Plasmacellular differentiation in extranodal marginal zone B cell lymphomas of the ocular adnexa: an analysis of the neoplastic plasma cell phenotype and its prognostic significance in 136 cases

S E Coupland, M Hellmich, C Auw-Haedrich, W R Lee, I Anagnostopoulos, H Stein

Aim: To determine (a) the expression of plasma cell related antigens in extranodal marginal zone B cell lymphomas (EMZL) of the ocular adnexa; and (b) the prognostic value of plasmacellular differentiation in these tumours.

Methods: A consecutive case series of 136 ocular adnexal EMZL obtained from three ocular pathology centres over 20 years was analysed retrospectively. An extensive immunohistochemical panel, including the plasma cell related antigens VS38c, CD38, CD138, multiple myeloma oncogene-1-protein (MUM1/IRF4), and CREB binding protein (CBP) was performed. EMZL were defined as "plasmacellular differentiated" on the basis of morphological features, evidence of cytoplasmic immunoglobulin, negativity for BSAP/PAX5, and expression of at least one of the investigated plasma cell related antigens. Controls included normal or hyperplastic lymphatic tissues. Detailed clinical data were collected for most patients, and compared with the results of immunohistochemistry. The end points considered for statistical analysis were development of local tumour recurrence, development of systemic disease, and lymphoma related death.

Results: 57 (42%) of the 136 ocular adnexal EMZL showed a plasmacellular differentiation; 45 of these plasmacytoid cases were primary tumours. In contrast with most admixed normal plasma cells, which displayed co-expression of MUM1/IRF4, VS38c, CD38, CD138, and CBP, the plasmacellular differentiated EMZL tumour cells demonstrated co-expression of all five plasma cell related antigens in only six of 57 (11%) plasmacellular differentiated ocular adnexal EMZL. The most commonly expressed plasma cell related antigen was MUM1/IRF4, immunoreactivity being seen in 56/57 (98%) plasmacellular differentiated EMZL examined. Although the association of plasmacellular differentiation in primary ocular adnexal EMZL and disseminated disease was statistically significant on univariate analysis (p = 0.042), this was weaker on multivariate analysis.

Conclusion: Plasmacellular differentiated tumour cells in EMZL demonstrate an aberrant immune profile for plasma cell related antigens when compared with normal plasma cells. On multivariate analysis, plasmacellular differentiation in ocular adnexal EMZL was not significantly associated with local recurrence, the development of systemic disease, or with lymphoma related death.

The clinical significance of plasmacellular differentiation of tumour cells in small cell B-NHL in general is unclear. Patients with "plasmacytoid" B cell chronic lymphocytic leukaemia (B-CLL) are proposed to have a better prognosis.15–17 In this study, we determined which ocular adnexal EMZL could be defined as "plasmacellular differentiated" on the basis of morphology and of their immunophenotype (including evidence of cytoplasmic immunoglobulin (Ig) with light chain (IgL) restriction, negativity for B cell specific activator protein (BSAP, also known as PAX5), as well as expression of the plasma cell related antigens VS38c, CD38, CD138, multiple myeloma oncogene-1-protein (MUM1, also known as IRF4), and of the CREB binding protein (CBP)). The results were compared with clinical
data, in particular, with the development of local tumour recurrence, of systemic disease, and of lymphoma related death to establish whether plasmacellular differentiation of ocular adnexal EMZL has any value in the prediction of these events.

**METHODS**

**Tissue samples**

A series of 136 consecutive cases of ocular adnexal EMZL were collected and slides retrospectively reviewed from the pathology departments of Charité-Medical Faculty Berlin, Campus Benjamin Franklin, Berlin, Germany, and of Western Infirmary, Glasgow, UK, as well as from the Department of Ophthalmology, University Hospital Freiburg, Germany. The cases, which were collected between the 1980 and 2000, were assessed on the basis of morphology and immunochemistry and classified according to the new classification of the World Health Organization (WHO) Classification of Tumors of Haematopoietic and Lymphoid Tissues. Only those cases with biopsies of sufficient size, adequate morphology, appropriate fixation (4% buffered formaldehyde), and clinical information were analysed. In addition, 10 cases of normal or reactivity hyperplastic lymphoid tissue (RLH)—for example, hyperplastic tonsil and lymphoplasmacellular infiltrates within lacrimal glands, were evaluated.

**Immunohistology**

Immunohistochemical investigations were performed using monoclonal and polyclonal antibodies reactive in paraffin sections. A pressure cooker antigen retrieval method was performed before immunohistochemical staining. The staining consisted of a first stage incubation with the following primary monoclonal antibodies against the following antigens: CD79a; CD20; BSAP/PAX5; CD5; CD10; CD21; CD23; CD43; BCL2; cyclin D1; BCL6; VS38c; CD138; MUM1/IRF4; CBP; MIB-1 (Ki-67 antigen); as well as anti-pancytokeratin. Polyclonal antibodies were used for the CD3 antigen, and for immunoglobulin light and heavy chains (IgL and IgH, respectively). The antibodies were obtained from Dako (Glostrup, Denmark), Santa Cruz (CA, USA), Serotec (UK), Transduction and Novostra (UK) (table 1), except for MUM1/IRF4, which was provided by Dr B Falini (Perugia, Italy). Bound antibodies were visualised using an indirect immunoperoxidase method for the antibodies to IgL and IgH, whereas the alkaline phosphatase anti-alkaline phosphatase (APAAP) method was used for the remainder. Double staining of some tumours was performed: the first antigen was detected by an avidin-biotin-peroxidase technique using diaminobenzidine/hydrogen peroxide as substrate, and the second antigen, by the APAAP procedure using naphthol AS-MX plus Fast Red TR (Sigma-Aldrich) as substrate. Incubation omitting the specific antibody was performed before immunohistochemical staining. The staining of MUM1/IRF4 was localised predominantly to the nucleus, although weak cytoplasmic staining was also present in most cases with nuclear positivity. Strong staining in more than 20% of plasmacellular differentiated tumour cells was scored as positive, as defined by Tsuboi et al. Strong staining localised to the nucleus was considered positive for CBP; that localised to the plasma membrane was considered positive for CD138 and CD38; and that to the cytoplasm was considered positive for Vs38c.

**Medical history**

Detailed clinical information was gathered in all patients; those cases where significant data were not traceable, were excluded from the study. In order to obtain a follow up period of at least 2 years in patients still alive at the final examination, cases of ocular adnexal EMZL diagnosed after July 2000 were excluded. “Stage at presentation” was defined according to the modified Ann-Arbor classification scheme at the time of first diagnosis. Patients who had bilateral OAL without systemic manifestations were considered to have stage IE disease. “Primary disease” was defined as primary involvement of the ocular adnexa, corresponding to stage IE. “Secondary disease” was defined as a lymphomatous infiltration of the ocular adnexa by an identical lymphoma of another primary site.

**Statistical analysis**

Three clinically relevant end points were considered: (a) the development of local tumour recurrence; (b) the development of systemic disease; and (c) death related to lymphoma (time to event). For the three end point analysis, we also categorised the percentage of tumour cell expression for the proliferation marker MIB1 as previously described. The association of categorical variables was assessed by cross tabulation, exact Pearson χ² tests, and multiple logistic regression. Corresponding relative risks and odds ratios (multivariable) with 95% confidence intervals were calculated. The equality of “time to event” distributions was investigated by Kaplan-Meier estimates of “survival curves,” log rank tests, and (multiple) Cox regression. Corresponding hazard ratios (univariable and multivariable) with 95% confidence intervals were calculated. p Values (two tailed) lower than alpha = 5% were considered statistically significant.

**Definition of plasmacellular differentiation of EMZL**

Ocular adnexal EMZL were defined as plasmacellular differentiated when 10% or more of the tumour cells displayed morphological features similar to plasma cells, such as eccentric nuclei, violet coloured or basophilic cytoplasm, the presence of Dutcher bodies, and/or Russell bodies, as defined in the literature (fig 1). Confirmation was achieved as proposed by other authors using immunohistochemical investigations for evidence of cytoplasmic (c) Ig and IgL restriction, negativity for BSAP/PAX5, and expression of at least one of the investigated plasma cell related antigens (VS38c, CD38, CD138, MUM1/IRF4, and CBP). A hypothesis that plasmacellular differentiated EMZL of the ocular adnexa which demonstrated immunoreactivity for a larger number (if not all) of the five investigated plasma cell related antigens possibly being better differentiated tumours than those which displayed positivity for only a few antigens, we repeated the statistical evaluations for all chosen end points by increasing the number of plasmacellular antigen expressed (for example, 2, 3, or 4) required in the inclusion criteria.

Normal plasma cells entrapped within the lymphoma were excluded from the evaluation. The distinction between such plasma cells and neoplastic cells with plasmacellular differentiation was based on features such as the maturity of cell morphology and the presence or absence of atypia (fig 1).

The staining of MUM1/IRF4 was localised predominantly to the nucleus, although weak cytoplasmic staining was also present in most cases with nuclear positivity. Strong staining in more than 20% of plasmacellular differentiated tumour cells was scored as positive, as defined by Tsuboi et al. Strong staining localised to the nucleus was considered positive for CBP; that localised to the plasma membrane was considered positive for CD138 and CD38; and that to the cytoplasm was considered positive for Vs38c.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD79a</td>
<td>JCB117</td>
<td>Dako, Denmark</td>
</tr>
<tr>
<td>CD20</td>
<td>L26</td>
<td>Dako, Denmark</td>
</tr>
<tr>
<td>BSAP/PAX5</td>
<td>Z4</td>
<td>Transduction Laboratories, Lexington, KY, USA</td>
</tr>
<tr>
<td>VS38c</td>
<td>D-4</td>
<td>Dako, Denmark</td>
</tr>
<tr>
<td>CD138</td>
<td>B-84</td>
<td>Serotec, UK</td>
</tr>
<tr>
<td>CD38</td>
<td>SPC32</td>
<td>Novostra, UK</td>
</tr>
<tr>
<td>CBP</td>
<td>C-1</td>
<td>Santa Cruz Biotechnology, CA, USA</td>
</tr>
</tbody>
</table>

*Table 1 B cell and plasma cell associated antibodies used with corresponding clones and commercial sources*
Figure 1  Illustration of normal and neoplastic plasma cells. (A) Normal plasma cells in lacrimal glands with their characteristic morphological appearance with eccentric nuclei with a "clock face" or "cartwheel" pattern distribution of chromatin, abundant cytoplasm, and distinct perinuclear holes. (B) Extranodal marginal zone B cells with a plasmacellular differentiation demonstrated violet coloured or basophilic cytoplasm, the presence of Dutcher bodies, and/or Russell bodies (see also fig 4B) as well as atypical nuclear features.

Figure 2  Expression of the five investigated plasma cell related antigens in reactive lymphoid tissue. Subepithelial reactive plasma cells in tonsils were positive for (A) Vs38c (cytoplasmic), (B) MUM1/IRF4 (nuclear), (C) CD138 (membranous), (D) CD38 (membranous), and (E) CBP (cytoplasmic), with polytypical expression for the light immunoglobulin chains kappa (F) and lambda (G). The tonsillar epithelium also demonstrates positivity for CD138 (C); while the germinal centre cells display mild to moderate expression of CD38 (D). Original magnification, ×1000.
RESULTS
Clinical features and anatomical distribution
Most patients with ocular adnexal EMZL were female (F = 100; M = 36; F:M 2.7:1), with a median age of 64 years (range 19–91 years) at first diagnosis. The ocular adnexal EMZL were distributed as follows: orbit (n = 47 stage IIE and seven stage II, III, or IV) with lacrimal gland involvement in 8+2 cases; conjunctiva (n = 41 +7) and lid (n = 21+3). Synchronous bilateral EMZL were seen in 14+2 patients. In 43 cases, clinical information on this aspect was missing; however, subsequent treatment would indicate that these ocular adnexal EMZL must have been unilateral. Most ocular adnexal EMZL (117 of 136 cases, 86%) represented primary OAL (that is, stage I, IE lymphoma). Nineteen (14%) of the EMZL patients had disseminated disease at the initial examination (that is, stage II, III, or IV), with the ocular adnexal tumours in 10 (53%) of these cases representing secondary manifestations of systemic disease. In some patients with ocular adnexal EMZL, concurrent disease (representing stage IIE and above) was observed in other extranodal locations.

Histology and immunohistology
Normal or reactive hyperplastic tissues
The palatine tonsils and the interstitial tissue of the lacrimal glands contained an infiltrate of small bland mixed T and B lymphocytes with reactive lymphoid follicles. In the mantle zones, weak positivity for MUM1/IRF4 and CBP but not for Vs38c, CD38, or CD138 was observed (fig 2). Most germinal centre cells demonstrated weak to moderate positivity for CD38 (membranous), and CBP (nuclear strong, cytoplasmic weak), Vs38c (cytoplasmic), CD38 (membranous), CD138 (membranous), and CBP (nuclear strong, cytoplasmic weak), as well as polyclonal staining for IgH and IgL (fig 2). In some cases, occasional plasma cells failed to express CD138 and CBP, varying immunoreactivity for Vs38c, CD138 (fig 2), and CBP but not for MUM1/IRF4 or CD38 was seen in the adjacent epithelium of the tonsils and lacrimal acini.

Extranodal marginal zone B cell lymphoma
Briefly, the EMZL consisted of small cells resembling centrocytes, monocytoid B cells, or small lymphocytes, with weak nuclear immunoreactivity for MUM1/IRF4 and CBP, but were negative for Vs38c, CD38, and CD138.

Admixed plasma cells were seen in the subepithelial areas in the tonsils, in the interstitial tissue of the lacrimal glands, and occasionally in the reactive germinal centre. These cells demonstrated mature cytology with basophilic cytoplasm, eccentric nuclei, clumped “clock faces” like chromatin and perinuclear halos. Virtually all plasma cells, which were negative for BSAP, demonstrated strong co-expression of CD79a, MUM1/IRF4-protein (nuclear strong, cytoplasmic weak), Vs38c (cytoplasmic), CD38 (membranous), CD138 (membranous), and CBP (nuclear strong, cytoplasmic weak), as well as polyclonal staining for IgH and IgL (fig 2). In some cases, occasional plasma cells failed to express CD138 and CBP. Varying immunoreactivity for Vs38c, CD138 (fig 2), and CBP but not for MUM1/IRF4 or CD38 was seen in the adjacent epithelium of the tonsils and lacrimal acini.

Table 2 Immunophenotype of normal plasma cells as well as of non-plasmacytoid and plasmacytoid cells in EMZL

| Normal plasma cells | CD79a+, CD20+, BSAP/PAX5−, polyclonal IgL, MUM1−, CD138+, CD38−, Vs38c−, CBP+* | CD79a−, CD20−, BSAP/PAX5+, CD38+, CD138+, CD38+, MUM1+, CD79a+, CD20+, BSAP/PAX5+, CD43+*, monocular IgH (usually IgM−) |
|-------------------|---------|----------------|----------------|
| Non-plasmacytoid cells in EMZL | MUM1−, CD138−, CD38−, Vs38c−, CBP− | MUM1−, CD138−, CD38−, Vs38c−, CBP−, Cyclin D1−, CD5+, CD23−, CD10+, BCL6−, CD79+, CD20−, BSAP/PAX5−, CD43+, BCL2−, monocular IgL (kappa−>lambda+), monocular IgH− (usually IgM+) |
| Plasmacytoid cells in EMZL | MUM1−, CD138−, CD38−, Vs38c−, CBP+ | MUM1−, CD138−, CD38−, Vs38c−, CBP+, cyclin D1−, CD5−, CD23−, CD10−, BCL6− |

* + = positive; − = negative; ** in most cases: IgL, immunoglobulin light chain; IgH, immunoglobulin heavy chain.
occasional blasts surrounding reactive B cell follicles (fig 3). Lymphoepithelial lesions were seen in some conjunctival specimens and in the orbital cases with lacrimal gland involvement. In most cases, the tumour cells expressed CD20, BSAP, CD79a, CD43, and BCL2 with absence of staining for CD10 and CD23 (fig 3) (table 2). Furthermore, scattered extrafollicular blasts stained for BCL6 or MUM1/IRF4 protein (fig 3). The MIB1 growth fraction ranged from 2–50%, median 10%. In cases where an increased number of blasts were present in the marginal zone, an increased percentage of tumour cells (>10%) positive for either BCL6 or MUM1/IRF4, together with an increased growth fraction, was observed.

Fifty seven of 136 (42%) EMZL demonstrated plasmacellular differentiation, fulfilling the criteria as mentioned above (tables 2 and 3); 45 (33%) of these “plasmacytoid” tumours represented stage I, IE EMZL. The tumour cells of the plasmacellular differentiated EMZL were positive for CD79a but negative for CD20 and BSAP (table 2). They demonstrated clg and a monotypical expression for IgL or IgH, as well as a variable positivity for MUM1/IRF4, VS38c, CD38.
CD138, and CBP (fig 3) (tables 2 and 3). The most consistently expressed plasma cell related antigen of the plasmacytoid tumour cells was MUM1/IRF4, its immuno-reactivity being seen in 56/57 (98%) plasmacellular differentiated EMZL examined (fig 4). The other plasma cell related markers showed a variable expression with CD138 protein positivity in 52/57 (91%) cases, CD38 in 51/57 (89%), V$\alpha$38c in 41/57 (72%), and CBP in 10/57 (18%) cases. Co-expression of all plasma cell related antigens by the “plasmacytoid” EMZL tumour cells was observed in only six of the 136 (4%, table 3).

**DISCUSSION**

Plasma cells are derived from B cells during immune responses and can be essentially divided into short and long lived plasma cells. Short lived plasma cells, with a life span of a few days, are generated following the response and are, therefore, not listed.

### Table 3 Immunophenotype of the tumour cells in the plasmacellular differentiated ocular adnexal EMZL (BSAP negative, kappa, or lambda positive)

<table>
<thead>
<tr>
<th>Immunophenotype*</th>
<th>EMZL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stage I, IE</td>
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<tr>
<td>MUM1+/V$\alpha$38c+/CD138+/CD38+/CBP-</td>
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<tr>
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</tr>
<tr>
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</tr>
<tr>
<td>MUM1+/V$\alpha$38c+/CD138+/CD38+/CBP-</td>
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</tr>
<tr>
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<tr>
<td>MUM1+/V$\alpha$38c+/CD138+/CD38+/CBP+</td>
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</tr>
<tr>
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<td>1</td>
</tr>
<tr>
<td>MUM1+/V$\alpha$38c+/CD138+/CD38+/CBP+</td>
<td>1</td>
</tr>
<tr>
<td>MUM1+/V$\alpha$38c+/CD138+/CD38+/CBP+</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
</tr>
</tbody>
</table>

*The remaining plasma cells related antigen combinations were not represented in this sample of 136 lymphomas and are, therefore, not listed.

A statistical significance could not be demonstrated using univariate or multivariate analysis between plasmacellular differentiation of the tumour cells and either local tumour recurrence or lymphoma related death. For the latter, the parameters which reached statistical significance on multivariate analysis were (a) age at diagnosis (in years, p = 0.058, hazard ratio with 95% confidence interval: 1.07 (1.00 to 1.15)) and (b) an increased growth fraction (p = 0.006, HR with 95% CI: 6.01 (1.65 to 21.80)). As described above, alternative definitions of plasmacellular differentiation were investigated—that is, simultaneous negativity for BSAP, positivity for kappa or lambda and positivity of at least one, two, three, or four of the markers MUM1, CD138, V$\alpha$38c, CD38, and CBP. Even when the tumour cells expressed more than one plasma cell related antigen, there was no significant alteration in the results for any of the end points (table 4).

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**Figure 5** Kaplan-Meier estimate of survival curve for stage I, IE patients with (solid line) and without (broken line) plasmacellular differentiation defined as simultaneous negativity for BSAP, positivity for kappa or lambda and positivity of at least one of the markers MUM1, CD138, V$\alpha$38c, CD38, and CBP (p value from log rank test: 0.404; hazard ratio with 95% confidence interval: 1.69 (0.49 to 5.84); tick marks correspond to censored observations).
transformation of IgM+/IgD+ “naive mature” B cells into extrafollicular B blasts after antigen contact. In contrast, long lived plasma cells, with a life span of possibly years, are derived either from germinal centre cells or from memory B cells, which have undergone the germinal centre reaction. Normally, long lived plasma cells are to be found only in peripheral lymphoid organs, the bone marrow, and at sites of immune responses, and are not detected in the peripheral blood. In their mature form, these cells have a typical morphological appearance, and are immunophenotypically characterised by positivity for CD79a, CD138, CD38, and VS38c by negativity for CD20 and BSAP.27 28

Whereas CD79a is expressed during most stages of B cell differentiation, both CD38 and CD138 are present on pre-B cells, lost during differentiation, and are re-expressed at the plasma cell stage in normal cells.29 30 VS38c is also expressed at this terminal stage of B cell differentiation, when CD20 and BSAP antigens are lost. Recently, plasma cells have also been described as expressing MUM1/IRF4,31 known to be active in the control of plasma cell differentiation. The co-activator of the cyclic AMP responsive element binding protein (CREB)—namely the CREB binding protein (designated CBP), was included in the present study as we have observed a strong cytoplasmic expression of this transcriptional co-activator in plasma cells in our investigations of normal and neoplastic lymphatic tissues (unpublished results). At what point CBP is expressed during normal B cell differentiation is yet to be established.

In the current investigation, 136 ocular adnexal EMZL were analysed for their degree of plasmacellular differentiation on the basis of morphology, evidence of clg, BSAP negativity, and the expression of five plasma cell related antigens. The results were subsequently correlated with three clinically relevant end points. Despite plasmacellular differentiation being described in up to 30% of EMZL,2 its clinical significance has not been subject to thorough investigation in any anatomical location to date. Furthermore, although some of the plasma cell related antigens investigated in the current study have been the subject of examination using both FACS and immunohistochemical analysis in haematopoietic malignancies,15 24 31–36 there are only limited (or no) analyses of the prognostic value of these markers.

Similar to previous studies,24 31 virtually all plasma cells in normal or hyperplastic lymphoid tissues demonstrated co-expression of VS38c, CD138, and MUM1/IRF4. Many but not all mature peripheral plasma cells also displayed strong membranous and cytoplasmic reactivity for CD38 and CBP, respectively; 57/136 (42%) EMZL examined demonstrated features of plasmacellular differentiation. In contrast with their non-plasmacellular differentiated counterparts, the “plasmacytoid” EMZL tumour cells were negative for the B cell marker BSAP, and demonstrated Ig light chain restriction as well as expression of at least one of MUM1/IRF4, VS38c, CD38, CD138, or CBP. In contrast with normal plasma cells, the neoplastic tumour cells of only six plasmacellular differentiated ocular adnexal EMZL demonstrated immunoreactivity for all five plasma cell related antigens (table 3). MUM1/IRF4 protein was the most stable plasma cell related antigen in the “plasmacytoid” EMZL of the ocular adnexa, being observed in 56/57 (98%) tumours (table 3). That MUM1/IRF4 expression is not exclusively observed in cells with plasmacellular differentiation has been previously reported by other authors.24 31 37 However, only those cells with the corresponding cytomorphology, clg/IgL, and BSAP negativity were considered in the interpretation of these stains. The remaining plasma cell related markers showed considerable variability with CD38 protein expression in 52/57 (91%) cases, CD38 in 51/57 (89%), VS38c in 41/57 (72%), and CBP in 10/57 (18%) cases (table 3). Therefore, most plasmacellular differentiated tumour cells of ocular adnexal EMZL demonstrate an aberrant plasma cell related antigen profile when compared to normal plasma cells. This aberrant immune profile of “plasmacytoid” EMZL, which we described recently in uveal EMZL,38 may aid the establishment of malignancy in ocular adnexal lymphoproliferative lesions, particularly in those EMZL where the interpretation of light chain expression can be difficult.

Various parameters have been described to be of prognostic value in patients with OAL, including anatomic location,14–62 stage at diagnosis,1 2 41 lymphoma subtyping according to the REAL classification1 4 7 41 and new WHO lymphoma classification;51 tumour cell growth fraction and increased blast percentages positive for BCL-6;15 21; as well as serum lactate dehydrogenase.6 The results of the current data suggest, however, that the clinical significance of the presence and degree of plasmacellular differentiation in EMZL of the ocular adnexa is not large. When defining plasmacellular differentiation of the tumour cells of the EMZL as described above, the relation between this tumour parameter and the development of systemic disease was statistically significant only on univariate analysis (p = 0.042). This significance was lost, however, on multivariate analysis when other factors of previously described importance,1 2 31—namely age >60 years at diagnosis and an increased MIB1 growth fraction of the tumour cells—were also considered.

Following a hypothesis that “plasmacytoid” EMZL of the ocular adnexa which demonstrated immunoreactivity for an increased number of the five investigated plasma cell related antigens (for example, ≥3) possibly being better differentiated than those which displayed positivity for only a few of the antigens (for example, <3), we repeated the statistical evaluations for all chosen end points. However, there was no major alteration in the results for any of the end points investigated on multivariate analysis.

In summary, most plasmacellular differentiated tumour cells in ocular adnexal EMZL demonstrate an aberrant

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**Table 4** Stage I, IE lymphoma (n = 117) and association of alternative definitions of plasmacellular differentiation and development of local tumour recurrence, systemic disease, and death related to lymphoma

<table>
<thead>
<tr>
<th>Number of markers positive for definition of plasmacellular differentiation</th>
<th>Development of local tumor recurrence</th>
<th>Development of systemic disease</th>
<th>Death related to lymphoma</th>
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<tbody>
<tr>
<td><strong>Univariate</strong></td>
<td><strong>Multivariate</strong></td>
<td><strong>Univariate</strong></td>
<td><strong>Multivariate</strong></td>
</tr>
<tr>
<td>RR (95% CI)</td>
<td>OR* (95% CI)</td>
<td>RR (95% CI)</td>
<td>OR* (95% CI)</td>
</tr>
<tr>
<td>≥1 or 2t</td>
<td>1.10 (0.56 to 2.15)</td>
<td>0.93 (0.36 to 2.40)</td>
<td>1.77* (1.06 to 2.98)</td>
</tr>
<tr>
<td>≥3</td>
<td>0.98 (0.49 to 1.94)</td>
<td>0.77 (0.29 to 2.02)</td>
<td>1.65 (0.99 to 2.78)</td>
</tr>
<tr>
<td>≥4</td>
<td>1.17 (0.55 to 2.46)</td>
<td>1.08 (0.38 to 3.05)</td>
<td>1.73* (1.04 to 2.90)</td>
</tr>
</tbody>
</table>

RR, relative risk (from cross tabulation); OR, odds ratio (from logistic regression); HR, hazard ratio (from Cox regression).

*Adjusted for age at diagnosis and MIB1 (<, > 10%).
**Statistically significant (p value ≤ 5%).

These definitions did not yield different results on this dataset.

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immune profile for plasma cell related antigens when compared to normal plasma cells. On multivariate analysis, plasmacellular differentiation in ocular adnexal EMZL had no (or very little) value in the prediction of local recurrence, the development of systemic disease, or for lymphoma related death. Strong predictors of (a) systemic tumor dissemination and (b) lymphoma related death included advanced age at diagnosis and an increased MIB-1 growth fraction. None of the investigated parameters in the current study seem to be predictive of local tumour recurrence.

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