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Outcome and Prognostic Factors in Ocular Adnexal Lymphoma

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Aim. To classify ocular lymphomas in patients treated at the Zagreb University Hospital Center according to the new classification of the World Health Organization (WHO) and to determine factors with prognostic significance.

Methods. From 1986 to 2003, histological diagnosis of ocular lymphoma was made in 24 patients. The median age of patients was 62 years, with 2:1 female predominance. The patients underwent staging procedures and clinical evaluations prior to the date of the initial therapy. Histopathologic slides were reviewed and tumors were classified according to the new WHO classification. Additional immunohistochemical studies were performed on 35 available specimens. The antibodies used were CD3, CD5, CD10, CD20, CD43, and bcl-6; and in a few cases cyclin D1, bcl-2, CD23, CD79a, and CD138. The main outcome measures were development of distant recurrence after new presentation with solely ocular adnexal disease, and death attributable to widespread lymphoma.

Results. Ocular adnexal lymphomas were found in orbit in 20 patients, in eyelid in two, and conjunctiva in two patients. Twenty patients had lymphoma stage IE, one had IIE, and three had stage IV. Three patients had prior or concurrent systemic disease and 21 patients had primary lymphoma. The main subtypes of non-Hodgkin lymphoma according to the WHO classification were extranodal marginal zone B-cell lymphoma (n = 20), diffuse large cell B-cell lymphoma (n = 2), mantle cell lymphoma (n = 1), and plasmacytoma (n = 1). Six lymphomas were CD43 positive and five of them were extranodal marginal zone B-cell lymphomas. Radiotherapy was given to 11 patients, chemotherapy in 8 patients, whereas radiotherapy and chemotherapy were implemented in three patients. Two patients underwent only surgical excision of the tumor. Local relapse was found in three and distant recurrence in four patients. Distant recurrence was found in four patients with stage IE (two of them also had a local relapse). In the group of patients with extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (B-EMZL), the estimated 5-year overall survival was 92.9 ± 6.6% (mean ± standard deviation) and the 5-year failure-free survival was 80.1 ± 10.3%. Age, sex, side of involvement, anatomic localization of the lesion, clinical stage of disease, and mode of therapy did not have any prognostic significance during the follow-up period (median, 53; range, 9-131 months). Immunohistochemical marker CD43 was the only parameter of prognostic significance (p = 0.035). Patients with B-EMZL had almost 14 times higher chance for an unfavorable outcome if the tumor cells expressed CD43 on their surface, than the CD43-negative cases.

Conclusion. Most ocular adnexal lymphomas usually have a B-cell immunophenotype, the morphologic and immunohistochemical features of extranodal marginal zone B-cell lymphoma, and a favorable prognosis. Our data suggest that CD43 could be useful to separate the group of patients with extranodal marginal zone B-cell lymphomas with unfavorable prognosis from those that have a good prognosis. CD43 positive ocular lymphomas are associated with a higher rate of subsequent distant recurrence and the rate of lymphoma-related death.

Key words: *antigens, CD; classification; lymphoma, B-cell; lympohoma, mucosa-associated lymphoid tissue; orbital neoplasms; survival*

The majority of ocular adnexal lymphomas present usually as a primary disease of the orbital soft tissue, conjunctiva, and eyelid. These tumors originate from B-cells in the eye and related tissues. The most common type is an extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (B-EMZL), although diffuse large cell B-cell lymphomas (B-DLCL), mantle cell lymphomas (B-MCL), follicular lymphomas, and plasmacytomas also occur (1-7). Ocular adnexal B-EMZL is a rare disease. With a few exceptions (1,6,8), almost all reported studies have included a relatively small number of patients (3-5,7,9-11), so information regarding the prognosis and adequacy of tumor control is limited.

Most ocular adnexal lymphomas present as primary localized disease, whereas others occur as a

part of systemic disease. If a patient has a lymphoma limited to the ocular adnexa, after systemic evaluation a local therapy is recommended (2,4,6,9,10). The prognosis for ocular adnexal lymphomas is closely related to the age of patients (12,13), clinical stage at presentation (1,2,12,13), localization (2,12,13), blood lactate dehydrogenase values (12), signs and symptoms of disease (13), response to treatment (12), histologic type (1,2), and immunohistochemical markers such as MIB-1 (Ki-67) and p53 (1,2,4).

The development of immunohistochemistry and molecular biology techniques, such as the polymerase chain reaction (PCR), has allowed better understanding of B-EMZL. The tumor cells express B-cell-associated antigens CD19, CD20, CD22, and CD79a, and usually lack others, such as CD5, CD10, and CD23. Rare cases of apparently CD5 positive B-EMZL have been described (1,2). Some of these patients had reached the advanced stage of the disease from which they died within 5 years after diagnosis. Another immunohistochemical marker, which is also helpful in diagnosing B-EMZL, is CD43. Essentially, all white blood cells, except resting mature B-cells, express CD43 (14). Consequently, the immunohistochemical demonstration of CD20 and CD43 co-expression can be extremely useful in distinguishing a B-cell non-Hodgkin lymphoma from reactive lymphoid hyperplasia. The prognostic impact of CD43 expression on tumor cells of orbital adnexal B-EMZL is unknown.

The aim of this study was to re-examine the orbital adnexal lymphomas found in patients treated at our hospital, classify them according to the new WHO classification, and correlate clinical, histopathological, and immunohistochemical parameters with the clinical outcome.

Patients and Methods

Patients

Thirty-five biopsy specimens of ocular lymphomas in 28 patients were collected between 1986 and 2003 at the Department of Pathology, Zagreb University Hospital Center. Four cases were eliminated because of the following reasons: a) one of them had intraocular lymphoma; b) a diagnosis other than non-Hodgkin lymphoma was made upon re-review (the patient had only lymphoid hyperplasia); c) slides of primary tumor were no longer available for review; or d) clinical data were not available. This left 24 patients and 31 biopsy specimens for further analysis.

The medical records of patients with orbital lymphoma were reviewed. Clinical data analyzed for each patient included age at lymphoma presentation, sex, side of involvement, anatomic localization of the lesion, presenting signs and symptoms, duration of orbital symptoms, clinical stage of disease at diagnosis, type and extent of therapy, course of disease, disease-free period, and duration of survival. The data were available for all patients, except for duration of symptoms in 7 cases, and orbital signs and symptoms in 4 of them. The anatomic localization of the lesions was defined as proposed by Knowles et al (2). The patients were staged according to the Ann Arbor system (15). *Primary lymphoma* was defined as primary involvement of the ocular adnexa at the time of presentation, and *secondary lymphoma* as a secondary involvement of ocular adnexa in a patient who had a history of prior nonorbital lymphoma. Patients with bilateral but no extraorbital involvement were included in stage IIE category. In most cases, orbital imaging studies included ultrasonography, orbital computerized tomography (CT), and/or magnetic resonance imaging (MRI). Systemic staging workup included complete blood count, chest X-ray, abdominal sonography or CT, and bone marrow biopsy. The patients underwent staging procedures and clinical evaluations prior to the date of the initial therapy. The treatment was not uniform and many different therapies were used in this group of patients.

Methods

The tissue biopsy specimens had been fixed in 10% formaldehyde solution and embedded in paraffin. Conventional histologic stains included hematoxylin and eosin. Two pathologists (N.M. and B.M) reviewed the histological and immunohistochemical pattern of each lesion. They were not aware of the clinical outcome. The lymphomas were further classified on the basis of morphologic features and immunophenotpye according to the WHO classification. The antibodies used were CD3, CD5, CD10, CD20, CD43, and bcl-6. In a few cases we also used cyclin D1, bcl-2, CD23, CD79a, and CD138. All antibodies were obtained from DAKO (Glostrup, Denmark) and Novocastra (Newcastle upon Tyne, UK).

Statistical Analysis

The Kaplan-Meier method was used to estimate the overall and failure-free survival. Overall survival time was calculated as the time from the diagnosis to the date of death (endpoint reached) or last contact (censored). Failure-free survival was calculated as the time from the date of diagnosis to either date of distant relapse or death (endpoint reached) or last contact (censored). Cox proportional-hazards regression was used to analyze the effect of several parameters as possible risk factors on overall and failure-free survival. Cox regression statistics is presented with regression coefficient (b), standard error of regression coefficient (SE(b)), and odds ratio (OR) with 95% confidence intervals (95% CI). Only p-values lower than 0.05 were considered significant. Statistical analysis was performed by using MedCalc statistical package (Version 7.3, Frank Schoonjans, Mariakerke, Belgium).

Results

Among 24 patients with ocular adnexal lymphoma, there were 16 women and 8 men, aged between 34 and 77 years (median, 62 years; Table 1). Right side involvement was present in 13 patients, and 20 lymphomas were located in the orbital soft tissue. Visible mass was present in 15 patients and this was the most frequent clinical sign. Duration of symptoms was less than 12 months in 11 patients, with a median of 6 months (range, 1-40 months). According to Ann Arbor system, 20 patients had stage I (stage IE), one patient had stage II (bilateral tumors), and three patients had stage IV disease. There were 20 cases diagnosed as B-EMZL, two cases as B-DLCL, one case as B-MCL, and one case as plasmacytoma. In the group of patients with B-EMZL, the tumor cells in all cases were positive for CD20 and negative for CD3, CD5, CD10, cyclin D1, and bcl-6. The tumor cells were positive for CD43 in 5 B-EMZL cases.

Two patients with orbital adnexal lymphoma were treated with surgical excision only. Relapse did not occur in either patient. Eleven patients were treated with localized radiotherapy alone. Chemotherapy was the initial treatment in 8 patients, and 3 patients underwent combined localized radiation therapy and systemic chemotherapy. All patients treated with radiotherapy received a total dose (TD) of 30-40 Gy.

At the time of analysis, follow-up information was obtained for all 24 patients, out of whom 20 were alive at the time of the last contact and four had died. The median follow-up time of surviving patients was 52 months (range, 9-131 months). Overall, five patients exhibited disease recurrence at 16-91 months. In the group of patients with primary disease, local re-

Table 1. Clinical characteristics of 24 patients with ocular adnexal lymphoma

Patient characteristics	No. of patients
Age:	
<60 years	14
>60 years	10
Sex:	
female	16
male	8
Side of involvement:	
right	13
left	11
Anatomic localization:	
orbital soft tissue	20
eyelid	2
conjunctiva	2
Orbital signs and symptoms:	
visible mass	15
ptosis	5
decreased vision	4
exophthalmia	4
motility restriction	4
pain	2
Duration of orbital symptoms:	
<12 months	11
>12 months	6
Clinical stage:	
I	20
II	1
III	0
IV	3
Histological type:*	
B-EMZL	20
B-DLCL	2
B-MCL	1
Plasmacytoma	1
CD43:	
negative	18
positive	6
Therapy:	
surgical excision only	2
radiotherapy only	11
chemotherapy only	8
radiotherapy and chemotherapy	3
Relapse:	
local	3
distant	4
Survival:	
alive	20
dead	4

*B-EMZL – extranodal marginal zone B-cell lymphoma; B-DLCL – diffuse large cell B-cell lymphoma; B-MCL – mantle cell lymphoma.

lapse was found in three and distant disease was found in four out of 21 patients (two of them had also a local relapse). Local relapse was observed on average after a median latency period of 24 months (range, 16-52 months), whereas distant recurrence occurred on average after a median latency period of 63 months (range, 28-91 months). In the group of patients with clinical stage IV, only a single patient survived and two other died. One of these patients had B-EMZL, and the other had B-DLCL. The other patient with B-DLCL was diagnosed as stage IE disease and was alive at the time of last contact.

Cox analysis of risk factors affecting overall and failure-free survival was performed for 20 patients with B-EMZL. In the group of patients with extranodal marginal zone B-cell lymphoma of mucosa associated lymphoid tissue, the estimated 5-year overall survival was 92.9±6.6% (mean±standard deviation) and the 5-year failure-free survival was 80.1±10.3% (Fig. 1). In Cox analysis of risk factors for overall survival, there were no parameters that influenced the

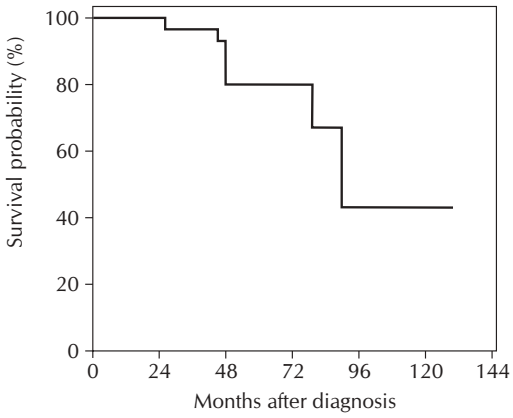

Figure 1. Failure-free survival of 20 patients with extranodal marginal zone B-cell lymphoma (B-EMZL).

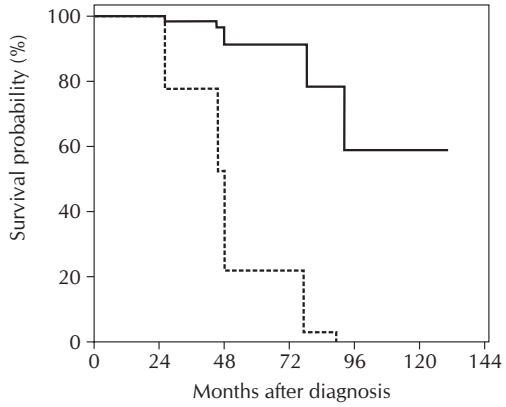

Figure 2. Failure-free survival of 20 patients with extranodal marginal zone B-cell lymphoma according to CD43-positive (dashed line, n=5) and CD43-negative (full line, n=15) cases.

prognosis (data not shown). Patient age, sex, side of involvement, anatomic localization of the lesion, clinical stage of disease at diagnosis, and mode of therapy did not influence failure-free survival (Table 2). Immunohistochemical marker CD43 was the only parameter of prognostic significance (p=0.035, Table 2) in the group of patients with B-EMZL according to failure-free survival (Fig. 2). The five patients with B-EMZL had almost 14 times higher chance for occurrence of distant relapse or death if the tumor cells expressed CD43 marker than did CD43 negative patients (odds ratio=13.78, Tables 2 and 3).

Table 2. Cox regression analysis of risk factors affecting failure-free survival of 20 patients with extranodal marginal zone B-cell lymphoma (B-EMZL)

	Regression analysis*			
Patient characteristic	b	SE (b)	p	OR (95% CI)
Age	-0.518	0.925	0.575	0.59 (0.01-3.65)
Sex	0.262	0.916	0.775	1.30 (0.21-7.82)
Side of involvement	-0.433	1.162	0.710	0.65 (0.07-6.32)
Anatomic localization	1.364	1.233	0.268	3.91 (0.35-43.84)
Clinical stage	1.108	1.228	0.367	3.03 (0.27-33.61)
CD43 positivity	2.624	1.243	0.035*	13.78 (1.22-155.66)
Mode of therapy	-0.323	0.924	0.726	0.72 (0.11-4.42)

*SE – standard error of b; OR – odds ratio; CI – confidence interval.

Table 3. Patient characteristics, immunohistochemical data, and treatment techniques of 5 patients with CD43+ extranodal marginal zone B-cell lymphoma*

	Patient characteristics		Cancer characteristics											Relapse	
No. of patient	age (years)	sex	site	stage	CD3	CD5	CD10	CD20	bcl 6	cyclin D1	therapy	status	survival (months)	local	distant
1	43	F	Conj	I	-	-	-	+	-	-	R	alive	56	-	+
2	71	M	Orb	I	-	-	-	+	-	-	C	alive	31	-	-
3	62	M	Orb	I	-	-	-	+	-	-	C+R	alive	20	-	-
4	58	M	Orb	IV	-	-	-	+	-	-	C+R	dead	48†		
5	76	F	Orb	I	-	-	-	+	-	-	R	alive	9	-	-

*Abbreviations: F – female; M – male; Conj – conjunctiva; Orb – orbital soft tissue; R – radiotherapy; C – chemotherapy.
†Secondary lymphoma, ie, secondary involvement of ocular adnexa in a patient who had a history of prior nonorbital lymphoma.

Discussion

Similar to other series (1,3,4,6,7,11), the most common lymphoma subtype in our collection of ocular adnexal lymphomas was extranodal marginal zone B-cell lymphoma (more than three-quarters), which is characterized by the presence of neoplastic centrocyte-like cells distributed in a marginal zone pattern around reactive follicles and frequently attenuated rims of mantle zone lymphocytes. Pseudofollicular proliferation centers and starry-sky appearance were absent in all cases. Tumor cells were positive for CD20 but negative for CD3, CD5, CD10, bcl-6, and cyclin D1. On the basis of these morphological and immunohistochemical characteristics, we excluded the B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma, follicular lymphoma, mantle cell lymphoma, and Burkitt lymphoma.

CD43 is expressed by essentially all white blood cells, except resting mature B cells (14). This includes activated B-cells, plasma cells, NK cells, granulocytes, and monocytes/macrophages. Immunohistochemical detection of CD43 expression by non-neoplastic B-cells is extremely uncommon. Consequently, CD43 expression on B cells is an immunophenotypic feature suggestive of malignancy (14,15). CD43 is expressed by most lymphoblastic lymphomas/ leukemias, T-cell lymphomas, and 30% of all non-lymphoblastic B-cell lymphomas (15). CD43 positive cells in our group of B-EMZL displayed strong immune reactivity in all five cases. In the group of B-EMZL, tumor cells positive for CD43 were seen in a quarter of patients, a finding similar to the percentage reported by Lai et al (16). According to these authors, positivity for this marker in the group of B-EMZL is between 20-40%.

In the analysis of prognostic factors, our study disclosed that age, sex, side of involvement, anatomic localization of the lesion, stage of disease at diagnosis, and mode of therapy did not have prognostic significance during a follow-up period. Immunohistochemical marker CD43 was the only parameter of prognostic significance for failure-free survival in the current study. To our knowledge, the prognostic significance of this maker in the group of B-EMZL of ocular adnexa has not been reported so far.

Seventeen patients with B-EMZL stage I represented the largest subgroup in our sample. Similar data were obtained in other studies (1,2,4,6). In many reports (1,2,4,12,13), clinical stage of disease was the most important prognostic parameter. We could not prove statistical significance of this parameter in our study due to the small number of patients (we did not make a multiinstitutional research).

The second most common lymphoma subtype in our series was the B-DLCL. Coupland et al (1) showed that orbital B-DLCL was frequently a part of systemic disease in contrast to B-EMZL, which occurred mostly as a primary orbital lymphoma (1). This was true in our study as well: three patients presented with clinical stage IV and one of them had B-DLCL. The second patient with B-DLCL was diagnosed in stage IE disease and was alive at the time of last contact, but his follow-up lasted only 28 months.

In the group of patients with ocular adnexal lymphoma, the most common sign was a visible tumor mass. This observation is similar to that reported by others (6,10,13). In contrast to some reports (2,13) but in agreement with others (1,4,9,10), there was no difference in the clinical course of the disease between patients with lymphomas located in the conjunctiva and those in the lid or orbit. In our study, the tumors were designated as orbital if any aspect of the retrobulbar tissues was infiltrated with the tumor. This can partially explain the fact why we had more orbital tumors than reported in some other studies (2). Despite this minor difference, the treatment outcome, ie, the 5-year overall survival and failure-free survival in our group of patients is similar to the treatment outcome from other centers worldwide (6,9,10,17).

There was no evidence that the clinical stage, age of patients, anatomic localization of the lesion, or mode of the therapy made an impact on the failure-free survival. Yet, the number of patients was quite small in our study. In this study we could not investigate the value of some other well-known prognostic factors such as lactodehydrogenase value, immunohistochemical markers, such as MIB-1 (Ki-67) and p53, and cytogenetic or molecular features (1,2,4,11, 18). A larger, collaborative study with implementation of new biological markers is warranted to identify prognostic factors that influence overall and failure-free survival of patients with B-EMZL. However, immunohistochemical marker such as CD43 has the advantage of being inexpensive and easy to evaluate in comparison with other methods. It would also be of interest to examine the differences in the patient's outcome within a group of patients with other extranodal marginal zone lymphoma from other localizations.

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