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Frequency and clinical association of *NY-ESO-1* gene expression in diffuse large B-cell lymphoma

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Abstract

Objective: *Our objective was to evaluate the frequency of expression and determine the expression levels of the NY-ESO-1 gene in patients with DLBCL as well as to examine its relationship with clinical parameters and survival.* **Methods:** *We analyzed NY-ESO-1 gene expression levels using real-time quantitative RT-PCR (RT-qPCR) in 112 patients with DLBCL. The associations between the expression of the NY-ESO-1 gene and the clinical variables were evaluated using the Chi-square test and Fisher's exact test. Overall survival (OS) was determined using the Kaplan–Meier method.* **Result:** *The results showed that the NY-ESO-1 gene was expressed in 46.4% (52/112) of patients with DLBCL, and NY-ESO-1 gene expression was associated with clinical parameters such as LDH, clinical stage, and International Prognostic Index (IPI) ($p \leq 0.05$). High levels of NY-ESO-1 gene expression were associated with advanced disease stages, and the survival rates after 5.3 years of tracking were lower in the patients expressing the NY-ESO-1 gene (66.4%) than in those not expressing the gene (23.1%).* **Conclusion:** *The expression levels of the NY-ESO-1 gene in patients with DLBCL may be of great utility for diagnosing and determining the prognosis of this disease.*

Keywords: *DLBCL. NY-ESO-1. Lymphoma.*

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma, and it is responsible for approximately 30-50% of all new cases[1,2]. DLBCL represents a heterogeneous group of tumors with highly variable genetic abnormalities, clinical characteristics, responses to treatment, and prognosis[3-5]. The diagnosis of DLBCL is accomplished through histopathological studies and immunophenotyping. The following clinical criteria are currently used for determining the prognosis of this disease: clinical stage, functional state (ECOG), International Prognostic Index (IPI), LDH levels, and β2 microglobulin levels. Despite advances in immunotherapy (anti-CD20 therapy) as well as the incorporation of new cytotoxic agents (bendamustine), a select group of patients continues to have an unfavorable prognosis[6].

The subdivision of DLBCL into two major biological categories based on their presumed cell of origin: germinal center B cell (GCB), and activated B cell (ABC)[7].

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Several molecular alterations have been identified in DLBCL, such as the expression of the *NY-ESO-1* gene (New York esophageal squamous cell carcinoma-1), which is part of the group of cancer-testis antigen (CTA)[8-9]. This gene encodes a protein that is overexpressed in many cancers, but absent in normal tissue except for testicular[10-11]. This gene is found in a duplicated region of the X-chromosome and, therefore, has a neighboring gene of identical sequence. It has been used to diagnose and assess the prognosis of various types of cancer[11-12], and its expression is restricted solely to immune-privileged germinal cells, which are the most immunogenic of this family[13-14]. It is abnormally expressed in a variety of cancers and is associated with the unfavorable evolution of cancer of the cervix and breast, as well as multiple myeloma and non-small cell lung cancer[15-19].

Levels of expression of the *NY-ESO-1* gene were analyzed in patients with DLBCL using quantitative RT-PCR (RT-qPCR) in real time and relationship between the clinical parameters and survival rate, the detection of *NY-ESO-1* by RT-qPCR could be useful for disease prognosis and follow-up.

Materials and methods

Type of study

This was a prospective, descriptive observational, clinical study between April 2018 and June 2020.

Study population

This was a prospective clinical study with 112 patients with DLBCL who had previously provided signed informed consent forms. The histological diagnosis was established according to the World Health Organization (WHO) classification (SH, 2020). Approval for the present study was provided by the Ethics Committee of the Hospital General de Mexico "Dr. Eduardo Liceaga." The informed written consents were collected from all enrolled patients and the entire study was performed based on the Declaration of Helsinki.

The study population was characterized according to their clinical parameters, including prior medical history, disease stage, and levels of lactate dehydrogenases (LDHs). The average age was 45 years (range 18-69), and 46.4% were male and 53.5% female. The patients were treated with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone). Patients who showed a partial response to treatment were treated with dexamethasone, etoposide, and cisplatin as second-line chemotherapy at the discretion of the treating doctor. The survival global analysis was conducted after 5.3 years.

Lymph node biopsies

Lymph nodes from the patients were frozen in liquid nitrogen immediately after surgical excision and stored until RNA extraction.

Testicular tissue

Testicular tissue from a 60-year-old patient with prostate cancer was used to determine the levels of relative expression basal of the *NY-ESO-1* gene.

RNA extraction and cDNA preparation

Total cellular RNA was extracted from the frozen tissue and the controls using TRIzol® Reagent (Life Technologies, Paisley, UK). The RNA was stored at –80°C until needed. A total of 2 μg of RNA was used for the synthesis of cDNA by means of the reverse transcriptase M-MLV (Life Technologies, Paisley, UK).

Quantitative real-time PCR assay

The mRNA expression levels of the *NY-ESO-1* (Hs00265824_m1)[19] and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Hs00985689) genes were measured using the TaqMan® gene expression assay (Applied Biosystems, Foster City, CA, USA). The *GAPDH* gene was used as an endogenous control, and each sample was analyzed in triplicate.

The relative gene expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method. We used the median as cutoff between high and low expression.

Statistical analysis

The analyses between *NY-ESO-1* gene expression and the clinical variables were performed using Chi-square test and Spearman test. The survival data were analyzed using the Kaplan–Meier method and compared with the log-rank test, considering $p \leq 0.05$ to be statistically significant. The statistical program S.P.S.S. version 20 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, USA) was used for the analyses.

Results

Frequency of NY-ESO-1 expression at the mRNA level in DLBCL patients

The frequency of *NY-ESO-1* gene expression was 46.4% (52/112). The levels of relative expression with respect to control (testicular tissue) were 0.2 times in Stages I/II, while in Stages III/IV, they were 1.5 times and 2.2 times, respectively, (Fig. 1). The expression levels were significantly different between Stages III and IV in comparison with Stage I/II, revealing a relation hip between the level of expression and advanced-stage disease (p = 0.007).

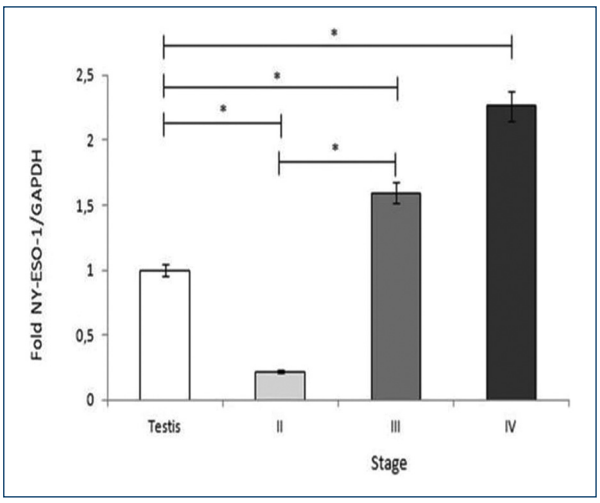

Figure 1. mRNA expression levels of *NY-ESO-1* in DLCBL stages. The expression results were obtained from four samples from Stages I and II, 48 from Stages III and IV. Significant differences between results ($p \leq 0.5$) were obtained means of parametric test. T, testicular tissue.

Association of NY-ESO-1 expression with clinical variables

The statistical analysis showed significant values for the parameters of LDH, clinical stage, and IPI ($p \leq 0.05$). Elevated LDH levels in serum and a high IPI were associated with gene expression in 39.2% (p = 0.001) and 32.1% (p = 0.019) of the patients, respectively. In addition, 42.8% of the positives were associated with clinical Stage III or IV (p = 0.001) (Table 1).

Expression of NY-ESO-1 and its relation to the survival rate

The study was performed over 5.3 years, and survival median at 3 years was 23.1% for the positive patients and 66.4% for the negative patients (Fig. 2). During this period, we observed that 76.9% (40/52) of the patients expressing *NY-ESO-1* died. In contrast, 33.3% (20/60) of the negative patients died. In the statistical analysis, a log-rank value of p = 0.001 was calculated.

Discussion

Patients with DLBCL exhibit heterogeneous clinical characteristics, as well as variability in their responses to treatment and prognoses[20-22]. Although survival can be estimated based on clinical parameters (age, LDH levels in serum, extranodal site involvement, disease stage, and immunophenotype B), as well as molecular abnormalities (*p53, BCL-2, BCL6, MUM.1, and Ki67*), controversy exists regarding their utility as prognostic and survival markers[23]. As a result, it is of paramount importance to find new markers that could be incorporated to determine the prognosis of this disease.

We evaluated the clinic pathological relevance of *NY-ESO-1* gene expression in patients with DLBCL at diagnosis who were admitted to the hematology service of the Hospital General de México. We decided to examine the expression of the *NY-ESO-1* gene in patients with lymphoma, as it is a CTA present in various types of cancer and is associated with clinical factors such as poor prognosis and lower survival[24-25]. We confirmed that *NY-ESO-1* gene expression is associated with the advanced stage of the disease, changes in the levels of LDH and the IPI, and survival rates. In DLBCL, there are no reports of an association between the expression of this gene and clinical parameters. Hudolin et al.[26] analyzed the expression of the *NY-ESO-1* gene in 24 samples of testicular tissue with DLBCL; expression was observed in 54.1% and was not correlated with clinical parameters or survival. We observed that the percentage of expression of the *NY-ESO-1* gene in the stages of the disease in patients with DLBCL was 3.5% in Stages I-II and 42.8% in Stages III-IV. These results are consistent with the previous reports on melanoma in which a percentage of 3.34% was observed in Stage I and 9.52% in Stage II, and up to 45% in Stage III[27]. Similar results were reported in bladder and prostate cancer, where the frequency of expression increases with respect to the stage of the disease[28-30].

Only two studies have measured the expression levels in metastatic esophageal squamous cell carcinoma

Table 1. Associations between clinical characteristics and NY-ESO-1 expression in DLBCL patients

	Expression	No expression
Median (range)	58 (18-69)	37 (19-65)
Sex Male Female	 (%) 20 (17.8) 32 (28.5)	 (%) 32 (28.5) 28 (25)
More than 1 extra nodal site Yes No	 32 (28.5) 20 (17.8)	 20 (17.85) 40 (35.7)
ECOG performance status greater 0 1 2 3 4	 0 28 (25) 24 (21.4) 0 0	 4 (3.5) 48 (42.8) 8 (7) 0 0
Serum LDH level > normal Yes No	 44 (39.2) 8 (7)	 12 (10.7) 48 (42.8) 0.001*
IPI Low Low-intermediate High	 4 (3.5) 12 (10.7) 36 (32.1)	 28 (25) 20 (17.8) 12 (10.7) 0.019*
Stage I-II III-IV	 4 (3.5) 48 (42.8)	 40 (35.7) 20 (17.8) 0.001*

*Chi-square value significance level $p \leq 0.05$.
LDH: lactate dehydrogenase.

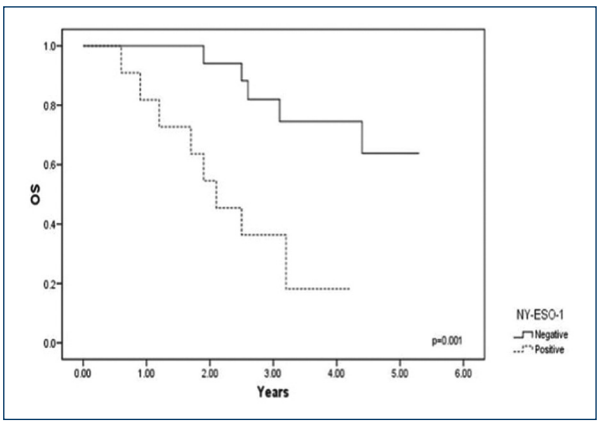

Figure 2. Overall survival in DLCBL patients based on the *NY-ESO-1* expression. The 5.3 years overall survival rate is analyzed in patients. Survival median at 3 years was 23.1 for the positive and 66.4% for the negative patients ($p = 0.001$).

and non-small-cell lung cancer using RT-qPCR, and elevated transcription levels were associated with advanced disease stages[31-32].

The increase in the level of *NY-ESO-1* gene transcription in patients with DLBCL is a finding of great importance; it could be a prognostic marker for this disease. In addition, the increase in advanced stages of the disease may explain its oncogenic role and the proliferative advantage it confers to tumor cells[33-34].

Global survival is lower in patients who express *NY-ESO-1*, and these results concur with those reported for lung cancer, demonstrating that the expression of *NY-ESO-1* is significantly associated with an adverse prognosis[35]. Similar data associating the expression of this gene with decreased disease-free survival have been reported for gastrointestinal and bladder cancer[36]. Other reports have examined the associations between the expression of the *p53, bcl-2, and ki67* genes and global survival in patients with DLBCL and did not observe an association[37]. Rearrangements of the *BCL-6* gene have been associated with 50% survival at 5 years in patients treated with R-CHOP, although the reported expression frequency was only 19%[38]. We have reported that the *MAGE-A3* gene is associated with a decrease in survival in patients with DLBCL[39].

NY-ESO-1 gene expression in patients with DLBCL may be helpful for identifying and stratifying risk groups, with other molecular marker of this disease that may benefit from new or intensified therapies.

Conclusion

Our results demonstrate that expression of the *NY-ESO-1* gene is associated with a poor prognosis of patients with DLBCL, and it is highly important to incorporate this gene into panels of existing molecular markers.

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Conflicts of interest

The authors declare that do not exist conflicts of interest.

Ethical disclosures

Protection of people and animals. The authors declare that no experiments have been performed on humans or animals for this research.

Data confidentiality. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the corresponding author.

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