



CLINICAL RESEARCH

Molecular markers (*PECAM-1*, *ICAM-3*, *HLA-DR*) determine prognosis in primary non-Hodgkin's gastric lymphoma patients

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Abstract

AIM: To investigate the prognostic significance of *PECAM-1*, *ICAM-3* and *HLA-DR* antigens in patients with primary non-Hodgkin's gastric lymphoma.

METHODS: We immunohistochemically studied *PECAM-1*, *ICAM-3* and *HLA-DR* antigen expression in 36 B-cell MALT-type primary gastric lymphoma patients. Ten non-malignant and ten healthy gastric tissue specimens were used as controls. Clinicopathological and survival data were correlated with the staining results.

RESULTS: *HLA-DR* antigen expression was detected in 33 gastric lymphoma patients (91.7%) and 6 non-malignant patients (54.5%). *PECAM-1* stained tumor cells of 10 patients (27.8%), endothelial cells of 9 patients (25%) and inflammatory infiltrate of 4 patients (40%) with benign gastric disease. *ICAM-3* expression was observed on the tumor cells of 17 patients (47.2%), while 5 non-malignant patients (50%) were stained positive as well. None of the healthy controls was stained for any of the genes studied. In the multivariate analysis, *HLA-DR* antigen and *PECAM-1* were proved to be statistically significant independent prognostic factors associated with a favourable and an unfavourable prognosis respectively ($P=0.009$ and $P=0.003$). In the univariate analysis, *PECAM-1*(+)/*ICAM-3*(-) and *HLA-DR*(-)/*ICAM-3*(-) patients exhibited a significantly decreased overall survival compared to those with the exactly opposite gene expression patterns ($P=0.0041$ and $P=0.0091$, respectively). Those patients who were *HLA-DR*(+)/*ICAM-3*(+)/*PECAM-1*(-) ($n=8$) had a significantly higher survival rate compared to the rest of

the group ($n=24$) ($P=0.0289$).

CONCLUSION: *PECAM-1*, *ICAM-3* and *HLA-DR* are representative markers of tumor expansion potential and host immune surveillance respectively. Their combined use may help us to identify high-risk patients who could benefit from more aggressive therapeutic protocols.

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Key words: *PECAM-1*; *ICAM-3*; *HLA-DR*; Non-Hodgkin's gastric lymphoma; Prognosis

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INTRODUCTION

Primary gastric B-cell non-Hodgkin's lymphomas are uncommon tumors, constituting less than 2% of all primary gastric malignancies^[1]. Numerous clinicopathologic studies have identified stage and grade as the most important prognostic factors^[1,2]. Still there are a number of patients with favourable stage and grade that exhibit an aggressive phenotype. A second line of molecular prognostic markers has been introduced lately to better describe this clinical entity.

Several different molecules (*bcl-2*, *p53*, *PCNA*, *c-fos*, *c-myc* and *Ki67*) and biologic pathways have been implicated in the initiation and progress of primary non-Hodgkin's gastric lymphoma^[3]. Immunohistochemical tracing of these molecules in gastric lymphoma patients has already been employed not only for the diagnosis but for the determination of prognosis as well^[4].

HLA-DR antigen is a class II MHC membrane-bound glycoprotein, which plays an important role in the regulation of the immune response^[5]. Although it is normally expressed exclusively by antigen presenting cells of the immune system^[6], it shows variable expression in malignancies^[7,8]. During oncogenesis, it is modified to affect tumor

Table 1 Demographics and clinicopathological characteristics of 36 patients with primary gastric lymphoma

Characteristics	Frequency	Percentage (%)
Age of disease presentation (yr)	58.39 ± 15.39 (median: 59, range: 33-82)	
Gender		
Male	21	58.4
Female	15	41.6
Type of Surgery		
Total gastrectomy	10	27
Subtotal gastrectomy	20	55.5
Unknown	6	17.5
Microscopic Margins		
Positive margins	5	13.9
Negative Margins	31	86.1
Stage		
I	17	47.2
II	7	19.4
III	6	16.7
IV	6	16.7
Histologic Grade		
Low grade	15	41.7
Intermediate grade	7	19.4
High grade	14	38.9
Tumor Surface (cm ²)	50.04 ± 47.9 (median 29.15, range 1.5-180)	
Tumor Thickness (mm)	7.05 ± 4.07 (median: 6, range: 1.5- 15)	
Tumor Diameter (cm)	6.66 ± 3.8 (median: 5.75, range: 1-15)	
Adjuvant Chemotherapy		
Yes	25	69.4
No	8	22.3
Unknown	3	8.3

cell behavior by decreasing or enhancing specific anti-tumor immune mechanisms^[9].

Cell adhesion molecules are membrane glycoproteins that play a major role in neoplastic disease by interfering with cell-matrix and cell-cell interactions. They are also believed to participate in host immune surveillance by providing antigen non-specific recognition between Th-cells, APCs, Tc-cells, NK-cells and their potential targets. *ICAM-3* and *PECAM-1* are considered to be representative members of the immunoglobulin Ig superfamily of cell adhesion molecules (*CAMs*). *ICAM-3* is constitutively highly expressed by leukocytes especially in lymphomas and myelomas^[10]. It is postulated that it may constitute the critical ligand for the initiation of lymphocyte immune responses^[11] with possible antitumoral properties. Tumor expansion is angiogenesis dependent^[12,13], a function which is orchestrated by a constant interaction between tumor cells and host cells. This "cross talk" can lead either to cell cycle arrest and tumor regression or to tumor progression^[14,15]. *PECAM-1* (platelet/endothelial cell adhesion molecule) is considered to be an accurate measure for the assessment of vascular proliferation on tumor sections^[12] determining prognosis in a variety of tumors^[16,17]. Moreover, *PECAM-1* expression by immunocompetent cells^[11] as well as its regulatory role in the extravasation of leukocytes^[18], suggests a potential pathway through which lymphoid tumors

may escape immune surveillance.

Although the above molecules appear to represent interacting pathways responsible for the antitumoral response and tumor progression, up to day very little information is available on their role in the pathogenesis and prognosis of primary gastric lymphoma^[19-21]. The aim of this study was to analyze the immunohistochemical expression of *ICAM-3* (*CD50*), *PECAM-1* (*CD31*) and *HLA-DR* antigens in a group of patients operated for primary non-Hodgkin's gastric lymphoma with respect to their clinicopathological characteristics and clinical outcome.

MATERIALS AND METHODS

We retrospectively recruited 36 B-cell MALT-type non-Hodgkin's primary gastric lymphoma patients (76.6%). All of them were diagnosed and treated during the period from 1991 to 1997 at the First Department of Propaedeutic Surgery of the Hippokration Hospital, Athens Medical School. Follow-up time measurements were specifically interrupted by death of the affected individuals from any cause. In this regard, follow-up time ranged between 15 d and 135.5 mo, with a mean of 60.5 ± 38.8 mo and a median of 68.5 mo. Favorable treatment outcome was defined as undetectable disease at the most recent follow-up. Unfavorable treatment outcome was defined as tumor recurrence, either locally or distantly, or death due to the tumor. Four patients had inadequate 5-year survival data and were excluded from the survival analysis. All reported deaths were attributed to the underlying disease, thus the overall survival corresponded to tumor-associated survival. Our study also included 10 gastric tissue specimens of non-malignant origin (Analytically this group consisted of 4 patients with gastritis and 6 patients with non malignant ulcer of the stomach, none of whom was submitted to any kind of surgery) and 10 healthy control tissue specimens. The characteristics of gastric lymphoma patients as well as information regarding adjuvant therapy are summarized in Table 1.

All patients were diagnosed by endoscopic biopsy and preoperatively evaluated by bone marrow biopsy, chest radiography, abdominal CT and indirect laryngoscopy. The mean age of disease presentation was 58.39 ± 15.39 years (median: 59 years).

Staging was performed according to the Japanese Classification of Gastric Carcinoma by the Japanese Gastric Cancer Association^[22]. Seventeen patients (47.2%) were stage I, 7 (19.4%) stage II, 6 (16.7%) stage III and 6 (16.7%) stage IV. Histopathologic examination was undertaken according to the working formulation^[23]. Fifteen (41.7%) were found to be low grade lymphomas (grade I), 7 (19.4%) intermediate grade lymphomas (grade II) and 14 (38.9%) high grade lymphomas (grade III). The presence of *H pylori*-associated MALT-type lymphoma was not determined in the majority of cases and this information was not included in our statistical analysis. Ten patients (27.7%) received a total gastrectomy and 20 (55.5%) a subtotal gastrectomy, while there was no information regarding the type of surgery for 6 (16.8%) patients. All patients had macroscopically clear margins, while microscopically

involved resection margins were detected in 5 patients (13.9%). Intraoperative staging consisted of biopsies of the liver and any enlarged abdominal lymph nodes. Splenectomy was performed only if the spleen was directly invaded.

Adjuvant chemotherapy was administered to 25 patients (69.4%), 3 of whom (8.3%) received a combination of adjuvant chemo/radiotherapy. Eight patients (22.3%) did not receive any kind of supplemental therapy, while no data were retrieved for three more (Table 1).

Immunohistochemistry

Immunohistochemical studies were performed on formalin-fixed and paraffin-embedded sections using the streptavidin-biotin-peroxidase method (Novostain Super ABC Novocastra laboratories Ltd, Newcastle, UK) with monoclonal antibodies specific for *HLA-DR* (DAKO, Glostrup, Denmark, dilution 1:70) and *PECAM-1* (Oncogene Research Products, Calbiochem, Boston, dilution 1:50) and *ICAM-3* (Oncogene Research Products, Calbiochem, Boston, dilution 1:1000). The assignment was performed on surgical specimens obtained during the surgical excision of the tumors.

The staining technique used was the same as previously described^[24, 25]. The sections were deparaffinized in xylene and rehydrated in graded ethanol. Antigenic determinants masked from the formalin-fixation and paraffin-embedding were exposed to saponin. Briefly, the endogenous peroxidase activity was blocked using a hydrogen peroxide solution. The primary antibody was then applied overnight at 4°C followed by incubation with a polyvalent antibody for 30 min and then a streptavidin-peroxidase reagent for 30 min at room temperature. Diaminobenzidine tetrahydrochloride (DAKO) was used as the chromogen, and hematoxylin for counterstaining. Appropriate positive and negative controls were used.

All slides were evaluated by two independent reviewers. The percentage of *HLA-DR* (+) tumor cells was estimated, compared to the total area covered by the tumor in 10 randomly selected low power fields (x40). The proportion of *HLA-DR* (+) stromal cells close to and far from the tumor (macrophages, leukocytes, activated T-cells, necrotic tissue) was also assessed, as well as *HLA-DR* expression in normal gastric mucosa and stroma, for all the slides containing benign gastric tissue. *HLA-DR* antigen expression was located mainly in the cytoplasm but on some occasions membrane staining was seen. According to our initial estimation paraffin sections expressing *HLA-DR* in less than 5% of the tumor were considered negative. Tumor sections in which most of the tumor was found positive for *HLA-DR* expression in 10 randomly selected low power fields, were characterized as >75% positive.

A semi-quantitative grading system for *ICAM-3* and *PECAM-1* was used with the following criteria:

Negative (-): no immunoreaction or < 5% of tumor cells stained; (+) : 5-10% of tumor cells stained; (+ +) : 10-50% of tumor cells stained; (+ + +) : >50% of tumor cells stained.

Tumor vascularity was assessed using the method described by Horak *et al*^[12] and penfold *et al*^[26].

Microvessels identified with *PECAM-1* staining were counted on three 400 × fields ($A=0.302\text{ mm}^2$) within areas of maximum vascularity and the mean microvessel count was calculated for each area.

Statistical analysis

Data were expressed as mean ±SD. Survival analysis was performed using the Kaplan-Meier method with the log rank test. To determine independent prognostic factors we used the Cox proportional hazards model, which estimates the odds ratio in 95% confidence intervals. Univariate analyses comparing subgroups of patients were performed with the chi-square test (Pearson, Mantel-Haenzel test for linear association). The results of the morphometric examination were studied in various levels of the variables examined by the Student's *t*-test or the one-way ANOVA. The Levene test for homogeneity of variance was performed before the application of *t*-test or ANOVA. Non-parametric tests such as the Mann-Whitney test and the Kruskal-Wallis one-way ANOVA were applied in every case where the requirements of the *t*-test or the one-way ANOVA were not met. For all statistical tests, $P < 0.05$ was considered statistically significant.

RESULTS

PECAM-1 was immunohistochemically expressed on the tumor cells of 10 gastric lymphoma patients (27.8%) (mean expression = $26.5\% \pm 8.8\%$ for *PECAM-1* positive cells) (Figure 1A). *PECAM-1* (+) endothelial cells were found in the vessels of 9 gastric lymphomas (25%) (Figure 1B). The tumor stromal *PECAM-1* (+) vessel counts/mm² varied between 1.987/mm² and 8.609/mm² (mean ± SD: 4.6358 ± 2.0679) and were significantly higher ($P=0.027$) than *PECAM-1* (+) vessel counts located at the tumor margin (mean ± SD: 2.1341 ± 2.7460 , range: 0 - 8.609). Similarly, the number of centrally located *PECAM-1* (+) vessels/mm² was significantly higher in gastric lymphoma patients with lymph node involvement (7.285 vs 3.753 , $P=0.002$). Four gastric lymphoma patients (11.11%) expressed *PECAM-1* on their tumoral and neovascular endothelial cells. *PECAM-1* was also expressed by the inflammatory infiltrate of 2 (50%) gastritis and 2 (33.33%) non-malignant ulcer patients. Nevertheless, its immunohistochemical expression was not detected on the endothelial cells of any of the above patients. *PECAM-1* upregulation did not correlate with patients' age, gender, tumor stage and grade or type of surgical treatment, as well as tumoral diameter, surface or thickness.

ICAM-3 positive staining was observed in 17 gastric lymphoma patients (47.2%) (mean expression = $34.118\% \pm 14.5\%$ for *ICAM-3* positive lesions) (Figure 2A). *ICAM-3* was also stained in 1 (25%) gastritis and 4 (66.66%) non-malignant ulcer specimens. It was mainly expressed in patients with more advanced local disease as demonstrated by the increased tumor surface (70.074 cm^2 vs 29.7 cm^2 , $P=0.033$) and thickness (8.786 mm vs 5.531 mm , $P=0.034$) (Table 2). Furthermore, *ICAM-3* expression level differed significantly in gastric lymphomas with different histologic grade ($F=4.833$, $P=0.014$) (Figure 2B and Table 3). *ICAM-3* expression level correlated with

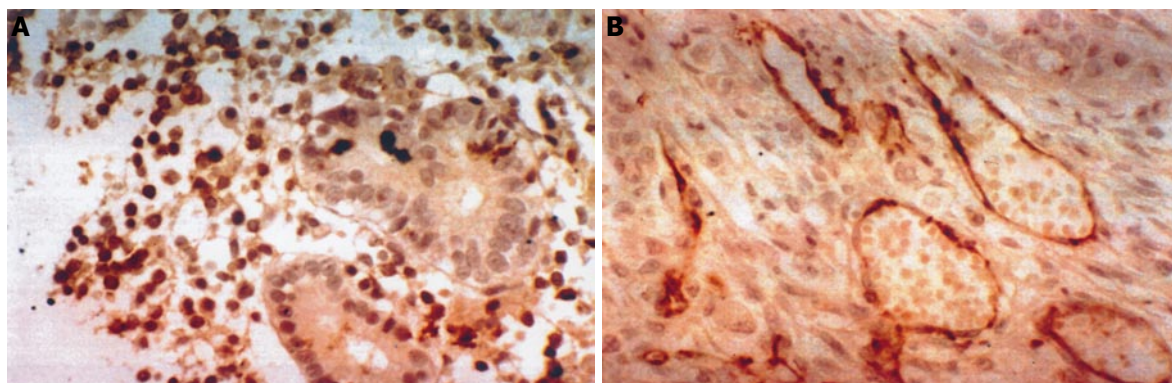


Figure 1 Positive membrane immunostaining (A) and endothelial staining (B) for *PECAM-1* in diffusely growing lymphocytes (magnification 1X400). Gastric glands were identified as negatively stained.

Table 2 Differences in tumor thickness, surface and diameter according to *ICAM-3* expression status in patients with non-Hodgkin primary gastric lymphoma (mean \pm SD)

	<i>ICAM-3</i> Tumor Expression		<i>P</i>
	<i>ICAM-3</i> (+) (<i>n</i> = 14)	<i>ICAM-3</i> (-) (<i>n</i> = 16)	
Mean tumor thickness (mm)	8.79 (\pm 4.67)	5.53 (\pm 2.81)	<0.05
Mean tumor surface (cm ²)	70.07 (\pm 9.18)	29.70 (\pm 28.96)	<0.05
Mean tumor diameter (cm)	7.89 (\pm 7.89)	5.59 (\pm 2.78)	NS ¹

¹ NS: Non-significant.

Table 3 *ICAM-3*, *PECAM-1* and *HLA-DR* antigen quantitative expressions according to tumor grade (mean \pm SD)

Gene expression (%)	Grade I (<i>n</i> = 15)	Grade II (<i>n</i> = 7)	Grade III (<i>n</i> = 14)	<i>P</i>
<i>ICAM-3</i> (+) tumor cells	26.67 \pm 22.09	3.57 \pm 9.45	11.07 \pm 5.95	< 0.05
<i>PECAM-1</i> (+) tumor cells	6 \pm 10.55	10.71 \pm 13.36	7.14 \pm 5.28	NS ¹
<i>HLA-DR</i> (+) tumor cells	40 \pm 25.98	55.71 \pm 26.21	31.43 \pm 6.63	NS ¹

¹ NS: Non-significant

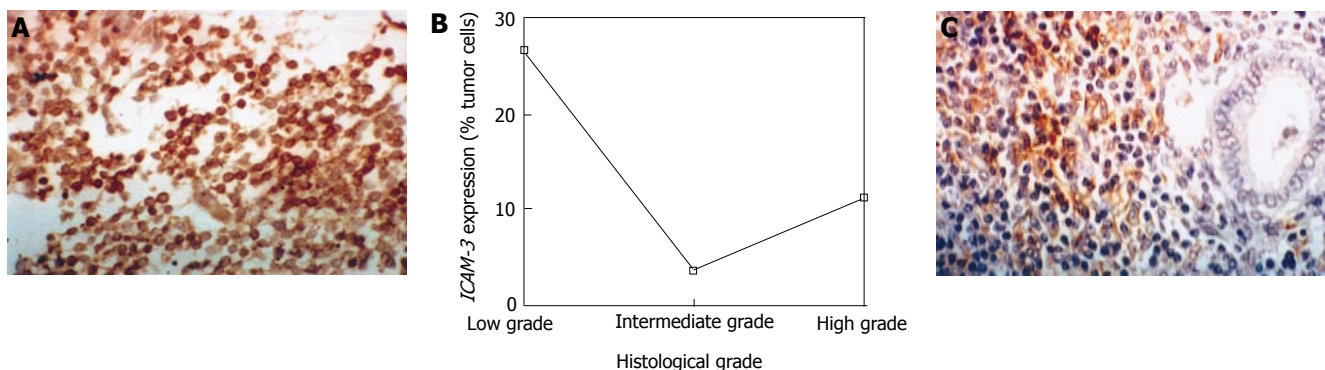


Figure 2 Positive membrane immunostaining for *ICAM-3* in diffusely growing lymphocytes (magnification 1X400) (A) and fluctuations of the mean *ICAM-3* expression level according to tumor grade (B) and positive intracytoplasm immunostaining for *HLA-DR* antigen in gastric lymphoma lymphocytes (magnification 1X200) (C) with gastric glands indicated as negatively stained.

increased tumor diameter ($r=0.400$, $P=0.028$), tumor surface ($r=0.462$, $P=0.012$), tumor thickness ($r=0.526$, $P=0.003$) and higher tumor grade ($r=-0.362$, $P=0.030$), but not with age, gender, stage or type of surgical treatment.

HLA-DR antigen expression on tumor and peritumoral stromal cells was observed in 91.7% ($n=33$) of gastric lymphoma patients (Figure 2C). The proportion of *HLA-DR* positive tumor and peritumoral stromal cells was equally distributed in gastric lymphoma patients with different stage, grade, gender and type of surgery. *HLA-DR* was also expressed on stromal cells in 2 (50%) gastritis and 4 (66.6%) non malignant gastric ulcer control cases.

Several patients co-expressed two or more of the markers studied. The patterns of different protein co-expression are demonstrated in Table 4. None of the 10 healthy controls expressed any of the molecules studied.

Survival analysis

Univariate analysis showed that *ICAM-3* expression was associated with improved 5-year survival rate (78.6% *vs* 55.6%, $P=0.1701$). *PECAM-1* expression on gastric lymphoma tumor cells was associated with significantly decreased 5-year survival rate (28.6% *vs* 76%, $P=0.0078$) (Figure 3A). Although the presence of *PECAM-1* (+) vessels within the tumor was associated with decreased overall

Table 4 *ICAM-3*, *PECAM-1* and *HLA-DR* antigen expressions in gastric lymphoma tumor cells¹

Gene expression	Frequency	Percentage (%)
<i>ICAM-3</i> (+)/ <i>PECAM-1</i> (+)/ <i>HLA-DR</i> tumor(+)	4	11.1
<i>ICAM-3</i> (+)/ <i>PECAM-1</i> (+)/ <i>HLA-DR</i> tumor(-)	1	2.8
<i>ICAM-3</i> (+)/ <i>PECAM-1</i> (-)/ <i>HLA-DR</i> tumor(-)	4	11.1
<i>ICAM-3</i> (+)/ <i>PECAM-1</i> (-)/ <i>HLA-DR</i> tumor(+)	8	22.2
<i>ICAM-3</i> (-)/ <i>PECAM-1</i> (+)/ <i>HLA-DR</i> tumor(+)	4	11.1
<i>ICAM-3</i> (-)/ <i>PECAM-1</i> (+)/ <i>HLA-DR</i> tumor(-)	1	2.8
<i>ICAM-3</i> (-)/ <i>PECAM-1</i> (-)/ <i>HLA-DR</i> tumor(+)	10	27.8
<i>ICAM-3</i> (-)/ <i>PECAM-1</i> (-)/ <i>HLA-DR</i> tumor(-)	4	11.1

¹While *PECAM-1* and *ICAM-3* were positive when more than 5% of the tumor cells were stained positive, *HLA-DR* antigen positivity depicted in Table 4 represents the 15% cut-off level used in our survival analysis.

survival rate (55.6% *vs* 69.6%), this difference was not proved to be statistically significant ($P=0.4067$). Nevertheless, patients who showed *PECAM-1* expression both on their endothelial cells ($n=4$), presented a decreased overall survival rate compared to the rest of the group (25% *vs* 71.4%, $P=0.0403$).

Different levels of proportional *HLA-DR* antigen expression were sequentially evaluated for their prognostic value. *HLA-DR* antigen expression in more than 15% of the tumor cells ($n=26$, 72.2%) was associated with an increased 5-year survival rate (75% *vs* 37.5%, $P=0.0469$) (Figure 3B). A corresponding though non-significant increase in the overall survival rate was also observed in those gastric lymphoma patients expressing *HLA-DR* antigen in more than 15% of their peritumoral stromal cells ($n=19$, 52.8%) (66.67 % *vs* 64.29 %, $P=0.8593$).

A univariate analysis was also performed to identify high-risk groups of patients with regard to gene co-expression. For the needs of this analysis, the 15% *HLA-DR* antigen expression level was considered a measure of *HLA-DR* positivity between tumor cells. Those patients who were *PECAM-1*(+)/*ICAM-3*(-) ($n=5$) presented a significantly decreased overall survival rate compared to those who were *PECAM-1*(-)/*ICAM-3*(+) ($n=12$) (20% *vs* 83.3%, $P=0.0041$) (Figure 4A). Furthermore, gastric lymphoma patients who were *HLA-DR* (+)/*ICAM-3*(+) ($n=10$) presented a significantly improved overall survival rate compared to those who were *HLA-DR*(-)/*ICAM-3*(-) ($n=4$) (90% *vs* 25%, $P=0.0091$) (Figure 4B). When all three genes were studied together, all *HLA-DR*(+)/*ICAM-3*(+)/*PECAM-1*(-) gastric lymphoma patients ($n=8$) were alive 5 years postoperatively (100% 5-year survival rate), compared to a 54.2% survival rate for the rest of the group ($n=24$) ($P=0.0289$) (Figure 5).

Univariate analysis revealed that patients' gender, tumor stage, histologic grade and marginal status were not associated with their overall survival rate as demonstrated in Table 5.

To identify the independent prognostic factors that would predict survival, multivariate analysis was performed. The analysis included *HLA-DR* antigen and *PECAM-1* upregulation (which was proved to be statistically significant prognostic markers in univariate analysis),

Table 5 Five-year survival rate according to patients' gender, tumor stage, histologic grade and microscopic resection margins

Characteristics	Number of cases	5-yr survival rate (%)	P
Gender			NS ¹
Male	20	65	
Female	12	66.7	
Stage			NS
I	16	62.5	
II	9	66.7	
III	1	100	
IV	6	66.7	
Histologic grade			NS
Low	13	76.9	
Intermediate	7	71.4	
High	12	50	
Microscopic margins			NS
Positive	5	60	
Negative	27	66.7	

¹ NS: Non-significant.

age of the patients, tumor stage and tumor grade. Four patients were excluded from the process due to inadequate 5-year survival data. Multivariate Cox regression analysis revealed that *PECAM* and *HLA-DR* antigen expressions were the only statistically significant independent prognostic variables in the group of gastric lymphoma patients ($P=0.005$ and $P=0.016$, respectively). Similar results were obtained when *PECAM-1* and *HLA-DR* antigen expressions were the only covariates submitted to multivariate analysis ($P=0.003$ and $P=0.009$, respectively). The results of multivariate analysis are depicted in Table 6 and Table 7.

DISCUSSION

Primary non-Hodgkin's gastric lymphoma represents a rare malignant tumor comprising 2-5% of all cases of malignant gastric tumor^[27, 28]. The 5-year survival rate ranges between 57%^[29] and 96% for IIE and IIE patients^[30], while it has been considered as low as 25% when all stages are grouped together. Several molecular markers like *P27*, *cyclin E*^[31] and *bcl-6*^[32] have been recently assessed for their prognostic value. Still, most of them have no independent prognostic value.

In malignant lymphoma patients, clinical outcome and prognosis appear to depend largely on host immune response and vascular invasion. The current study attempted to clarify the prognostic value of *HLA-DR* antigen, *ICAM-3* and *PECAM-1* since they represent specific markers of regional immune reactions, cell-cell interactions and transendothelial migration.

HLA-DR antigen expression is a marker of host immune response in human malignant neoplasms. Its expression in tumor cells has been reported to be related with a favorable prognosis in patients with different types of cancer such as breast cancer^[33] and squamous cell laryngeal carcinoma^[34]. In our multivariate analysis, *HLA-DR* antigen expression was found to be a statistically significant independent prognostic factor associated

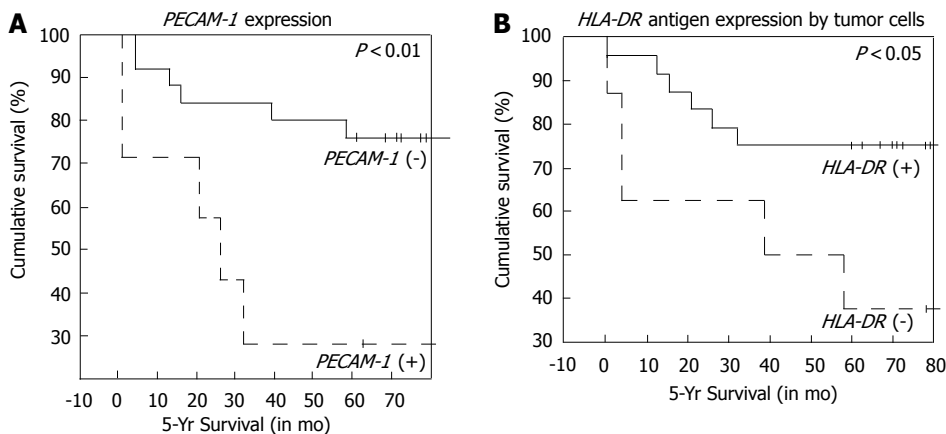


Figure 3 Five-year survival according to *PECAM-1* (A) and *HLA-DR* antigen (B) expression (*HLA-DR* positive patients had expression of *HLA-DR* antigen in more than 15% of their tumor cells).

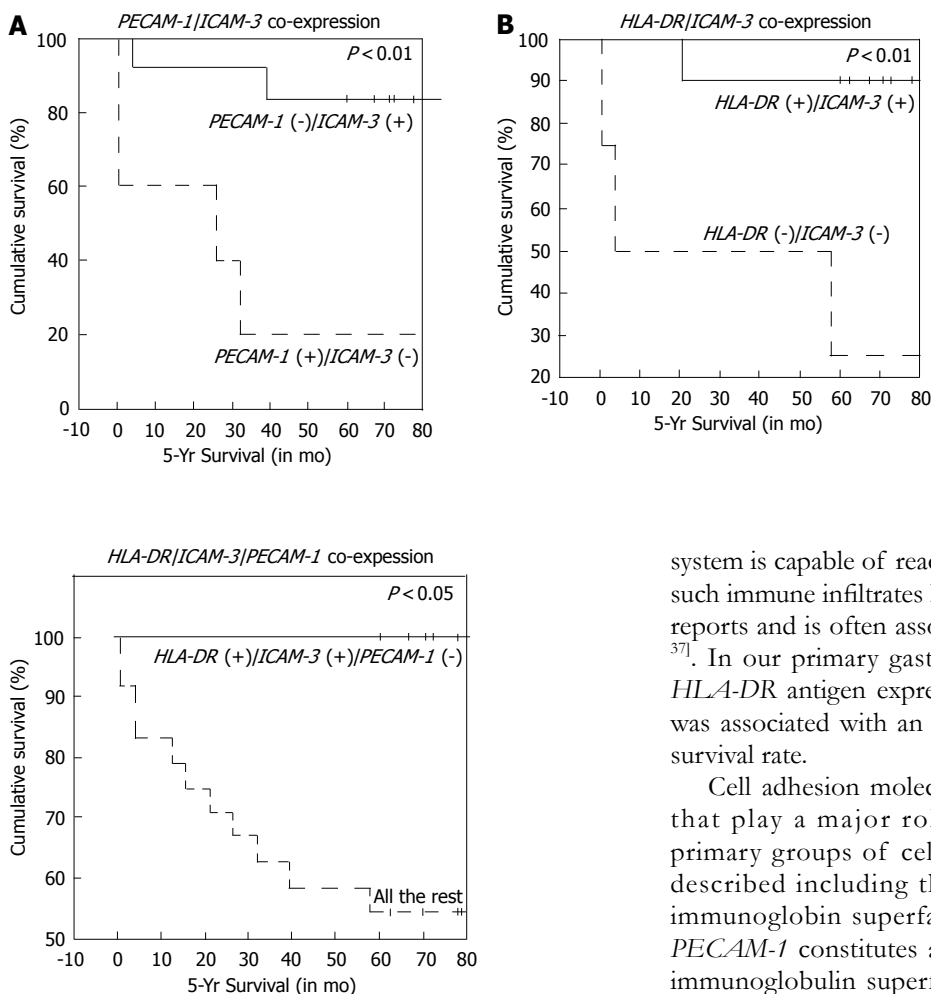


Figure 4 Decreased 5-year survival rate in *PECAM-1*(+)/*ICAM-3*(-) (A), and increased 5-year survival rate in *HLA-DR*(+)/*ICAM-3*(+) (B) gastric lymphoma patients.

Figure 5 One hundred percent 5-year survival rate in *HLA-DR*(+)/*ICAM-3*(+)/*PECAM-1*(-) gastric lymphoma patients.

with a favorable clinical outcome ($P=0.0199$). This is in agreement with previous reports^[33, 34]. The expression of *HLA-DR* antigen in tumor cells has a modulating effect on the host immune response, possibly by helping in the presentation of tumor associated antigens to T-lymphocytes^[35], thus enabling the immune system to inhibit cancer growth. Regarding peritumoral stroma cells, the presence of tumor infiltrating leukocytes (TIL) invading the site of malignancy, suggests that the immune

system is capable of reacting with tumors. The presence of such immune infiltrates has been documented in numerous reports and is often associated with improved prognosis^[36, 37]. In our primary gastric lymphoma group of patients, *HLA-DR* antigen expression in peritumoral stromal cells was associated with an increased (though non-significant) survival rate.

Cell adhesion molecules are membrane glycoproteins that play a major role in neoplastic disease. Four primary groups of cell adhesion molecules have been described including the integrin receptor family, the immunoglobulin superfamily, selectins and cadherins^[38]. *PECAM-1* constitutes a significant representative of the immunoglobulin superfamily and has been applied with considerable accuracy as an angiogenesis marker in several kinds of human neoplasia^[16, 17, 39, 40].

A significant number of gastric lymphoma patients expressed *PECAM-1* in their tumor cells ($n=10$, 27.8%). Since *PECAM-1* is constitutively expressed not only on the surface of endothelial cells, but on platelets, leukocytes, monocytes, neutrophils and selected T cell subsets as well^[11], our findings can be attributed to the lymphoid origin of the malignancy. *PECAM-1* positive staining on gastric lymphoma tumor cells was found to be a statistically significant independent prognostic factor, associated with unfavorable prognosis ($P=0.0029$). Although there are several reports^[17, 39] correlating increased angiogenesis with an unfavorable prognosis, this is the first report

Table 6 Multivariate Cox regression analysis I (Age, grade, stage, *PECAM-1* and *HLA-DR* antigen expression were included)

Variable	P value	Exp (B)	95% CI for Exp (B)
Age	NS	1.0085	0.9541-1.0660
Stage	NS	2.0919	0.2317-18.8828
Grade	NS	0.2272	0.0475-1.0855
<i>PECAM-1</i> tumor expression	$P < 0.01$	19.9490	2.7865-142.8178
<i>HLA-DR</i> tumor expression	$P < 0.05$	0.1373	0.0258-0.7299

on the clinical outcome of gastric lymphoma patients based on *PECAM-1* expression by tumor cells. As a key participant in cell adhesion cascade, *PECAM-1* can lead to the extravasation of leukocytes^[18], constituting a possible regulator of the metastatic process in lymphoid tumors. Its presence on the surface of tumor cells can be therefore associated with altered cellular adhesivity, enabling malignant cells to dissociate from their primary sites, leading to tumor growth and metastasis^[41-43].

The detection of *PECAM-1* (+) vessels within the tumor was related to an unfavorable, though non-significant clinical outcome. This difference reached statistical significance only for gastric lymphoma patients ($n = 4$) who showed *PECAM-1* expression both in their tumor cells and in their endothelial cells ($P = 0.0403$). Although the fact that *PECAM-1* expression in tumor endothelial cells has been associated with unfavorable prognosis in several tumors^[2, 16, 17, 40, 44-46], tumors with *PECAM-1* (+) endothelia exhibit an extended^[47] overall survival rate.

Vascular density at the tumor margins is significantly lower than that within tumor stroma^[12]. Additionally, gastric lymphoma patients with lymph node involvement present a significantly higher number of *PECAM-1* (+) stromal vessel counts (7.285 vs 3.753, $P = 0.002$). It appears that the density of *PECAM-1* (+) stromal microvessels/mm² correlates with the lymphatic metastatic pathway. Several studies have reported that *PECAM-1* is a non-specific angiogenesis marker, which stains both lymphatic (weaker staining) and blood vessel endothelial cells^[48, 49]. New proliferating capillaries in the tumor stroma have fragmented basement membranes^[50], while endothelial cells at the tips of growing capillaries secrete collagenases and plasminogen activators^[51]. These properties facilitate the metastatic process and may explain the association between tumor stromal vessel counts and lymph node metastasis as observed in our study.

Antigen-dependent and/or independent interactions between target cells and lymphocytes are required for the initiation of a specific immune response. In addition to antigen-specific interactions, "accessory" cell-surface molecules, ICAMs^[52,53], mediate an antigen-independent, non-specific adhesion between the reactant cells, which is considered the primary step in activation of lymphocytes^[54]. These events often take place in a host against malignant cells. Recent work has indicated that resting leukocytes express a third ligand, ICAM-3, which appears to be the major ligand for *LFA-1* in initiating phases of immune response^[10,55]. Although it is poorly investigated, *ICAM-3* upregulation has been well documented in lymphoid tumors^[56,57]. In our gastric

Table 7 Multivariate Cox regression analysis II (Only *PECAM-1* and *HLA-DR* antigen expressions were included, since they were the only statistically significant factors found in the univariate analysis)

Variable	P value	Exp (B)	95% CI for Exp (B)
<i>PECAM-1</i> tumor expression	$P < 0.01$	10.3520	2.2196-48.2812
<i>HLA-DR</i> tumor expression	$P < 0.01$	0.1308	0.0286-0.5989

lymphoma group of patients, *ICAM-3* upregulation was associated with a non-significantly improved 5-year survival rate. The fact that *ICAM-3* expression level is associated with increased tumor surface and thickness provides evidence that its regulation is directly proportional to the tumor burden.

Furthermore, *ICAM-3* expression when studied in combination with *PECAM-1* and/or *HLA-DR*, improved their prognostic accuracy. From our univariate analysis, three distinct gene expression patterns were distinguished. Two of them [*PECAM-1*(+)/*ICAM-3*(-) and *HLA-DR*(-)/*ICAM-3*(-)] were associated with a significantly decreased 5-year survival rate, when compared to the exactly opposite gene expression patterns [*PECAM-1*(-)/*ICAM-3*(+) with $P = 0.0041$ and *HLA-DR*(+)/*ICAM-3*(+) with $P = 0.0091$, respectively]. Furthermore, gastric lymphoma patients who were *HLA-DR* (+) / *ICAM-3* (+) / *PECAM-1* (-) had 100% 5-year survival rate. Similar results have been reported by Hosch *et al*^[58], who observed that the co-expression of *HLA* class I molecules and *ICAM-1* is a significant predictor of increased disease-free survival in patients with primary esophageal carcinomas. It appears that the lack of expression for both *HLA-DR* and *ICAM-3* [*HLA-DR*(-)/*ICAM-3*(-)] in gastric lymphoma patients is associated with a compromised host immune response against the tumor as well as with an unfavourable prognosis compared to those who were *HLA-DR*(+)/*ICAM-3*(+) ($n = 10$, $P = 0.0091$). On the contrary, gastric lymphomas expressing both *HLA-DR* and *ICAM-3* but not *PECAM-1* have not only a more potent host immune surveillance, but a negative expansion potential and a growth disadvantage as well.

In our study, *PECAM-1* and *HLA-DR* antigen expressions were proved to be statistically significant independent prognostic factors in a group of patients with primary B-cell MALT-type non-Hodgkin's gastric lymphoma, suggesting that upregulation of both *PECAM-1* and *HLA-DR* antigen is closely related to the clinical phenotype exhibited by the affected cases. Furthermore, it appears that *ICAM-3*, *PECAM-1* and *HLA-DR* represent complementary biologic pathways associating host immune surveillance with non-specific intercellular interactions and endothelial transmigration. Their combined study in gastric lymphoma patients may amplify their prognostic accuracy and provide a better description of the biologic behaviour of these tumors.

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