Osteopontin expression and its relationship with prognostic factors in diffuse large B-cell lymphoma

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Abstract

The aim of this study was to explore the expression of osteopontin (OPN) and its relationship with prognostic factors and survival in diffuse large B cell lymphoma (DLBCL). A tissue microarray was performed for immunohistochemical evaluation. Contingency tables were analyzed for trends; chi-square test was used to determine differences between groups. Univariate and multivariate Cox proportional hazards-regression analyses were performed to evaluate the impact of prognostic factors on survival. Expression of OPN was observed in 28%. It was different in non-germinal center DLBCL (P=0.04). The mean overall survival (OS) was lower in patients with positive OPN expression (19.762; CI 95% 14.269-25.255) it was not significant (P=0.123). It is not possible to establish a clear relationship between the expression by immunohistochemistry of osteopontin and a poor prognosis but it would be important to complement the analysis with other techniques as PCR or NGS that allow us to assess the influence of the isoforms and post-translational modifications of OPN on the biological behavior of DLBCL. Our findings indicate that OPN expression could be associated with a more aggressive variant of lymphoma: non-germinal center DLBCL.

Introduction

Diffuse large B cell lymphoma (DLBCL) is a heterogeneous disease which prognosis depends on clinical and biological factors.1 Advanced age, low performance status, advanced Ann Arbor stage, elevated lactate dehydrogenase (LDH) and extranodal disease have shown to be predictors of survival.2 The origin of the neoplastic cell (germinal center vs. non-germinal center), MYC, BCL6 and BCL2 translocations (double-hit or triple hit lymphoma) also influence the prognosis.

Osteopontin (OPN) is a non-collagenous extracellular matrix (ECM) protein with cytokine activity, expressed by various cell types, and is involved in multiple biological processes, both physiological and pathological; different isoforms (a, b, c) can be produced by alternative splicing.3-7 The OPN was found intracellular and could also be secreted by an alternative translation mechanism and undergoes post-translational modifications (cleavage, glycosylation, etc.).8-11 OPN exerts its function binding to integrins and CD44.12,13 The biological function that OPN produces depend on the type of cell, isoforms and receptors that recognize the proteins.3 In cancer, OPN induces the inhibition of apoptosis, favors tumor invasion, metastasis, angiogenesis and deregulation of cellular energetics, avoiding immune destruction and tumor-promoting inflammation.14

The increase in the production of osteopontin in different types of neoplasias has been associated with tumor aggressiveness and poor prognosis.13,15-22

A retrospective cohort study was conducted among patients with DLBCL; the purpose was to evaluate the expression of osteopontin in order to analyze its association with known prognostic factors and its influence on the overall survival.

Materials and Methods

Patients and tissue specimens

The present study was approved by the IRB Committee (Rev/93/16), Instituto Nacional de Cancerologia Mexico (INCan). Data were obtained from DLBCL database at INCan, between November 2014 and March 2016. All patients included in the analysis meet the following criteria: (i) histologically diagnosed as DLBCL; (ii) tumor specimens with available quality for tissue microarray construction; (iii) complete data parameters to calculate the IPI and NCCN-IPI scales on diagnosis (age, performance status, Ann Arbor stage, LDH levels and extranodal disease); (iv) Patients who were followed-up at the INCan. Clinical stage was determined according to the Ann Arbor staging system. Cellular origin was determined according to the Hans algorithm.23 As a result, 80 cases met the inclusion criteria and were incorporated in our study. The survival time was measured from the date of diagnosis to the date of death, or last follow-up.

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Key words: osteopontin, lymphoma, prognosis.

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Contributions: GB, SR, CL, RQ, and DN performed diagnosis and follow-up, analyzed data, and wrote the manuscript. EF and GB performed immunohistochemistry and wrote the manuscript. GB collected samples and clinical data. ER, JA, AG, HA designed the study, supervised research, and wrote the manuscript. All authors contributed to the preparation of the draft, and approved the final version for submission.

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Immunohistochemistry detection of osteopontin

The paraffin-embedded primary tumor tissues of 80 patients were used to construct the DLBCL tissue microarray and were cut into 4 mm thick slices. For the IHC analysis, the slides were hydrated and antigenically reactivated using a citrate buffer (0.01 M citric acid, 0.01 M sodium citrate) for 10 min at 90˚C. Endogenous peroxidase was blocked using Bloxall solution (Cat. SP6000. Vector), and after three washes with PBS 1X, nonspecific antigenic sites were blocked using 1% bovine serum albumin (BSA)/Triton X-100 0.1% dissolved PBS 1X during 30 min at 37˚C. Blocked solution was discarded and samples were incubated with OPN 1:200 (Cat. Sc-21742. Santa Cruz Biotechnology) dissolved in BSA 0.1%/Triton X-100 0.01% overnight at 4˚C. The slides were washed 3 times with PBS 1X, and the secondary antibody was used as specified by the manufacturer (mouse and rabbit specific HRP/AEC detection IHC kit, ab94705. Abcam). The slides were counterstained with Mayer’s haematoxylin (Cat. HK100-9K. Bio Genex) and were mounted using Aqua Mounter Solution (Cat. BSB0091). Digital images of tissue sections (40X) were captured using a light microscope and a color AxioCam MRc5 camera (Zeiss).

Three different pathologists, blinded to the clinical information, determined the immunoreactivity of OPN. To evaluate the immunohistochemical expression of OPN, we used a score corresponding to the sum of both staining intensities (0=negative; 1=weak; 2=intermediate; 3=strong) and the percentage of positive cells (0=0% positive cells; 1=25% positive cells; 2=26–50% positive cells; 3>50% positive cells). The sum of a+b reached a maximum score of 6. A score equal or greater than 3 represented a positive immunohistochemical survey.24

Statistical analysis

The SPSS statistical software package version 20 was used for data analysis. Contingency tables were analyzed for trends; chi-square test was used to determine the significance of differences between groups for categorical variables. Univariate survival analysis was based on the Kaplan-Meier product limit estimator. Univariate and multivariate Cox proportional hazards-regression analyses were performed to evaluate the impact of prognostic factors on survival.

Results

Patients

In total, 48 females and 32 males were enrolled in our study, with a mean age of 59.64 years (range, 22–88 years); 85% were treated with R-CHOP or CHOP-like chemotherapy. OPN was expressed in 43.8% of the cases, primarily presenting a nuclear and cytoplasmic staining pattern, however when applying the score only 28% were considered positive; in some cases, OPN also presented little expression in macrophages and interstitial tissue (Figure 1).

Correlation of OPN positivity with prognostic factors

Positivity of OPN was not significantly correlated with age, performance status, Ann Arbor stage, LDH levels and extranodal disease but was significantly different in cases of non-germinal center lymphoma. Table 1 summarizes the detailed information of the included cases.

Long-term survival

The mean OS time for the entire cohort of patients was 24.04 months (95% confidence interval [CI]: 21.4–26.6 months), with a 30-months OS rate of 75.8%; the median was not reached. Table 2 and Figure 2 summarize the survival details according to the expression of OPN.

Factors that impact survival

Univariate analysis, using the Cox proportional hazards regression model, demonstrated that performance status was the only factor that adversely affected OS (Table 3). Multivariate analysis did not identify any independent factor that adversely affected OS (Table 3).
Discussion and Conclusions

DLBCL is an aggressive disease with a heterogeneous clinical behavior, while some patients may respond in a great way to treatment with immune-chemotherapy; however about 30% of cases do not achieve an adequate response. Although risk factors have been identified, it is important to identify targets that influence the prognosis and may be susceptible to treatment.

Osteopontin has been identified as a prognostic factor in hematologic and non-hematologic malignancies. Aggressive cases of DLBCL expressing osteopontin have been reported. In our study, we did not find a significant association between OPN expressions and known risk factors. But, we found a significant difference of OPN expression between germinal center and non-germinal center (NGC) lymphomas, that until today never has been reported, and this finding have clinical relevance since this last variant (NGC) is associated with a lower overall and progression-free survival.

An association between the expression of osteopontin and extranodal disease has been also reported. OPN has been linked to the tropism of neoplastic B cells to the central nervous system (CNS) and it has been found that the OPN gene is up-regulated in the primary nervous system lymphoma. Although the characteristics of our study do not allow us to identify a relationship between OPN expression and CNS involvement by lymphoma, we consider that it is important to deepen the investigations to know if the expression of OPN in the neoplastic cell can predict an infiltration, posterior to the central nervous system, as it would help to discern which patients with DLBCL would benefit from prophylaxis to prevent CNS involvement.

In our study, a deceased overall survival was found in patients with positive OPN expression; however, this was not significant. In the multivariate analysis, all the risk factors evaluated (age, PS, Ann Arbor stage, elevated LDH, extranodal disease, increased ECOG, stage III, IV, normal LDH) have been reported. In our study, we did not find a significant association between OPN expressions and known risk factors. But, we found a significant difference of OPN expression between germinal center and non-germinal center (NGC) lymphomas, that until today never has been reported, and this finding have clinical relevance since this last variant (NGC) is associated with a lower overall and progression-free survival.

Table 1. Correlation of osteopontin expression with clinical-pathological characteristics.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (%)</th>
<th>Positive (%)</th>
<th>Negative (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60y</td>
<td>35 (43.8)</td>
<td>6 (7.5)</td>
<td>29 (36.3)</td>
<td>0.152</td>
</tr>
<tr>
<td>&gt;60y</td>
<td>45 (56.2)</td>
<td>14 (17.5)</td>
<td>31 (37.7)</td>
<td></td>
</tr>
<tr>
<td>ECOG PS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0/1</td>
<td>57 (71.3)</td>
<td>12 (15)</td>
<td>45 (56.3)</td>
<td>0.199</td>
</tr>
<tr>
<td>&gt;/=2</td>
<td>23 (28.7)</td>
<td>8 (10)</td>
<td>15 (18.7)</td>
<td></td>
</tr>
<tr>
<td>Ann Arbor stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I, II</td>
<td>25 (31.3)</td>
<td>8 (10)</td>
<td>17 (21.3)</td>
<td>0.330</td>
</tr>
<tr>
<td>III, IV</td>
<td>55 (68.7)</td>
<td>12 (15)</td>
<td>43 (53.7)</td>
<td></td>
</tr>
<tr>
<td>LDH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>31 (38.7)</td>
<td>7 (8.7)</td>
<td>24 (30)</td>
<td>0.691</td>
</tr>
<tr>
<td>Increased</td>
<td>49 (61.3)</td>
<td>13 (16.3)</td>
<td>36 (45)</td>
<td></td>
</tr>
<tr>
<td>Extranodal disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>30 (37.5)</td>
<td>6 (7.5)</td>
<td>24 (30)</td>
<td>0.424</td>
</tr>
<tr>
<td>Positive</td>
<td>50 (62.5)</td>
<td>14 (17.5)</td>
<td>36 (45)</td>
<td></td>
</tr>
<tr>
<td>Cell origin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germinal center</td>
<td>42 (52.2)</td>
<td>8 (11.3)</td>
<td>34 (47.9)</td>
<td>0.040</td>
</tr>
<tr>
<td>Non-germinal center</td>
<td>29 (40.8)</td>
<td>12 (16.9)</td>
<td>17 (23.9)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Survival comparison between groups with positive and negative OPN expression.

<table>
<thead>
<tr>
<th>OPN</th>
<th>Mean (CI 95%)</th>
<th>Median (CI 95%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>24.961 (22.898-27.823)</td>
<td>Not reached</td>
<td>0.123</td>
</tr>
</tbody>
</table>

Table 3. Univariate and multivariate Cox regression analysis prognostic factors for OS.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate Hazard ratio (95% IC)</th>
<th>P-value</th>
<th>Multivariate Hazard ratio (95% IC)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age&gt;60y</td>
<td>2.204 (0.765-6.345)</td>
<td>0.143</td>
<td>3.275 (0.906-11.840)</td>
<td>0.070</td>
</tr>
<tr>
<td>ECOG &gt;/=2</td>
<td>3.738 (1.385-10.096)</td>
<td>0.009</td>
<td>2.081 (0.610-7.094)</td>
<td></td>
</tr>
<tr>
<td>Ann Arbor stage III, IV</td>
<td>3.364 (0.764-14.814)</td>
<td>0.109</td>
<td>2.966 (0.572-15.378)</td>
<td>0.195</td>
</tr>
<tr>
<td>LDH increased</td>
<td>2.730 (0.880-8.473)</td>
<td>0.082</td>
<td>1.763 (0.465-6.677)</td>
<td>0.404</td>
</tr>
<tr>
<td>Extranodal disease</td>
<td>2.338 (0.753-7.255)</td>
<td>0.142</td>
<td>1.449 (0.422-4.969)</td>
<td>0.555</td>
</tr>
<tr>
<td>Non-germinal center</td>
<td>1.307 (0.458-3.727)</td>
<td>0.617</td>
<td>1.277 (0.370-4.408)</td>
<td>0.699</td>
</tr>
<tr>
<td>Osteopontin expression</td>
<td>2.161 (0.785-5.952)</td>
<td>0.136</td>
<td>2.337 (0.677-8.064)</td>
<td>0.179</td>
</tr>
</tbody>
</table>
cell origin and OPN expression) confer an increased risk of mortality, but were not statistically significant, probably due to size of our cohort and the losses in the follow-up of the patients.

In other types of neoplasms, the identification of the different OPN isoforms has allowed to relate some of them to the prognosis, an example could be breast cancer, since the identification of the mRNA of the C isoform in tumor cells is associated to a poor prognosis, recurrence and decrease in disease-free survival, on the other hand, there is a decrease in the mRNA of isof orm A as it increases the clinical stage by TMN; another example would be pancreatic cancer, since mRNA detection of isof orm C is associated with metastatic disease while mRNA of isof orm B is associated with decreased overall survival.18

In conclusion, the expression of osteopontin in DLBCL is relatively frequent, in our series it was observed in 28% of the cases, with a slight predominance in those cases of non-geminal center origin. Although it is not possible to establish a clear relationship between the expression by immunohistochemistry of osteopontin and a poor prognosis it would be important to complement the analysis with other techniques as PCR or NGS that allow us to assess the influence of the isoforms and post-translational modifications of OPN on the biological behavior of DLBCL.

References