REVISTA DE INVESTIGACIÓN CLÍNICA

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Rev Inves Clin. 2016;68:171-5 BRIEF COMMUNICATION

Expression of HER2/Neu in B-Cell Acute Lymphoblastic Leukemia

Sergio Rodriguez-Rodriguez[1,2], Alan Pomerantz[1,2], Roberta Demichelis-Gomez[1], Georgina Barrera-Lumbreras[1], Olga Barrales-Benitez[1] and Alvaro Aguayo-Gonzalez[1]*

[1]Department of Hematology and Oncology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Tlalpan, Mexico City; [2]Faculty of Health Sciences, Universidad Anáhuac México Norte, Huixquilucan. Mexico

ABSTRACT

Background: The expression of HER2/neu in B-cell acute lymphoblastic leukemia has been reported in previous studies. **Objective:** The objective of this research was to study the expression of HER2/neu on the blasts of patients with acute leukemia from the Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran. **Methods:** From June 2015 to February 2016, a HER2/neu monoclonal antibody was added to the panel of antibodies that we routinely use in patients with acute leukemia. An expression of ≥ 30% was considered positive. **Results:** We studied 33 patients: 19 had *de novo* leukemia (57.6%), three (9.1%) were in relapse, and in 11 (33.3%) their status could not be specified. Seventeen patients (51.5%) were classified as B-cell acute lymphoblastic leukemia with a median expression of HER2/neu of 0.3% (range 0-90.2). Three patients with B-cell acute lymphoblastic leukemia were positive for HER2/neu: 89.4%, 90.9%, and 62.4%. The first and third patient had *de novo* B-cell acute lymphoblastic leukemia. The second patient was in second relapse after allogeneic stem cell transplant. All three patients were categorized as high-risk at the time of diagnosis. **Conclusions:** In the studied Mexican population, we found a positive expression of HER2/neu in 17% of the B-cell acute lymphoblastic leukemia patients, similar to previous studies in which the expression was found in 15-50%. (REV INVES CLIN. 2016;68:171-5)

Key words: Acute leukemia. Acute lymphoblastic leukemia. HER2/neu. Trastuzumab. Monoclonal antibody.

INTRODUCTION

Human epidermal growth factor 2 (HER2/neu) is a transmembrane tyrosine kinase receptor that belongs to the ErbB family[1]. Overexpression of HER2/neu has been found in 15-25% of patients with breast cancer, as well as in other carcinomas (i.e. stomach, colon, ovary, lung and cervix)[2-4]. In breast cancer, its presence has been correlated with a worse outcome (i.e. shorter survival time or lower response to chemotherapy)[5,6]. The use of trastuzumab, a humanized 4d5 monoclonal antibody that interferes with the HER2/neu receptor, in combination with chemotherapy has improved the overall response and survival in patients with breast cancer[7-10].

Corresponding author:
*Alvaro Aguayo-González
Departamento de Hematología y Oncología
Instituto Nacional de Ciencias Médicas y Nutrición
Salvador Zubirán
Vasco de Quiroga 15
Col. Belisario Domínguez Seccion XVI, Del. Tlalpan
C.P. 14080, Ciudad de México, México
E-mail: alvaroaguayo@hotmail.com

Received for publication: 09-03-2016
Accepted for publication: 20-05-2016

In 1995, Bühring, et al. reported for the first time the presence of HER2/neu on leukemic blasts of the B-lymphocytic lineage[11]. Researchers analyzed the blasts of 18 pediatric and 86 adult patients with chronic or acute leukemia and found the presence of HER2/neu in 47.6% (n = 10/21) of the adult patients with B-cell acute lymphoblastic leukemia (ALL) and 13.3% (n = 2/15) of the pediatric patients with B-cell ALL. Additionally, 75% (n = 3/4) of patients with chronic myeloid leukemia in B-lymphoid blast crisis expressed high levels of HER2/neu. No expression was found on the blast cells of patients with T-cell ALL, acute myeloid leukemia (AML), or chronic lymphocytic leukemia. After these results were published, other authors have corroborated the presence of HER2/neu on blast cells of pediatric and adult patients with B-cell ALL[12-16]. The purpose of this study was to demonstrate the presence of HER2/neu on the blast cells of adult Mexican patients with acute leukemia (AL) from the Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran.

MATERIALS AND METHODS

Study design

We included all the patients that according to the immunophenotypic analysis were diagnosed with AL (whether they had *de novo* or relapse AL) in our institution between June 2015 and February 2016. Anti-HER-2/neu (Neu 24.7/phycoerythrin) (Beckton Dickinson, San José, CA, USA) monoclonal antibodies were added to the panel of antibodies that are routinely used in patients with AL in the Hematology and Oncology Department.

Flow cytometry

An eight-color flow cytometry panel was used, which included the following fluorochromes: phycoerythrin (PE), fluorescein isothiocyanate (FITC), peridinin chlorophyll (PerCP), allophycocyanin (APC), V450, V500, PE and cyanine die 7 (PE-Cy7), and PerCP-Cy5.5. The panel of antibodies included were: HER2/neu PE, CD2-FITC, cytoplasmic CD3 (cCD3-FITC), CD5-PE, CD7-APC, CD10-PE or CD10-APC, CD11b-Cy5, CD13-PE, CD14-PE, CD15-FITC, CD19-FICT, CD20-FITC or CD20-V450, CD22-PE or CD22-APC, CD33-PE or CD33-APC, CD34-PE, CD41-PE, CD56-FITC, CD61-FITC, CD64-PE or CD64-APC, cCD79a-PE, CD117-APC, CD235a-FITC, anti-MPO-FITC, HLA-DR-V450, IgM-PE or IgM-APC. Data were obtained and analyzed in a FACSCanto™ II flow cytometer (Becton Dickinson Immunocytometry Systems, San José, CA, USA) with the aid of FACSDiva™ software (Becton Dickinson, San José, CA, USA). Samples were analyzed via a CD45 gating technique, which allowed the laboratory to analyze only the blast population. Positive expression was considered when a marker was present in 10% or more of the blast population in cytoplasmic markers (cCD3, cCD79a, cIgM, and MPO) and CD34, 20% or more in myeloid markers and HLA-DR, and 30% or more in lymphoid markers and HER2/neu.

RESULTS

Patients

The HER2/neu antibody had a median expression of 0.3% (range 0-90.2). The median age of the patients was 41 years (17-80), and 17 patients were male (51.5%). Nineteen patients had *de novo* leukemia (57.6%), three (9.1%) were in relapse, and in 11 patients (33.3%) the relapsed or *de novo* status was not specified due to the fact that they had not been followed-up at our institution. Seventeen patients (51.5%) were diagnosed with B-cell ALL, one (3%) with T-cell ALL, 13 (39.4%) with AML, and two (6.1%) with acute leukemia of ambiguous lineage. Of the 17 B-cell ALL patients, four (23.5%) were sub-classified as pro-B cell ALL, 11 (64.7%) as common B-cell ALL, and two (11.8%) as pre-B cell ALL.

HER2/neu expression

HER2/neu was not expressed on the blast population of the patients with AML, T-cell ALL, or acute leukemia of ambiguous lineage. Among the 17 patients with B-cell ALL, the median expression of HER2/neu was 0.3% (0-90.2) and three patients (3/17, 17.6%) were positive for HER2/neu. The demographic and clinical characteristics of the patients with B-cell ALL are shown in table 1.

Patient 1 had *de novo* leukemia and was classified as a high-risk individual due to the presence of the

Table 1. Demographic and clinical characteristics of patients with B-cell acute lymphoblastic leukemia

	HER2/neu+ (n = 3)	HER2/neu– (n = 14)
Gender, no. patients (%)		
Male	2 (66.7)	6 (42.9)
Female	1 (33.3)	8 (57.1)
Age, years (range)	26 (22-44)	34 (17-65)
Immunophenotype, no. patients (%)	2 (66.7)	8 (57.1)
De novo	1 (33.3)	1 (7.1)
Relapse	–	5 (35.7)
Not specified		
Immunological subtype, no. patients (%)		
Pro-B (I)	–	4 (28.6)
Common B-cell (II)	3 (100)	8 (57.1)
Pre-B (III)	–	2 (14.3)
Mature B-cell (IV)	–	–
Sample origin, no. patients (%)		
PB	2 (66.7)	5 (35.7)
BM	1 (33.3)	7 (50.0)
Not specified	–	2 (14.3)
Blasts in PB or BM, % (range)	77.4 (61- 87)	66.6 (3.0-90.6)
Marker expression, % (range)		
HER2/neu	89.4 (62.4-90.2)	0.15 (0-6.6)
CD34	99.4 (25.6-99.6)	94.5 (0.1-99.8)
CD10	66.4 (61.9-72)	69.8 (1.0-99.5)
CD19	95.3 (42.9-99.8)	97.8 (94.2-98.5)
CD20	13 (4.3-18.3)	5.7 (0.2-97.6)
CD22	85.6 (76.1-98.0)	92.3 (0.8-99.0)

ALL: acute lymphoblastic leukemia; PB: peripheral blood; BM: bone marrow.

Philadelphia chromosome and a high white blood cell count at diagnosis; HER2/neu expression on the blast population of the patient was 89.4%. Patient 2 was in second relapse after allogeneic stem cell transplant, and was classified as high-risk due to the presence of a high white blood cell count at diagnosis; HER2/neu expression on the blast population in this patient was 90.2%. Patient 3 had *de novo* leukemia, and was classified as high-risk due to a high white blood cell count at diagnosis; HER2/neu expression on the patient's blast cells was 62.4%.

Expression of other markers

Patients 1 and 2 expressed CD34, CD19, and CD22 on > 90% of the blast population. CD10 was expressed on > 70% of the blast population in patient 1, while patient 2 expressed CD10 on > 60% of the blasts. Patient 3 expressed CD19 on > 90% of the blast population, CD22 on > 70% of the blast population, CD10 on > 60% of the blast population, and CD34 on > 20% of the blasts. Additionally, this patient was the only one that expressed CD20.

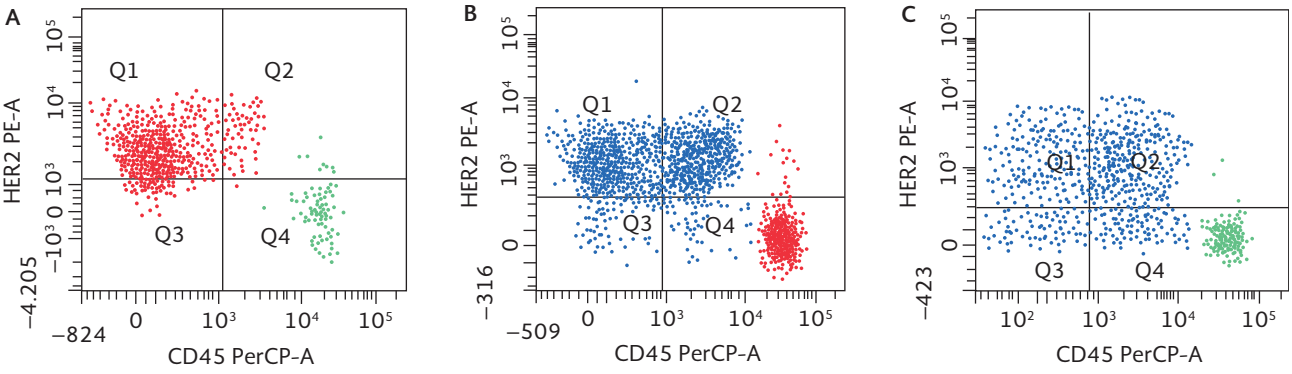
Outcome of patients

Patient 1 achieved complete remission after treatment with a hyper-cyclophosphamide, vincristine, doxorubicin (also known by its trade name, Adriamycin), and dexamethasone (CVAD) regimen. When we were writing this report, patient 2 was starting treatment with a modified hyper-CVAD regimen that included trastuzumab and excluded doxorubicin (the patient was in a dire state and, due to the cardiotoxicity of both trastuzumab and anthracyclines, we decided to exclude doxorubicin). Patient 3 achieved complete remission after treatment with hyper-CVAD and rituximab.

DISCUSSION

In previous studies, HER2/neu was positive in 15-50% of the patients with B-cell ALL[11-16]. Our results confirm the existence of HER2/neu in AL of a B-lymphoblastic lineage, and that the percentage of patients with B-cell ALL positive for HER2/neu in our population (17.6%)

Figure 1. Flow cytometry dot plots representing HER2/neu expression in B-cell acute lymphoblastic leukemia blasts. **A.** Patient 1 with 87% of blasts (red dots); 89.4% of the blasts expressed HER2/neu and 4.5% co-expressed HER2/neu and CD45. **B.** Patient 2 with 61% of blasts (blue dots); 90.9% of the blasts expressed HER2/neu and 47.5% co-expressed HER2/neu and CD45. **C.** Patient 3 with 76.4% of blasts (blue dots); 62.4% of the blasts expressed HER2/neu.



falls within the range reported by other authors. Furthermore, the expression of HER2/neu on the blast population of these patients (60-90%) was similar to that found by previous authors[13,14] (Fig. 1).

As mentioned before, the expression of HER2/neu in breast cancer is usually associated with a poor prognosis[5,6]. Some authors have stated that the expression of HER2/neu on the blasts of patients with B-cell ALL may also be associated with a poor outcome[12,13,15]. However, Haen, et al. stated that, since the patients were followed for a short period of time, these studies were underpowered to determine a valid prognostic significance[16]. In their study, which gathered information in a retrospective manner from patients that were followed-up to 15 years, they found no negative correlation between HER2/neu expression and chemoresistance, relapse rates, disease-free survival, or overall survival[16].

Furthermore, the antitumor activity of HER2/neu monoclonal antibodies was evaluated in three studies[12,15,16]. In two of these studies, the researchers demonstrated, *in vitro,* the lysis of blasts from patients with HER2/neu-positive B-cell ALL when exposed to an anti-HER2/neu antibody and either cytotoxic T-lymphocytes or NK cells[12,16]. To this day, only one study has used trastuzumab *in vivo* in patients with HER2/neu-positive B-cell ALL. The study included 15 adult patients with relapsed or refractory disease that received trastuzumab as their single treatment, and an overall response rate of 13% was achieved[15].

From the low overall response rate reported in the study that used trastuzumab in patients with HER2/neu-positive B-cell AL, it could be assumed that trastuzumab has no use in the treatment of these patients. However, in breast cancer, the use of trastuzumab as a single agent provides similar overall responses (15-26%)[17,18]. Consequently, we believe that in patients with HER2/neu-positive B-cell ALL, the rate of response to trastuzumab should be evaluated alongside other chemotherapeutic regimens.

Another factor that could impact the treatment strategies in these patients is the expression of other markers besides HER2/neu. A frequent association of HER2/neu with CD20 and CD34 has been observed[14]. In our study, we found that the markers more frequently expressed besides HER2/neu were CD19 and CD22. Therefore, in HER2/neu-positive B-cell ALL patients, trastuzumab and the usual chemotherapeutic regimens could be used in combination with other biological therapies (e.g. the anti-CD22 monoclonal antibody epratuzumab) in an attempt to improve the overall responses of these individuals[19].

Despite the relatively small number of samples, we proved the existence of HER2/neu in Mexican patients with B-cell ALL. Thus, we will continue to search for the expression of HER2/neu in patients with B-cell ALL to assess the clinical impact of this peculiar phenomenon. Additionally, we are planning to continue

the evaluation of trastuzumab in combination with other anticancer treatments in patients who present HER2/neu-positive B-cell ALL.

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