Endothelial hyperplasia and endothelial galectin-3 expression are prognostic factors in primary central nervous system lymphomas

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Summary

Recently, considerable attention has been focused on the identification of clinically relevant prognostic markers for primary central nervous system lymphomas (PCNSL). The present study investigated whether three morphological features, i.e. necrosis, reactive perivascular T-cell infiltrate and endothelial hyperplasia, and galectin-1 and galectin-3 immunohistochemical expression have prognostic roles in a series of 58 PCNSL samples from 44 immunocompetent and 14 immunocompromised patients. The presence of endothelial hyperplasia (identified in 21% of the assessable cases) was identified as a bad prognostic factor for immunocompetent PCNSL patients, whereas the other morphological features were not associated with any prognostic value. Lymphomatous cells of eight PCNSL cases expressed galectin-3 without any prognostic value, and lymphomatous cells did not express galectin-1. In contrast, endothelial expression of galectin-3 was identified (by means of uni- and multi-variate analyses) as a bad prognostic factor for immunocompetent PCNSL patients. In addition, a combination of endothelial hyperplasia and/or endothelial galectin-3 expression was shown to be an independent prognostic factor for immunocompetent PCNSL patients treated with methotrexate-based chemotherapy. In summary, this study suggests that endothelial-related markers can identify risk groups of PCNSL patients and indicates that galectin-3 could be involved in PCNSL angiogenesis.

Keywords: central nervous system, lymphoma, endothelial hyperplasia, prognosis, galectin.
phomas (Shipp et al., 2002). Both scores are based on clinical data. In contrast, very few histopathological PCNSL features with prognostic value have been identified or included in a prognostic stratification. About 90% of PCNSL are diffuse large B-cell lymphomas (DLBCL) (Batchelor & Loeffler, 2006) and the lymphomatous cells are typically localized in the perivascular areas (Hochberg et al., 2007). B-cell migration inside the CNS depends on a selective interaction with the specialized brain endothelial cells (Alter et al., 2003). These interactions would at least partially explain the perivascular location of the neoplastic lymphocytes. However, the interplay between lymphoma cells and tumour vessels is probably more complex, particularly when considering the maturity of vessels and the angiogenesis patterns. Angiogenesis is known to play a differential role in various B-cell NHL and aggressive lymphomas, like DLBCL, are characterized by a high-microvascular density (Tzankov et al., 2007). While the degree of vascularity of many human tumour types has been shown to be an independent prognostic factor (Vacca et al., 1999; Folkert, 2000), only a few studies have investigated microvascular density in PCNSL and these have provided controversial results (Roser et al., 2004; Sugita et al., 2007; Takeuchi et al., 2007). All these data and our observations on endothelial morphological features have motivated us to investigate the presence of endothelial hyperplasia and its prognostic value in PCNSL. Very recently, Ponzoni et al. (2007) showed that the presence of reactive perivascular T-cell infiltrate (RVPI) was associated with a significantly better overall survival in the case of immunocompetent PCNSL patients who underwent methotrexate-based chemotherapy. In contrast, the presence of tumour necrosis did not demonstrate prognostic significance (Ponzoni et al., 2007). This is why we also investigated the presence of RVPI and tumour necrosis in our series. Considerable attention was also focused on the identification of clinically relevant prognostic biomarkers. In our study, we thus investigated whether members of the galectin family could play such prognostic roles. Galectins are animal lectins defined by their shared consensus amino acid sequences and their affinity for β-galactose-containing oligosaccharides (Kaltner & Gabius, 2001). Fifteen galectins have already been identified in a wide variety of tissue from different species. Galectins are found in extracellular matrices, as well as in the different cellular compartments (nuclear, cytoplasmic and cell-surface) (Liu, 2000; Kaltner & Gabius, 2001; Leffler, 2001; Danguy et al., 2002; Rabinovich et al., 2002, 2004; Liu & Rabinovich, 2005; Thijssen et al., 2007). Different galectins are already known to play a number of important roles in lymphoma biology. A study relying on gene profiling expression has shown that LGALS3 mRNA levels are among the best genomic discriminators between DLBCL and follicular lymphomas (Shipp et al., 2002). In agreement with Hoyer et al. (2004), we previously reported that galectin-3 is expressed in about half of DLBCL patients, in contrast to normal lymphoid tissues and other NHL, which rarely express galectin-3 (D’Haene et al., 2005). Moreover, while the blood vessels walls of the lymphomas expressed galectin-1, those of normal lymphoid tissues did not. This expression of galectin-1 in blood vessel walls of systemic lymphomas was correlated with vascular density (D’Haene et al., 2005).

The present study analysed the immunohistochemical expression of galectin-1 and galectin-3 in PCNSL from immunocompetent and immunocompromised patients. In view of the results obtained in the case of systemic DLBCL (see above) we paid additional attention to the expression of galectin-1 and galectin-3 in endothelial cells.

Materials and methods

Clinical and histopathological data
A series of 58 PCNSL samples, collected between 1990 and 2006 and prepared as archival formalin-fixed and paraffin-embedded samples, was obtained from the Pathology Laboratory of the Erasme University Hospital (Brussels, Belgium) and this series included 44 immunocompetent and 14 immunocompromised patients. All the cases were classified by two pathologists according to the World Health Organization (WHO) classification (Kleihaus & Cavenee, 2000).

As detailed in Table I the available clinical data included patient age and gender, ECOG PS, immune status, magnetic resonance imaging features (tumour location and multiple versus single lesion), serum LDH levels, CSF cytological examination, CSF protein concentration, diagnostic procedure, pre- and postsurgecal adjuvant treatments and follow-up. For immunocompetent patients, Table I also reports the data obtained for the two prognostic scores (MSKCC and an adapted version of IELSG). The prognostic MSKCC model defines three prognostic classes: class 1 (patients <50 years), class 2 [patients ≥50 years; Karnofsky PS ≥70 (ECOG PS: 0–1)] and class 3 [patients ≥50 years; Karnofsky PS <70 (ECOG PS: >1)] (Abrey et al., 2006). The IELSG score is a 5-point scoring system based on age (≤60 years vs. >60 years), ECOG PS (≤1 vs. >1), serum LDH level (normal vs. elevated), CSF protein concentration (normal vs. elevated) and involvement of deep brain structures (periventricular regions, basal ganglia, brainstem and/or cerebellum) (Ferreri et al., 2003). Unfortunately, the CSF protein concentration was only assessed for 24 patients in our series. Thus, we used an adapted version of the original IELSG score, designated ‘IELSG/4’, which included the four remaining criteria of the original IELSG scoring system.

Immunohistochemistry
The 5-μm-thick sections were subjected to standard immunohistochemistry, as previously detailed (D’Haene et al., 2005; Mathieu et al., 2005). The immunohistochemical expression was visualized by means of streptavidin-biotin-peroxidase complex kit reagents (BioGenex, San Ramon, CA, USA) with diaminobenzidine/H2O2 as the chromogenic substrate. Finally,
the sections were counterstained with haematoxylin. Galectin-1 and galectin-3 expression was evidenced by means of two specific monoclonal antibodies (Novocastra, Newcastle, UK; dilution 1:100). Negative controls were had the primary antibodies replaced by non-immune serum (Dako, Glostrup, Denmark).

Evaluation of morphological and immunohistochemical features

We focused our morphological evaluation on the presence or absence of necrosis, reactive perivascular T-cell infiltrate (RVPI) and endothelial hyperplasia. As detailed by Ponzoni et al (2007), RVPI was defined as the presence of at least one small-to-medium sized vessel surrounded by a rim of small-to-intermediate sized cells that display a T-cell phenotype and occur as a continuous multilayered accumulation close to the vessel wall or interposed between the vessel wall and a concentric rim of neoplastic lymphocytes. Endothelial hyperplasia was defined as vessels showing an increased number of endothelial cells with prominent nuclei as compared with the vessels presenting a classic capillary-type aspect (Fig 1A and B).

Galectin-1 and galectin-3 expressions were evaluated by two independent observers. When discrepancies were encountered, the cases were settled by consensus with a third observer. The lymphomatous and endothelial galectin staining was assessed by two features: staining intensity (absent, moderate or strong) and labelling index (0, <30%, 30–60%, and >60%). The staining was considered positive if the proportion of positive cells was >60% and/or the staining intensity was strong in lymphomatous cells or endothelial cells respectively.

Statistical analyses

The categorical data were analysed by means of contingency tables. The significance of the potential associations was evaluated with either the chi-square or the exact Fisher test (in 2×2 cases). The comparison of independent groups of ordinal or numerical data was carried out by means of the non-parametric Mann–Whitney test. Survival data were analysed using the standard Kaplan–Meier analyses (together with the Gehan–Wilcoxon test and its generalization in the case of more than two patient groups) and the multivariate Cox regression. All the statistical analyses were carried out using Statistica (Statsoft, Tulsa, OK, USA), and P-values <0.05 were considered significant.

Results

Clinical series analysis

The main patient characteristics are listed in Table I. The median age of the 58 patients was 62 years (range: 10–86 years). Twenty-one patients (36%) had an ECOG PS
strictly higher than 2. An elevated LDH serum level was detected in 33 (59%) of the 56 patients assessed. Lymphomatous cells assessed by CSF cytological examination were found in seven (22%) of the 32 patients. Twenty-four patients were subjected to a stereotactic biopsy and 34 patients underwent a craniotomy procedure for an open

Fig 1. Illustrations of morphological features and galectin-3 and galectin-1 immunohistochemical expression patterns encountered in primary central nervous system lymphoma (PCNSL). (A–B) Two cases of PCNSL either (A) lacking or (B) presenting endothelial hyperplasia (arrows; H&E; original magnification ×200). (C–D) Illustration of two PCNSL expressing galectin-3 in the endothelial cells of a vessel (C) without endothelial hyperplasia or (D) with endothelial hyperplasia, both cases exhibit no galectin-3 expression in the lymphomatous cells (original magnification ×400). (E) Illustration of galectin-3 expression in the lymphomatous cells of a PCNSL (original magnification ×200). (F) Illustration of the absence of galectin-1 expression in both the lymphomatous and the endothelial cells of a PCNSL (original magnification ×200).
biopsy (nine patients) or a tumour resection (25 patients). All the patients had DLBCL. Treatment modalities included steroid therapy before surgery for 35 patients. After surgery, seven patients underwent radiotherapy alone, 19 methotrexate-based chemotherapy and 17 both.

In addition, our series of immunocompetent patients was characterized by the two prognostic scores proposed in (or adapted from) previous literature (Ferreri et al., 2003; Abrey et al., 2006). Table I shows that the data distribution was similar between the two highest classes of the MSKCC system whereas only few cases were classified in class 1. The IELSG/4 score could be evaluated for 34 cases, which were mainly distributed between the three highest scores. When comparing the 44 immunocompetent and the 14 immunocompromised patients, the sole feature in Table I that showed a significant difference was patient age, which was significantly higher for immunocompetent patients (median: 68 vs. 39 years; \( P < 10^{-6} \)). The median survival was 3·1 months for immunocompetent patients and 2·4 months for immunocompromised patients without any significant difference.

**Morphological analysis**

The presence of necrosis was observed in 35 (i.e. 62·5%; 26 immunocompetent and nine immunocompromised patients) of the 56 PCNSL cases for which this feature could be evaluated. RVPI was present in 16 (i.e. 28·1%; 15 immunocompetent and one immunocompromised patients) of the 57 cases for which this feature could be evaluated. In addition, we identified 11 cases (i.e. 21%; nine immunocompetent and two immunocompromised patients) presenting endothelial hyperplasia of the 52 cases for which this feature could be evaluated (Fig 1A and B). No glomeruloid-type endothelial hyperplasia was observed.

**Galectin-1 and galectin-3 expression**

The available materials enabled galectin-1 and galectin-3 expression to be evaluated in 46 cases (37 immunocompetent and nine immunocompromised patients). While the majority of PCNSL (i.e. 29 of 46) showed no galectin-3 expression in the lymphomatous or endothelial cells, we identified 11 cases (including one immunocompromised) with endothelial expression of galectin-3 (Fig 1C and D) and eight cases (including one immunocompromised) presenting galectin-3 immunostaining in lymphomatous cells (Fig 1E). Of the 17 positive samples, two showed galectin-3 expression in both cell types. Of the 11 cases with endothelial expression of galectin-3, five presented endothelial hyperplasia. However, no significant association was observed between these two endothelial-related features. As illustrated in Fig 1F, all PCNSL showed no galectin-1 expression in the lymphomatous or endothelial cells. We also determined that steroid therapy before surgery had no effect on the analysed morphological and the immunohistochemical features.

**Prognostic analysis**

The reduced number of immunocompromised patients did not enable any prognostic factor to be identified. We thus focused the prognostic study on the homogeneous series of immunocompetent patients only. Table II summarizes the results obtained by means of univariate analyses (Kaplan–Meier curves and Gehan-Wilcoxon test). As expected, the IELSG/4 score (and the MSKCC to a lesser extent) displayed a significant negative impact on patient survival period after grouping the IELSG/4 scores into three categories (0–1, 2 and 3–4, according to the value distribution reported in Table I). By considering patient age categories (<50, 50–70 and

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Impact* (+/−)</th>
<th>( P )-value</th>
<th>Morphological and immunohistochemical features</th>
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<tbody>
<tr>
<td>Age</td>
<td>−</td>
<td>0·02</td>
<td>Necrosis</td>
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<tr>
<td>PS</td>
<td>NS</td>
<td></td>
<td>RVPI</td>
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<tr>
<td>Location</td>
<td>NS</td>
<td></td>
<td>Endothelial hyperplasia</td>
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<tr>
<td>Serum LDH level</td>
<td>NS</td>
<td></td>
<td>Galectin-3 expression in lymphomatous cells</td>
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<tr>
<td>MSKCC score</td>
<td>−</td>
<td>0·04</td>
<td>Galectin-3 expression in endothelial cells</td>
</tr>
<tr>
<td>IELSG/4 score</td>
<td>−</td>
<td>0·003</td>
<td>Endothelial hyperplasia and/or galectin-3 expression in endothelial cells</td>
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<tr>
<td>Surgical resection</td>
<td>NS</td>
<td></td>
<td></td>
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<td>Steroid therapy</td>
<td>NS</td>
<td></td>
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<tr>
<td>Chemotherapy†</td>
<td>+</td>
<td>0·0004</td>
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*Negative (−) or positive (+) prognostic impact of the significant features.
†With or without radiotherapy.
NS = not significant; PS, performance status; LDH, lactate dehydrogenase; MSKCC, Memorial Sloan-Kettering Cancer Center classification; IELSG/4, International Extranodal Lymphoma Study Group (IELSG) prognostic score, modified as detailed in the Materials and methods section; RVPI, reactive perivascular T-cell infiltrate.

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we also identified a negative prognostic value for this clinical feature. It should be noted that a significant difference remained between the two older categories (i.e. 50–70 and >70 years, \( P = 0.04 \)). The last clinical feature for which we identified a significant prognostic value was the adjuvant treatment. The patients who underwent methotrexate-based chemotherapy (combined or not with radiotherapy) were associated with very significant longer survival periods than those who underwent either no adjuvant treatment or radiotherapy alone.

In addition to the clinical features, two features related to endothelial cells also contributed significant prognostic values. The presence of endothelial hyperplasia (nine of 41 patients with available survival data, i.e. 22%) and positive galectin-3 expression in endothelial cells (10 of 37 patients, i.e. 27%) were each associated with a significant negative impact on the patients’ survival periods (Figs 2A and B). In addition, the presence of endothelial hyperplasia and/or a positive expression of galectin-3 in endothelial cells enabled the identification of a larger group of patients (i.e. 15 of 37, i.e. 41%) associated with a poor prognosis (see Fig. 2C).

To determine independent prognostic factors, multivariate Cox regression analyses were carried out on the most significant features identified in Table II. However, to avoid data overfitting, the multivariate models were restricted to three features because of the reduced number of cases available. Table IIIA details the best Cox model obtained in these conditions (\( P = 0.0001 \)) and shows that galectin-3 expression in endothelial cells contributes a significant independent prognostic value to a model combining standard prognostic factors, such as chemotherapy treatment and IELSG/4 score. In contrast to the univariate analyses reported in Fig. 2, these multivariate analyses also showed that the use of the endothelial hyperplasia feature in place of, or in combination with, galectin-3 did not improve the prognostic model (data not shown).

Finally, to evaluate the actual prognostic impact of the endothelial-related features on a very homogeneous clinical group, we focused our analyses on the immunocompetent patients who underwent methotrexate-based chemotherapy. For this model, we excluded the IELSG/4 score, which was not

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<th>Table III. Multivariate Cox regression.</th>
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<td>( P )-value</td>
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<tr>
<td>(A) All immunocompetent patients ( N = 27^* )</td>
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<tr>
<td>Chemotherapy</td>
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<td>IELSG/4 score</td>
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<td>Galectin-3 expression in endothelial cells</td>
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<td>Model</td>
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<td>(B) Immunocompetent patients treated with chemotherapy ( N = 22 )</td>
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<tr>
<td>Age</td>
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<tr>
<td>Endothelial hyperplasia and/or galectin-3 expression in endothelial cells</td>
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<td>Model</td>
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\(^*\) For whom the IELSG/4 score was available.

IELSG/4, International Extranodal Lymphoma Study Group (IELSG) prognostic score, modified as detailed in the Materials and methods section.
available for a sufficiently large number of patients. In these conditions the best model \((P = 0.009)\) shown in Table III B involved the combination of the two endothelial-related features. The presence of endothelial hyperplasia and/or a positive expression of galectin-3 in endothelial cells (observed in 7/22 patients, i.e. 32%) was thus identified as a significant and independent factor of risk (in addition to patient age) in this reduced series of patients. This latter result should be confirmed on a larger series of immunocompetent patients who have undergone chemotherapy.

**Discussion**

Histologically, PCNSL is indistinguishable from systemic NHL and the majority of PCNSL are DLBCL (Lister et al, 2002). PCNSL and systemic DLBCL share many molecular features, including clonal rearrangement and somatic hypermutation of immunoglobulin genes (Hochberg et al, 2007). However, PCNSL differ in their clinical behaviour. None of the histological classification systems are considered clinically useful and it seems that that histotype is not a prognostic factor (Ferreri & Reni, 2007). Thus, it is critical to identify clinically relevant prognostic features. Currently, only age and PS are universally accepted prognostic factors (Ferreri & Reni, 2005). Two clinical prognostic scores were recently proposed in the literature for immunocompetent patients (Ferreri et al, 2003; Abrey et al, 2006). The IELSG score (Ferreri et al, 2003) is based on age, ECOG PS, LDH serum level, CSF protein concentration and involvement of deep brain structures whereas the MSKCC score (Abrey et al, 2006) only involves age and PS. The MSKCC score was derived from a retrospective analysis of 338 patients and has the advantage of simplicity (Abrey et al, 2006). While the IELSG score was derived from a multi-institutional retrospective study of 378 patients, only 103 patients had complete data for inclusion in the model (Ferreri et al, 2003). Similarly, in the present study, the available data did not enable an IELSG score to be assigned to 20 of the 44 immunocompetent patients of our series because the CSF protein concentration was missing in a large majority of these cases. However, we computed a related score (IELSG/4) for 34/44 patients. Despite our small series this score kept a very significant prognostic value whereas the value of results obtained for the MSKCC score (on a larger series) were not as significant.

Currently, very few histopathological features with prognostic value have been identified in PCNSL. In fact, only a few studies detailing the morphological features of PCNSL have been published (Henry et al, 1974; Ferracini et al, 1993; Miller et al, 1994; Camilleri-Broet et al, 1998; Choi et al, 2003; Ponzoni et al, 2007). In this context, the present study described the prevalence and the prognostic value of three morphological features, i.e. necrosis, RVPI and endothelial hyperplasia. Our study suggests that the presence of necrosis had no prognostic value in agreement with the results recently reported by Ponzoni et al (2007). Ponzoni et al (2007) also reported that immunocompetent PCNSL patients with RVPI-positive lesions exhibited a better overall survival, particularly in the case of patients treated with methotrexate-based chemotherapy. In our series, while we observed a similar percentage of patients with RVPI lesions, we did not observed such a prognostic role for RVPI. This could be because of the lower number of cases in our series. In contrast, our results agree with the more significant prognostic factors identified by Ponzoni et al (2007) on their entire series of immunocompetent PCNSL patients (i.e. methotrexate-based therapy and IELSG score). In addition, we were the first (to the best of our knowledge) to investigate the presence of endothelial hyperplasia in PCNSL. Twenty-one percent of the analysed PCNSL was associated with endothelial hyperplasia. Univariate and multivariate survival analyses indicated that the absence of endothelial hyperplasia in PCNSL is a favourable feature for immunocompetent patients. In brain tumours, and more particularly in malignant gliomas, endothelial hyperplasia (which is associated with a higher vascular density) depends on two pathophysiological mechanisms: vascular remodelling and angiogenesis (Kleihues & Cavenee, 2000; Sharma et al, 2006). However, while angiogenesis has prognostic significance in various neoplasms, including haematological malignancies (List, 2001; Moehler et al, 2003; Koster & Raemaekers, 2005), very little is known about the clinical and histopathological relevance of angiogenesis in PCNSL. Previous studies show that microvessel density increases along the nodal NHL progression and immature vessels are more frequently observed in high-grade B-cell NHL (Ribatti et al, 1996; Arias & Soares, 2000). Consequently, an increased vascular density was associated with higher malignant variants of NHL (Vacca et al, 1999; Tzankov et al, 2007). Today, the prognostic significance of microvessel density in PCNSL remains controversial. A recent study investigated microvessel density in PCNSL by means of immunohistochemical expression of CD105, a transforming growth factor-beta receptor overexpressed on proliferating endothelial cells that participate in angiogenesis, and showed that the survival of the lower microvessel density group was higher that that of the higher microvessel density group (Sugita et al, 2007). However, this difference was not observed when CD34 expression was considered rather than CD105 expression (Sugita et al, 2007). In parallel, Takeuchi et al (2007) investigated microvessel density using an anti-CD31 monoclonal antibody as well as vascular endothelial growth factor (VEGF) immunoreactivity in a series of 19 PCNSL (Takeuchi et al, 2007). This study associated positive VEGF immunoreactivity in PCNSL with higher (CD31-positive) microvessel density, the absence of blood–brain barrier markers and higher survival times. As emphasized by Sugita et al (2007), angiogenesis investigations in neoplasms require the use of markers that specifically target newly forming vessels in place of the usual panendothelial antibodies, such as anti-CD31 and anti-CD34 antibodies, which also react with normal vessels trapped within tumour tissues. The present study also suggests that the morphological
characterization of tumour vessels, such as the presence of endothelial hyperplasia, might provide additional information on tumour angiogenesis.

To the best of our knowledge, no other study has described the immunohistochemical expression of galectin-1 and galectin-3 expression in PCNSL. While PCNSL are characterized by an absence of galectin-1 expression in lymphomatous cells and endothelial cells, lymphomatous cells of a few cases expressed galectin-3, with no prognostic value. While it is known that the majority of blood vessels in normal brain do not express galectin-3 (Bresalier et al, 1997), in this study 11 PCNSL samples showed endothelial expression of galectin-3. In contrast to lymphomatous cell expression, endothelial galectin-3 expression was associated with a negative and independent prognostic impact on our series of immunocompetent PCNSL patients. These results contrast with our previous results showing: (i) endothelial galectin-3 expression in both normal lymph nodes and nodal lymphomas, and (ii) differential galectin-1 expression in endothelial cells between these two tissue types (D’Haene et al, 2005). A recent review focusing on galectin expression in the tumour endothelium suggests that galectins contribute to different functions of activated tumour endothelial cells and might be involved in different steps of tumour progression, i.e. immune modulation, metastasis and angiogenesis (Thijsen et al, 2007). Galectin-3 is known to stimulate angiogenesis in vitro and in vivo (Nangia-Makker et al, 2000). Recently, one study that employed gene profiling expression showed that LGALS3 mRNA is up-regulated in endothelial progenitor cells compared with differentiated endothelial cells (Furuhata et al, 2007). In contrast with this latter result and the fact that endothelial hyperplasia is often associated with angiogenesis in brain tumours (Kleiheues & Cavenee, 2000), we found no statistical association between endothelial hyperplasia and endothelial galectin-3 expression. This result could have been because of the reduced number of cases in our series. Therefore, further investigations on larger series are needed to explore the possible relationships between endothelial hyperplasia and endothelial galectin-3 expression in PCNSL. We thus plan to systematically analyse galectin-3 expression in vessels either associated or not with endothelial hyperplasia (by means of microdissection or other techniques). This analysis could be usefully completed with parallel investigations on angiogenic markers [such as VEGF receptors, COX2 or endothelial progenitor cell markers (CD133)] and differentiation markers, such as Bcl-6. This latter marker was previously proposed as a favourable prognostic marker for immunocompetent PCNSL patents who received methotrexate-based chemotherapy (Braaten et al, 2003; Lin et al, 2006).

In summary, we propose that the evaluation of endothelial hyperplasia and endothelial galectin-3 expression, which can be both easily assessed histologically, should be integrated into the IELSG score for determining the prognosis of PCNSL patients. These results need to be confirmed on a larger prospective series of patients and compared with other markers, such as angiogenetic and differentiation markers.

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References


