Extranodal Marginal Zone Lymphoma in the Ocular Region: Clinical, Immunophenotypical, and Cytogenetical Characteristics

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Purpose. To evaluate clinical, immunophenotypical, and cytogenetical characteristics of 116 patients with a diagnosis of extranodal marginal zone lymphoma (EMZL) presenting primarily in the ocular region.

Methods. Specimens from all patients with a diagnosis of ophthalmic lymphoma in Denmark during the period 1980 to 2005 were reviewed and reclassified according to the World Health Organization (WHO) classification. Cases reclassified as EMZL were selected and reviewed with respect to clinical characteristics and outcome. The presence of translocations involving IGH and/or MALT1 was investigated in 42 specimens by fluorescence in situ hybridization (FISH).

Results. Median age was 69 years. Most lymphomas were located in the orbit. Approximately one fourth of the patients had disseminated disease at presentation. One third experienced a relapse or progression of disease after initial therapy, and relapses were frequently found at extraocular sites. Five-year progression-free survival and overall survival (OS) rates were 71% and 75%, respectively. Translocations involving the IGH- or MALT1-gene loci were detected in 2 (5%) of 42 specimens. In Cox regression multivariate analysis, IGH-translocation was the only factor associated with PFS, whereas a favorable International Prognostic Index (IPI) score was the most reliable predictor of OS.

Conclusions. EMZL presenting in the ocular region usually runs an indolent course, but relapses are frequently seen. The IPI-score was the most reliable independent parameter for estimating risk of death in our cohort of patients. Furthermore, we found that the frequency of translocations involving the MALT1- and IGH-gene loci is low in ocular region EMZL. (*Invest Ophthalmol Vis Sci.* 2009;50:516–522) DOI:10.1167/iovs.08-2539

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Lymphoma is the most frequent malignant tumor in the ocular region (i.e., eyelid, conjunctiva, lacrimal sac, lacrimal gland, and orbit).[1] Several different lymphoma subtypes are represented in the ocular region, but extranodal marginal zone lymphoma (EMZL, also termed MALT lymphoma, which should be restricted to lesions associated with epithelium) constitutes more than 50%.[2,3] The incidence of ocular region lymphoma in general and of EMZL in particular is increasing.[4–6]

The pathogenesis of EMZL is largely unknown. However, it is generally believed that both chronic antigen stimulation and acquired genetic alterations are involved.[7,8] For instance, in gastric EMZL *Helicobacter pylori* has been shown to be present in most cases.[9] More recently, a possible connection between ocular region EMZL and *Chlamydia psittaci* has been suggested.[10] However, these results have not been substantiated in subsequent studies, suggesting geographical variation.[3,11–13] Similarly, different chromosomal abnormalities have been demonstrated in EMZL with various frequencies, depending on the initial anatomic site of involvement.[14,15] These include three different translocations that seem to target the same signaling pathway,[16] resulting in increased NFκB activation and decreased apoptosis (i.e., t(11;18) (q21;q21) involving API2 and MALT1, t(14;18) (q32;q21) involving IGH and MALT1, and t(1;14) (p22;q32) involving Bcl-10 and IGH).[7] The t(11;18) is prevalent in pulmonary and gastrointestinal EMZL, whereas t(14;18) is more frequent in EMZLs involving other extranodal sites, including the ocular region.[15] Of interest, in gastric EMZL aberrant nuclear Bcl-10 occurs predominantly in cases carrying t(11;18),[17] and these cases have a more aggressive course and generally do not respond to *H. pylori* eradication.[18,19] More recently, a similar association between nuclear Bcl-10 and an aggressive course has been suggested for ocular region EMZL.[20]

Most previous studies of clinicopathologic characteristics and prognostic factors in ocular region lymphoma have been based on unselected groups of patients with different lymphoma subtypes.[21–25] In this study of a large series (116 patients), we present an analysis exclusively focusing on EMZL presenting in the ocular region. We characterize clinical, immunophenotypical, and cytogenetic features of this disease entity and correlate our data with the clinical outcome to identify prognostic factors.

Material and Methods

Patients and Biopsies

Patients were enrolled using three different population based registries: the Eye Pathology Institute Registry, the Danish Registry of Pathology, and The Danish Lymphoma Group Registry, searching for all patients with a lymphoma diagnosis in the ocular region in Denmark during the period from 1980 to 2005. All cases were reviewed by two of the authors (LDS, ER) and reclassified according to the World Health Organization (WHO) classification,[26] based on histology and immuno-

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TABLE 1. Characteristics of 116 Patients* with EMZL Presenting Primarily in the Ocular Region

Parameter	*n* (%)	Percentage with Systemic Disease after Work-up†	Percentage with Relapse/Progression at Follow-up
Localization			
Orbit	69 (60)	27	25
Conjunctiva	38 (33)	10	39
Lacrimal gland	5 (4)	33	60
Eyelid	4 (3)	100	50
Bilateral disease	12 (10)	25	50

* Sex: 61 female (53%), 55 male (47%); age, median (range): 69 years (8–90).

† Clinical staging after work-up was known in 95 patients: 60 with orbital, 29 with conjunctival, 3 with lacrimal gland, and 3 with eyelid EMZL.

histochemistry using standard panels of antibodies (i.e., CD3, CD5, CD10, CD20, CD23, CD79α, Bcl-2, Bcl-6, Cyclin D-1, and κ and λ light chain). Of 228 confirmed ophthalmic lymphoma cases, 126 were EMZL.[6] Ten patients had a history of EMZL at extraocular sites and were excluded from the study. Hence, this study was based on the remaining 116 patients with EMZL presenting primarily in the ocular region.

Clinical Data

Clinical records and prebiopsy pathology requisition forms were reviewed with particular reference to sex, age, prior history of lymphoma, sites of involvement, stage of disease at diagnosis according to the Ann Arbor staging classification,[27] symptoms and signs, treatment, International Prognostic Index (IPI),[28] site and date of relapse, and date and cause of death. From all patients SNOMED (Systemized Nomenclature of Medicine) codes registered during the period 1980 to 2006 were obtained from the Danish Registry of Pathology and reviewed to detect relapses. Additional information concerning date and cause of death was obtained from the Registry of Cause of Death and the Danish Cancer Registry. All death certificates were reviewed to validate the cause of death.

Overall survival (OS) was calculated from time of diagnosis to time of death from any cause or to time of last follow-up. Progression-free survival (PFS) was defined as the time period from time of diagnosis until the date of first relapse/progression or death.

Bcl-10

The presence of nuclear and cytoplasmic Bcl-10 was evaluated in a subset of 75 tumors, where stage of disease was known and adequate material obtainable. Before staining with Bcl-10 antibody (diluted 1:200; Dako, Glostrup, Denmark), the sections were heated in a microwave oven in a TEG buffer (pH: 9) for 18 minutes. The staining was performed (LV-1Autostainer; Laboratory Vision, Freemont, CA) with a secondary antibody (Envision K4007; Dako).

The specimens were scored positive for nuclear Bcl-10 if a minimum of 10% of nuclei in the neoplastic cells expressed the protein as proposed by Ye et al.[29] Furthermore, both nuclear- and cytoplasmic staining intensity was graded into subgroups with weak/moderate or strong expression. The specimens were scored independently by two of the authors (LDS, ER). Cases with disagreement were reviewed in consensus.

Fluorescence In Situ Hybridization

Locus-specific interphase fluorescence in situ hybridization (FISH) was performed as described[30] on a subset of 50 biopsies with adequate tissue. In brief, thin sections were deparaffinized and rehydrated. After pretreatment, the sections were incubated in pepsin solution (10 minutes) at 20°C to increase DNA accessibility. The sections were then fixed in 1% paraformaldehyde for 2 minutes, dehydrated through increasing ethanol concentrations, and hybridized with the appropriate probe. Both probe and target DNA were simultaneously denatured at 82°C for 5 minutes and incubated overnight at 45°C. The sections were counterstained with 4,6-diamidino-2-phenylindole (DAPI II; Abbott Laboratories, Abbott Park, IL) and examined by microscope (E1000; Nikon, Tokyo, Japan) equipped with filters for spectrum green (emission wavelength [wl] 538 nm), spectrum orange (emission wavelength 588 nm), spectrum red (emission wavelength 630nm), DAPI (emission wavelength 461 nm; all from Chroma, Rockingham, VT), and a triple bandpass filter.

The specimens were initially screened for rearrangements at the immunoglobulin heavy-chain gene cluster (*IGH*, 14q32), and at the mucosa-associated lymphoid tissue lymphoma translocation 1 (*MALT1*, 18q21) gene loci, using dual-color, split-signal probes for IGH (Dako) and MALT1 (DAKO). In cases positive for both the *IGH* and *MALT1* gene break, the IGH/MALT1 fusion product was confirmed by using an IGH/MALT1 dual-color, dual-fusion translocation probe (Vysis; Abbott Molecular, Des Plains, IL). In cases positive for an IGH gene break only, dual-color, break-apart probes for Bcl-2 (18q21), Bcl-6 (3q27), and Bcl-10 (1p22) (Dako) were applied.

In each case the hybridization signals for each probe were evaluated in at least 100 nuclei. A reactive tonsil was mounted as the negative control on each slide. The threshold for presence of a translocation was determined counting split signals in 100 nuclei in five different reactive tonsils. The highest number of false-positive nuclei plus three standard deviations was taken as the cutoff point of each aberration.

Statistical Analyses

Differences in patient characteristics in subgroups were tested by using the Fisher exact test. OS and PFS curves were estimated by the Kaplan-Meier method and compared by the log-rank test.

Factors with $P < 0.5$ in univariate analyses were selected for multivariate analysis using the Cox regression method to determine independently predictive variables. All statistical analyses were performed with commercial software (SPSS, ver. 15.0; SPSS, Chicago, IL).

Ethics

The investigation adhered to the tenets of the Declaration of Helsinki and was approved by the local Scientific Ethics Committee (journal no. KF 01 262201) and the Danish Protection Agency (journal no. 2005-41-5098).

RESULTS

Patients

One hundred sixteen Danish patients with a confirmed diagnosis of EMZL during the period from 1980 to 2005 were included in the study. Full clinical files were found for 105 of these patients. Table 1 shows the main clinical features at presentation. The patients constituted 61 (53%) females and 55 (47%) males aged 8 to 90 years (median, 69 years) at diagnosis.

TABLE 2. Signs and Symptoms of 105 Patients with EMZL in the Ocular Region

Localization	Orbit *n* (%)	Conjunctiva *n* (%)	Lacrimal Gland *n* (%)	Eyelid *n* (%)
Patients	60	37	4	4
Clinical findings				
Tumor	28 (47)	37 (100)	4 (100)	3 (75)
Proptosis	31 (52)	0	2 (50)	0
Conjunctival hyperemia	10 (17)	5 (14)	0	0
Ptosis	9 (15)	1 (3)	2 (50)	2 (50)
Symptoms				
Tumor	15 (25)	25 (68)	3 (75)	3 (75)
Proptosis	26 (43)	0	1 (25)	0
Red eye	12 (20)	12 (32)	0	0
Pain	12 (20)	2 (5)	0	1 (25)
B-symptoms	5 (8)	0	0	1 (25)
Symptom duration in months, median (range)	7 (2 to 24)	12 (2 to >36)	14 (12 to 24)	10 (8 to >36)

Most lymphomas were localized in the orbit ($n = 69$, 60%) and the conjunctiva ($n = 38$, 33%). Unilateral involvement of more than one ocular anatomic site was seen in 19 (16%) patients. Twelve (10%) patients had bilateral ocular involvement.

The staging procedures varied, depending on different centers and periods, consequently Ann Arbor stage at diagnosis was applicable in 95 patients. Ann Arbor stage I disease was present in 72 (76%) patients, 2 (2%) had disease involving regional lymph nodes (stage II), 3 (3%) had involvement of the spleen or lymph nodes below the diaphragm (stage III), and 18 (19%) presented with bone marrow involvement (stage IV). IPI was retrieved in 51 patients; 45(88%) of them ranked in the low/low-intermediate risk group (IPI score 0–2).

The presenting symptoms varied according to site of involvement (Table 2). A visible or palpable tumor was found in 69% (72/105). Only 14% (15/105) complained of pain. Six patients (6%) had B symptoms (fever, night sweats, and loss of weight.)

The duration of symptoms at presentation ranged from 2 months to more than 3 years with a median of 8 months. In five cases (4%) a preceding history of Sjögren syndrome ($n = 2$) or other autoimmune disease (Graves disease; $n = 1$), Hashimoto thyroiditis ($n = 1$), and systemic lupus erythematosus ($n = 1$) was reported in the clinical files.

Treatment

Most patients (58/105, 55%) were treated with radiation therapy alone, with a total dose ranging from 26 to 40 Gy (Table 3). Fourteen (13%) patients received both radiation and chemotherapy. Chemotherapy alone was given to 22 (21%) patients, either consisting of chlorambucil (Leukeran; GlaxoSmithKline, Research Triangle Park, NC) with or without prednisolone or anthracycline-based regimens. Radiation therapy as the only treatment was primarily given to patients in stage I (53/73 patients; 73%), whereas most patients with more widespread disease were treated with chemotherapy (15/18 of patients in stage IV; 83%; Table 3). Prednisolone alone was given to three (3%) patients. Three (3%) patients were treated with surgery only. Five patients (5%) refused treatment because of older age ($n = 2$) or lack of symptoms.

Treatment Outcome and Survival Data

Thirty-seven (32%) patients experienced a relapse or progression of disease during a follow-up period ranging from 5 to 236 months (median, 49 months). Median time to relapse or progression was 2.5 years (range, 8–192 months) after diagnosis. Among the 11 patients not treated with radiation or chemotherapy initially, four treated with surgery ($n = 2$) or prednisolone ($n = 2$) achieved complete remission (CR). One had stable disease without treatment, and six progressed or relapsed. Because of the relapse, two of these patients received radiation 6 and 9 months after diagnosis, respectively.

The site of relapse or progression was ophthalmic and on the same side as the primary lymphoma in 15 (41%) cases, ophthalmic with bilateral affect in 2 (5%) cases, and ophthalmic but contralateral in 1 (3%) case. Sixteen (43%) patients had a relapse at one or multiple other sites: lymph nodes ($n = 9$ cases), lung ($n = 6$), cerebrum ($n = 2$), testes ($n = 1$ bilateral), spleen ($n = 1$), skin ($n = 1$), or bone marrow (n = 3). Another three patients (8%) relapsed or progressed, both in the initial ophthalmic site and in other localizations: regional lymph nodes, ($n = 1$) cerebrum, ($n = 1$) skin ($n = 1$), or bone marrow ($n = 1$).

Estimated PFS rates of all patients were 85%, 71%, and 57% at 2, 5, and 10 years. In patients with stage I, the PFS rates at 2, 5, and 10 years did not differ significantly (i.e., 86%, 66%, and 50%, respectively). OS rates at 2, 5, and 10 years in all patients were 90%, 75%, and 48%, and in patients with stage I: 90%, 74%, and 45% (Fig. 1). Among the 56 patients who died during follow-up, 12 (21%) died of lymphoma progression 4- to 149 months after diagnosis (median, 64.5 months). All 12 patients were older than 60 years at time of diagnosis.

TABLE 3. Treatment According to Stage of Disease at Diagnosis of 105 Patients with EMZL in the Ocular Region

Stage	Radiation	Chemotherapy	Radiation + Chemo	Prednisolone	Surgery	No Treatment	Total
I	52	6	7	2	2	3	72
II	0	2	0	0	0	0	2
III	0	0	3	0	0	0	3
IV	2	13	2	0	0	1	18
Unknown	4	1	2	1	1	1	10

Data are the number of cases.

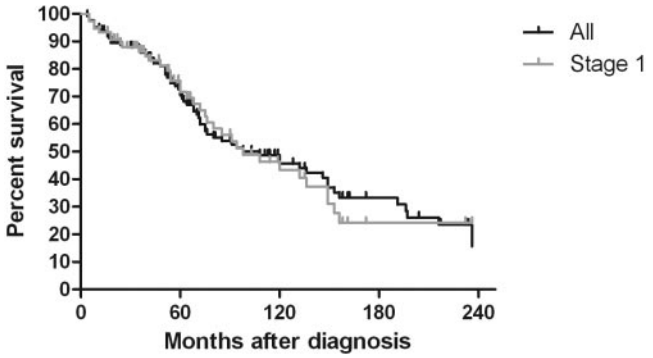

FIGURE 1. OS curves for all 116 patients and for patients in stage I (n = 72). Stage of disease at diagnosis had no effect on OS.

Histology and Phenotype

Morphologic features of the specimens were similar to EMZL in other sites (i.e., with infiltration of small B-cells: centrocyte-like cells, and monocytoid cells, with scattered larger centroblast-like cells). Plasma cells were seen with a varying frequency, commonly with prominent Dutcher bodies (Fig. 2).

Immunohistochemically, the tumor cells in all specimens expressed CD20, CD79a, and Bcl-2 and were negative for CD3 and cyclin-D1. CD5 positivity was seen in one case (1%), Bcl-6 in one case (1%), and CD23 in four cases (3%). MIB-1-positive cells ranged from 5% to 50%, with a median of 5%. Monotypic plasma cells with immunoglobulin light chain restriction were revealed in 35 (28%) cases. Nineteen cases were κ-positive and 16 cases were λ-positive.

Bcl-10 Expression

Bcl-10 protein expression was investigated in 75 cases (i.e., 57 patients in stage I, 2 in stage II, 2 in stage III, and 14 in stage IV). Strong nuclear and cytoplasmic Bcl-10 expression in 40% to 90% of the malignant cells was seen in four (5%) tumors. Eighteen (24%) tumors expressed weak/moderate nuclear Bcl-10, with strong cytoplasmic staining in 12 (67%) cases and weak cytoplasmic staining in 6 (33%). The remaining 53 (71%) cases displayed only cytoplasmic Bcl-10 staining, with strong expression in 16 (30%) and weak/moderate expression in 37 (70%). No clinical differences with respect to age, gender, stage of disease, IPI-score (0–2 vs. 3–5), or relapse/progression rate was found between patients with or without nuclear Bcl-10 expression.

FISH Analysis

Of the 50 specimens selected for FISH, 42 (25 with stage I, one with stage III, 11 with stage IV and 5 of unknown stage) showed satisfactory hybridization signals to be evaluated for rearrangements at the MALT1- and IGH loci. Evidence of IGH breakage was found in two cases (5%). One of these also showed MALT1 breakage (2%). In this case, the presence of a t(14;18)/IgH/MALT1 was confirmed by FISH with an IgH/MALT1 dual-color, dual-fusion translocation probe. The other case with an IGH-involved translocation showed evidence of breakage at the Bcl-6 gene locus, whereas MALT1, Bcl-10, and

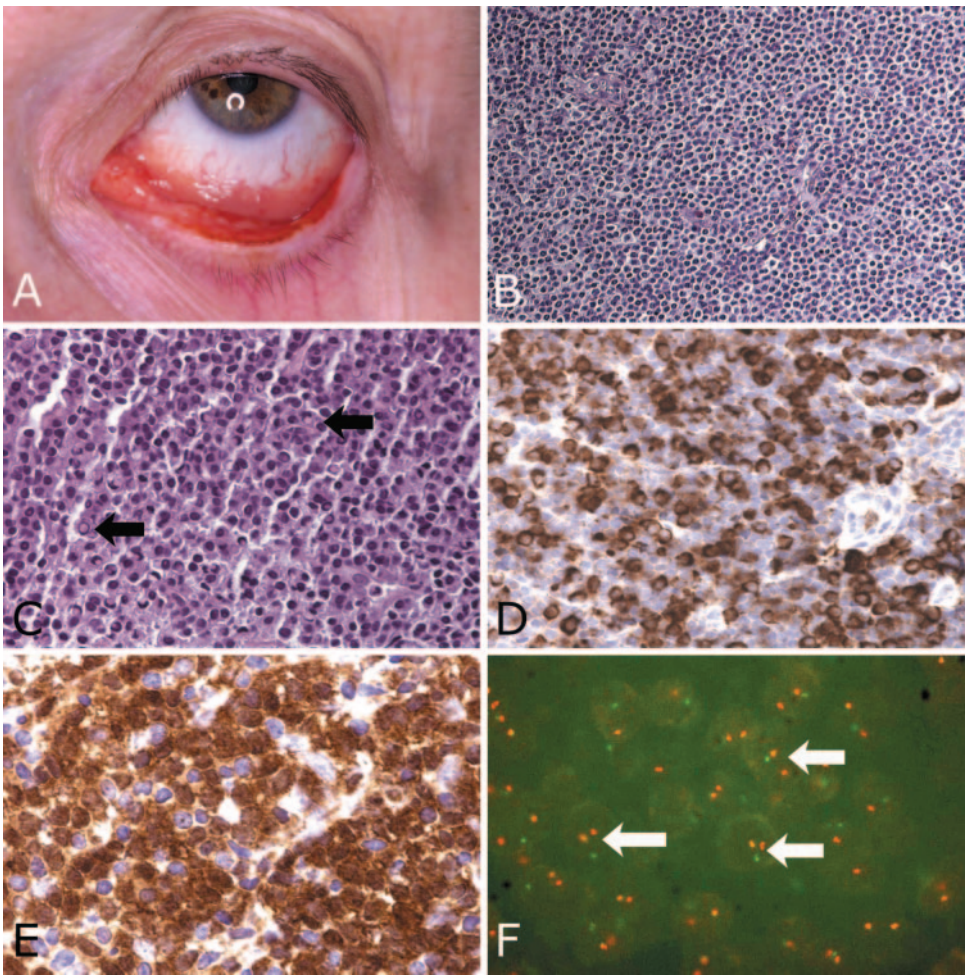

FIGURE 2. (**A**) A 63-year-old woman with conjunctival EMZL. The patient was in stage I at diagnosis, and received no treatment. She progressed after 4 years with bilateral disease. (**B**) Orbital EMZL characterized by a diffuse pattern of small centrocyte-like cells in a 67-year-old female patient (hematoxylin-eosin [HE]). (**C**) Conjunctival EMZL with plasmacytoid differentiation and multiple Dutcher Bodies (*black arrows*; HE). (**D**) Monotypic plasma cells in a conjunctival EMZL showing κ light chain restriction. (**E**) Nuclear Bcl-10 positivity in the neoplastic cells of a conjunctival EMZL. (**F**) Fluorescence in situ hybridization with a dual-color, split-signal probe in a specimen with a translocation at the IGH-gene locus. In the neoplastic cells (*white arrows*) there is one fusion product (*orange dot*) representing the normal chromosome and two split-signal products (one *green* and one *red*) from the chromosome with an IGH-associated translocation. Magnification: (**B**) ×200; (**C–E**) ×400.

Table 4. Clinical and Immunophenotypic Characteristics of Two Translocation Positive Patients

Translocation	Sex	Age	Site	Immunophenotype	Stage	Treatment	Relapse/ Progression (Mo after Diagnosis)	Cause of Death (Mo after Diagnosis)
IGH-MALT1	M	66	Conj, bilateral	nBcl-10neg MIB1: 5%	1	Radiation	Relapse (34)	Lymphoma (98)
IGH-Bcl6	F	71	Orbit	Bcl-6pos, nBcl-10pos, MIB1: 20%	1	Radiation	Relapse (21)	Unknown (60)

Conj, conjunctiva; mo, months; nBcl-10, nuclear Bcl-10 staining; neg, negative; pos, positive.

Bcl-2 showed no rearrangement. The specimen from the patient with Bcl-6 gene breakage showed also nuclear expression of Bcl-6 by immunohistochemistry (as the only one of 116 specimens).

Clinical and immunophenotypic characteristics from the two translocation-positive patients are shown in Table 4.

Analysis of Prognostic Factors

The prognostic significance of various factors was examined by univariate analysis (Table 5). Among these, the presence of a translocation at the IGH-locus was found to adversely affect PFS, whereas male sex, presence of B symptoms, and an IPI score >2 were poor prognostic factors for OS.

In Cox regression, multivariate analysis IGH translocation was the only factor associated with PFS ($P = 0.035$), whereas both male sex ($P = 0.039$), presence of B symptoms ($P = 0.023$), and an unfavorable IPI score ($P = 0.019$) retained independent adverse prognostic significance for OS.

Discussion

EMZL is the most frequent lymphoma subtype found in the orbit and ocular adnexa.[2,31,32] It is an extranodal B-cell lymphoma, considered to be the neoplastic counterpart of the marginal zone cells in reactive follicles in the lymph node. EMZL most commonly occurs in the stomach but may affect any organ of the human body. It only rarely arises from native organized MALT, as seen in the Peyer's patches of the ileum. Usually, EMZL arises from MALT that has been acquired as a result of a chronic inflammation caused by persistent infections

Table 5. Results of Univariate Analysis for Prognosis Evaluated by the Kaplan-Meier Method and Tested for Statistical Significance with Log-Rank Test

	Progression-Free Survival		Overall Survival	
Prognostic Factor	5-y (%)	*P*	5-y (%)	*P*
Sex				
Men ($n = 55$)	72		67	
Women ($n = 62$)	70	0.775	82	**0.040**
Clinical stage				
I ($n = 72$)	66		74	
II or more ($n = 23$)	76	0.736	74	0.877
Ocular region localization				
Orbit ($n = 69$)	74		74	
Conjunctiva ($n = 38$)	66		76	
Lacrimal gland ($n = 5$)	50		80	
Eyelid ($n = 4$)	50	0.162	75	0.464
B symptoms				
Yes ($n = 6$)	75		63	
No ($n = 99$)	57	0.487	75	**0.029**
Treatment				
None/surgery/prednisolone ($n = 11$)	46		74	
Radiation only ($n = 58$)	64		72	
Chemotherapy +/− radiation ($n = 36$)	76	0.264	75	0.951
Relapse/progression				
Yes ($n = 37$)			79	
No ($n = 79$)			72	0.578
Proliferation status				
MIB-1 < 30% ($n = 108$)	73		75	
MIB-1 ≥ 30% ($n = 7$)	50	0.075	63	0.912
Nuclear Bcl-10				
Positive ($n = 22$)	70		84	
Negative ($n = 53$)	70	0.816	65	0.208
IgH-involved translocation				
Positive ($n = 2$)	0		50	
Negative ($n = 40$)	74	**0.013**	75	0.390
IPI score				
Low (0–2, $n = 45$)	61		84	
High (3–5, $n = 6$)	83	0.852	44	**0.006**

Bold *P* indicates statistical significance.

or autoimmune disorders, and even in sites that do not contain epithelial structures.[32] In general, EMZLs at different extranodal sites share some common morphologic, phenotypic, and molecular features, yet the type of infectious agent differs with the primary site of involvement. In gastric EMZL *H. pylori* is the causative agent in almost all cases,[9] whereas a connection between ocular region EMZL and *C. psittaci* has been suggested in an Italian study of 40 patients with lymphoma in the ocular region.[10] However, various other investigators have failed to demonstrate the presence of *C. psittaci*, and results after first-line antibiotic treatment are variable.[33]

In this study, we analyzed the clinical, immunophenotypic, and cytogenetic characteristics of 116 patients with EMZL presenting primarily in the ocular region. Consistent with the study by Ferry et al.[31] of 168 patients with EMZL presenting in the ocular region, we found that EMZL in the ocular region is primarily located in the orbit, with bilateral presentation of disease occurring in 10 percent. Approximately one fourth of patients had disseminated disease at presentation, emphasizing the need of a complete staging procedure in patients with ocular region EMZL. In our cohort of patients one third had a relapse or progression of disease after initial therapy, and relapses were frequently found at extraocular sites. However, OS was not significantly poorer in patients with relapse. With a 5-year OS of 75% and only 10% of patients dying from their lymphoma, our data confirm that EMZL in the ocular region usually has a quite indolent course, even in cases presenting with widespread disease.

There are currently no generally accepted prognostic factors for primary EMZL in the ocular region. Most of the previous studies investigating prognostic factors in ocular region lymphoma have been influenced by the wide variety of lymphoma subtypes.[22–25] However, recently Tanimoto et al.[34] reported data concerning 114 Japanese patients with EMZL arising in the ocular region, and found, similar to our results, that PFS- and OS rates were not related to the initial stage of disease. In our cohort, the females had a significantly higher OS than did the males, contrary to the findings of Plaisier et al.,[24] who found that females had the more adverse prognosis. Their analysis, however, was based on 54 patients with a variety of lymphoma subtypes in the ocular region of which 27 patients had EMZL. In our analysis, the IPI score was the most reliable prognostic factor for OS. However, considering the limitations of applying Cox models to the small number of events and low death rate (75%, 5-year survival) observed in our study, the results of the multivariate analysis of prognostic factors for OS should be interpreted with caution.

Recurring structural abnormalities in EMZL include at least four balanced translocations resulting in API2/MALT1, IGH/MALT1, Bcl-10/IGH, and FOXP1/IGH rearrangements. All these, except the translocation involving FOXP1, lead to formation or upregulation of proteins (API2-MALT1, MALT1, and Bcl-10) that ultimately target the same signaling pathway (NFκB, a transcription factor with effect on a number of proliferation-related genes in B cells).[35]

The frequencies of the translocations seem to vary depending on the anatomic site at which EMZL arises.[14,15] We analyzed 42 EMZLs, presenting primarily in the ocular region, for the presence of split signals at the *IGH* locus (14q32) or the *MALT1* locus (18q21) and found that only 2 (5%) had a rearrangement. One was proven to be an IGH/MALT1 translocation, whereas the other showed evidence of *Bcl-6* gene breakage, suggesting the presence of an IGH/Bcl-6 fusion. Bcl-6 is a DNA binding transcription factor of germinal center B-cells that plays important roles in regulating differentiation, survival, and genetic stability of B-cells.[36] Translocations involving Bcl-6 are frequently seen in nodal diffuse large B-cell lymphoma and in grade 3 follicular lymphoma.[37,38] However, the occurrence in EMZL is rare. Recently, Ye et al.[39] found 7 of 392 EMZLs with a Bcl-6 translocation of which IGH was found to be the translocation partner in four.

The low frequency of translocations involving the IGH or MALT1 loci found in our study is in keeping with most other reports, describing frequencies from 0% to 10%.[14,17,40–43] However, Streubel et al.[15,44,45] have conducted three studies reporting frequencies of the IGH/MALT1 and FOXP1/IGH in 24 to 37% and 20%, respectively. Even the frequency of the API2-MALT1 translocation, although generally accepted to be almost nonexistent in ocular region EMZL, has been reported in up to 13% in two studies conducted in Europe and Japan.[46,47] The variable frequencies of these aberrations indicate that not only site, but also geographical and environmental conditions, may play an important role in the type of genetic abnormalities in EMZL. Although they vary, the frequencies of the known specific translocations are generally low in ocular EMZL, compared with gastric- or pulmonary EMZL, emphasizing the need for future studies elucidating the molecular pathogenesis of this disease. We found a statistically significant shorter PFS for patients with IGH translocation-positive tumors. Even though this should be interpreted with caution due to the low number of positive tumors, our results indicate that IGH translocation may be associated with a more aggressive course of disease.

The discovery of t(1;14)(p22;q32) in EMZL, resulting in deregulation of the Bcl-10 gene, has led to studies of the presence of Bcl-10 at the protein level by immunohistochemistry.[17,47] Bcl-10 is involved in development and function of B- and T-lymphocytes and is expressed exclusively in the cytoplasm. An aberrant nuclear Bcl-10 expression has been found in 30% to 60% of EMZL and is particularly evident in t(1;14)- and t(11;18)-positive tumors.[17,40,47] However, translocation-negative tumors may also show nuclear expression. Furthermore, two studies indicate that presence of Bcl-10 in the nucleus is related to prognosis.[18,20] We found nuclear Bcl-10-expression in 22 (29%) of 75 of our specimens, and these cases did not differ from the remaining cases with respect to clinical parameters. Thus, consistent with the report of Vejabhuti et al.,[48] the prognostic significance of nuclear Bcl-10 staining in ocular region EMZL could not be confirmed in our study.

In conclusion, we have performed a large population-based study focusing on the clinical, immunophenotypic, and cytogenetic characteristics of EMZL arising in the ocular region. We found that EMZL in the ocular region usually has a quite indolent course, despite presenting with stage IV disease in approximately one fourth of cases and despite a high frequency of relapse. The well-established IPI score was the most reliable independent parameter for estimating risk of death in our large cohort of patients. Furthermore, we found that the frequency of translocations involving the *MALT1* and *IGH* gene loci is low in ocular region EMZL, but may predict increased risk of relapse.

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