation between T-cell antigen positivity of tumor cells in ocular adnexal lymphomas and prediction of disease dissemination, other authors[25] subsequently have suggested that the presence of the CD5+ tumor cells in extranodal marginal-zone B-cell lymphoma at sites including the ocular adnexa is a prognostic marker for persistent or recurrent disease, for dissemination to the marrow and other extranodal sites, and for leukemic involvement of the peripheral blood. In the current study, tumor cells positive for CD5 were seen in approximately 15% of tumor cells in only 2 (3.27%) of 63 cases of extranodal marginal-zone B-cell lymphoma, a finding that is similar to the percentage reported by White et al.[14] These CD5+ extranodal marginal-zone B-cell lymphomas lacked the morphology and CD23 positivity of B-cell chronic lymphocytic leukemia. In addition, they lacked the morphology of mantle cell lymphoma as well as the usual cyclin D1 protein expression and IgD monoclonality, typical for this lymphoma subtype. Although the number of observed CD5+ extranodal marginal-zone B-cell lymphoma cases in the current study is too small to be statistically evaluated, it should be noted that both patients had stage III disease at the time of diagnosis and also died of their disease within 2 and 5 years after treatment commencement.

In this relatively large series of lymphomas of the ocular adnexa, some cases of particular interest were also observed. These included manifestation of hairy cell leukemia and three T-cell lymphomas in orbital tissues. Hairy cell leukemia, a rare lymphoid neoplasm, is characterized by peripheral blood cytopenias and splenomegaly. The involvement of other organs, including ocular tissues, has been reported infrequently.[48] Similarly, peripheral T-cell lymphomas in ocular adnexal tissue are rare and usually are manifestations of a systemic T-cell non-Hodgkin lymphoma or an extension of the "tumor" stage of mycosis fungoides.[49–55] Only one case of primary T-cell lymphoma of the orbit has been reported in the literature.[56] The three cases of T-cell lymphoma in this series were manifestations of a systemic middle- to large-cell peripheral non-Hodgkin T-cell lymphoma; one represented a T-cell lymphoma of the testis, with the initial presentation taking the form of an orbital tumor. The rare cases of hairy cell leukemia and T-cell lymphoma have been included in this series because the ophthalmic pathologist was required to make a diagnosis in the absence of relevant clinical information. Finally, one case of diffuse large cell B-cell lymphoma was observed in a patient who was positive for human immunodeficiency virus with evidence of Epstein–Barr virus in the tumor cells. Orbital lymphomas in association with acquired immune deficiency syndrome are uncommon and usually aggressive, as was seen in this patient.[57–63]

In conclusion, clinical, histomorphologic, immunohistochemical, and molecular biologic analyses of 112 cases of ocular adnexal lymphoid proliferations showed mainly that the REAL classification proved suitable for the subdivision of the ocular adnexal lymphomas. In addition, this classification was an accurate predictor of the evolution of the various lymphoproliferative tumors. The authors, therefore, recommend it as the standard classification of lymphomas of the ocular adnexa to allow future comparison between independent studies regarding therapy and prognosis and for the collaboration between centers. The study also established that complementary immunohistochemical markers such as MIB-1 and p53 were helpful in predicting the stage of disease and the outcome. Complementary techniques, such as PCR, confirmed the diagnoses and contributed to the reduction in the number of so-called histologically indeterminate lesions.

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