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Original article

NT-proBNP as predictor factor of cardiotoxicity during trastuzumab treatment in breast cancer patients

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ABSTRACT

Background: Trastuzumab is a drug used in HER2-positive breast cancer that increases patient survival. Due to cardiotoxicity is the most important side effect of trastuzumab treatment, cardiac monitoring should be a priority. The purpose of this study is to evaluate plasma NT-proBNP level and major cardiovascular risk factors as possible early predictors of trastuzumab-induced cardiotoxicity in HER2-positive breast cancer patients.

Methods: We conducted a retrospective observational study involving 66 patients with HER2-positive breast cancer treated with trastuzumab. Left ventricle ejection fraction (LVEF), NT-proBNP values, and the history of cardiovascular risk factors were collected. Cardiotoxicity was diagnosed considering a decrease of the LVEF from baseline or clinical manifestation of congestive heart failure. NT-proBNP cut-off points were considered to establish normal or abnormal values according to patient age.

Results: 27.3% of the patients suffered cardiotoxicity during trastuzumab treatment. Most cases were diagnosed due to the appearance of cardiac symptomatology (66.7%). Logistic regression analysis showed a significant association of diabetes mellitus (OR 5.9, 95% CI 1.2–28.5, $p = 0.028$) and high NT-proBNP levels (OR 22.0, 95% CI 5.7–85.4, $p < 0.0001$) with the development of trastuzumab-induced cardiotoxicity.

Conclusion: NT-proBNP levels above the upper limit of the normal range adjusted to age or diabetes mellitus seem to be associated with a higher risk of developing cardiotoxicity. However, some limitations of the present study make necessary further studies aimed to clarify whether NT-proBNP and diabetes-associated markers determinations can be useful in the monitoring of cardiotoxicity risk in breast cancer patients undergoing trastuzumab therapy.

1. Introduction

HER2/ErbB2 protein belongs to a group of transmembrane receptors with intracellular tyrosine kinase activity that is overexpressed in 20–25% of human breast cancers and is associated with aggressive tumor growth and poor prognosis [1]. However, the appearance in recent decades of targeted therapies against components of this family of receptors has provided a revolution in the treatment of this type of tumors. This is partially due to the possibility of combining targeted therapies with chemotherapy, hormone therapy, and/or other inhibitors. A wide variety of anti-HER2 drugs has been described including trastuzumab, pertuzumab, lapatinib, neratinib, margetuximab, zenocutuzumab (MCLA-128) and the antibody-drug conjugate trastuzumab-emtansine (T-DM1), among others [2,3]. Some of these drugs have provided extensive clinical experience in treating both early and advanced stages of breast cancer. Trastuzumab (Herceptin®) is a humanized monoclonal antibody that selectively binds to the extracellular domain of the human epidermal growth factor receptor-2 (EGFR-2, HER2) [4]. 1-Year treatment with trastuzumab in the adjuvant setting has

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been demonstrated to increase the disease-free period as well as the overall survival of these patients [5].

Despite the benefits of this type of therapy, trastuzumab has been shown to have a high number of cardiac side effects that can suppose a significant risk to patients. Similarly to other cytotoxic drugs such as anthracyclines, numerous cardiac side effects have been described associated to trastuzumab treatment, including: left ventricular dysfunction, congestive heart failure (CHF), conduction and rhythm disturbances, acute coronary syndrome, hypertension, pericardial pathology, vasospasm and dilated cardiomyopathy [6]. Dilated cardiomyopathy has the poorest prognosis, especially if it does not respond to conventional heart failure therapy, with a 2-year mortality of 60% [7]. Different clinical trials using trastuzumab as adjuvant therapy reported an incidence of clinical heart failure of 1.7%–4.1%, and a left ventricular dysfunction of 7.1%–18.6% [8]. Even so, the incidence of trastuzumab-induced cardiotoxicity in clinical practice may be even higher, especially when combined with other chemotherapy treatments.

To early diagnose trastuzumab-induced cardiotoxicity in patients with HER2-positive breast cancer, imaging tests are recommended for all patients undergoing treatment with trastuzumab. Currently, the method of choice is the measurement of the left ventricular ejection fraction (LVEF) by echocardiography or radionuclide ventriculography (MUGA scan) [9]. Transthoracic echocardiography is generally preferred due to its widespread availability, the absence of ionizing radiation exposure, and its ability to diagnose alterations in diastolic function. However, the main disadvantage of this technique is that the results present a high degree of observer-dependent variability, and also vary depending on the echocardiographic method chosen to calculate LVEF [10]. There are different protocols issued by various institutions about the clinical practice recommendations to be carried out in breast cancer patients being treated with trastuzumab, but all of them follow a similar scheme: evaluation of baseline heart function before trastuzumab administration, followed by LVEF monitoring every 3 months during the course of treatment [11–14].

Nonetheless, most authors agree that the drop of systolic function measured by LVEF is often a late phenomenon in the pathophysiological mechanism of trastuzumab-induced cardiotoxicity [15]. This means that when we detect a drop in LVEF by echocardiography there is a high likelihood that severe myocardial damage has already occurred, so that evaluation of cardiac function by this method alone substantially reduces the temporal window for the prevention and treatment of the cardiotoxic effects of chemotherapy [9,14]. Thus, this finding has led to the search for more sensitive techniques to detect minimal changes in heart function in breast cancer patients. In this way, we could achieve an improvement in the overall prognosis of patients, as well as optimal control of oncological treatment.

The main objective of this study is to analyze the correlation between the appearance of cardiotoxicity and the elevation of NT-proBNP plasma levels in comparison with LVEF measured by echocardiography, to identify this biomarker as an early predictor of trastuzumab-induced cardiotoxicity. Besides, we will try to demonstrate whether patients with certain cardiovascular risk factors such as smoking, diabetes, hypercholesterolemia, hypertension and increased body mass index, have a higher risk of developing cardiotoxicity during trastuzumab treatment.

2. Patients and methods

2.1. Study population

We performed a retrospective observational study that recruited women with breast cancer that were treated with trastuzumab from the San Cecilio University Hospital in Granada (Spain). The protocol was approved by the Provincial Ethics Committee of Granada following the principles set out in the Declaration of Helsinki. The information necessary for the elaboration of this study was extracted from the computerized medical records of each patient through the healthcare information system.

To be included in our study, all the patients had to present HER2-positivity according to the following two criteria: 3+ staining on immunohistochemistry (IHC), or a positive result on fluorescence in situ hybridization (FISH) analysis for HER2 in case of 2+ staining or less on IHC. The clinical stage of each patient was not an exclusion criterion and thus early or advanced breast cancer cases were admitted. Early disease was considered when the tumor was limited to the breast without invasion to other tissues/organs or lymph nodes, while advanced disease was diagnosed when any of these complications were present.

Trastuzumab was administered as long as the baseline LVEF was higher than 55%. General guidelines for its administration were the following: an intravenous infusion of an initial dose of 8 mg/kg, followed at 21 days by a maintenance dose of 6 mg/kg every three weeks; or an intravenous infusion of an initial dose of 4 mg/kg, followed 7 days after by a maintenance dose of 2 mg/kg weekly. If the drug was well tolerated, either of the two guidelines was maintained for an average period of 1 year. In those cases in which the patients had needed neoadjuvant chemotherapy, trastuzumab was administered together with taxanes (docetaxel or paclitaxel). The treatment was interrupted in those circumstances in which trastuzumab-induced cardiotoxicity was suspected, that is, when clinical manifestations of CHF or other clinical signs suggestive of cardiotoxicity appeared, as well as in those patients in whom a decrease in baseline LVEF $\geq$10% was detected by echocardiography, obtaining a LVEF value below 50%.

2.2. Clinical examination

Some clinical conditions that have been documented in previous studies as possible influencing factors on cardiotoxicity associated with breast cancer chemotherapy treatment were considered. Among them, we decided to gather information about 5 cardiovascular risk factors: smoking, hypertension, diabetes mellitus, hypercholesterolemia and body mass index (BMI). Regarding the latter, we decided to divide the patients into three groups: normal weight (BMI $\leq$24.99 kg/m^2), overweight (BMI = 25–29.9 kg/m^2), and obesity (BMI $\geq$30 kg/m^2).

2.3. Echocardiography

The echocardiographic method chosen to measure LVEF was the biplane method of disks (modified Simpson's rule), which is the current method recommended by the American Society of Echocardiography for the assessment of LVEF [15]. If this method was not available, the estimations were carried out using the Teichholz M-mode method. Measurements were made before chemotherapy and every 3 months after the introduction of trastuzumab. Cardiotoxicity was defined as an asymptomatic drop in baseline LVEF $\geq$10% with a final value < 50%, or any drop in LVEF accompanied by signs or symptoms of CHF or another cardiac clinic suggestive of cardiotoxicity [16–18]. Among the clinical manifestations that were considered for the appearance of trastuzumab-induced cardiotoxicity are the following: signs or symptoms of CHF, arrhythmias, pericarditis with or without pericardial effusion, and dilated cardiomyopathy with left ventricular systolic dysfunction.

2.4. Laboratory analyses

To obtain NT-proBNP plasma levels, sandwich-type ELISA (Roche Diagnostics®) were applied from blood samples extracted from the patients. Blood draws were taken about every 3 months after starting treatment with trastuzumab. The resulting values were expressed in pg/ml. Depending on the age group, different NT-proBNP cut-off points were considered to exclude the diagnosis of acute cardiac dysfunction [19,20], <125 pg/ml (<50 years), <300 pg/ml (≥50 and 75 years) or <450 pg/ml (≥75 years). Below 10 pg/ml, NT-proBNP plasma levels were considered undetectable. Since one of the objectives was to correlate NT-proBNP levels with the appearance of cardiotoxicity, the maximum time elapsed between each LVEF and NT-proBNP value that we admitted was 30 days with either parameter being measured first.

2.5. Statistical analysis

Qualitative variables were expressed in frequencies and percentages, while for quantitative variables both the mean and the standard deviation were calculated. Normality was checked by Lilliefors-corrected Kolmogorov-Smirnov test, and the correlation between NT-proBNP and LVEF was analyzed by Spearman's rho test considering all the patients or the association of both variables within two age groups (<50 and ≥ 50 years old). We performed a logistic regression model to evaluate the impact of cardiovascular risk factors and NT-proBNP levels (normal vs pathological) on trastuzumab-induced cardiotoxicity. Furthermore, we calculated ROC curves to predict the NT-proBNP cut-off points from which the risk of suffering trastuzumab-induced cardiotoxicity increases. $p < 0.05$ were considered statistically significant. All analyses were performed using IBM SPSS Statistics v.24 software.

3. Results

3.1. Patients

Our study sample consisted of 66 women with HER2-positive breast cancer diagnosed between 1995 and 2015. The baseline characteristics of patients are shown in Table 1. The mean time of clinical follow-up since the diagnosis of the primary tumor was 9.5 years. The mean age of the patients was 50.7 (with a range between 25 and 76), and more than half of them (54.5%) were over 50 years old. At the beginning of the study, 38 (57.6%) patients had localized disease while 28 (42.4%) had advanced disease. From the latter group, 27 patients had nodal involvement confirmed by biopsy, while 5 had distant metastases. 33 (50%) patients suffered one or more relapses during disease follow-up, and 19 (28.8%) ended up dying because of breast cancer.

A three-weekly regimen of trastuzumab (8 mg starting dose) was administered to 55 patients, and the remaining 11 patients received a weekly regimen (4 mg starting dose). 33 patients were treated with the drug at an early stage, whereas 33 were treated at an advanced stage. The advanced stage group included patients with localized disease that were initially treated with conventional chemotherapy but were treated with trastuzumab after nodal or distant metastases development. We found 6 (18.2%) and 12 (36.4%) cases of cardiotoxicity in the early and advanced stage groups, respectively. From the total number of women who received the three-weekly drug regimen, 14 (25.5%) had cardiac toxicity, and 13 of them presented NT-proBNP values in the pathological range adjusted to age. Moreover, in the weekly regimen group, 4 patients (36.4%) presented adverse cardiac events, and 5 NT-proBNP values above the upper limit of the normal range were registered.

The number of pairs LVEF and NT-proBNP that could be collected was not the same for each patient in the study. Table 2 shows the number of LVEF and NT-proBNP pairs and the number and proportion of patients that correspond to each value. A total of 174 pairs of LVEF and NT-proBNP values were obtained. The mean value (±SD) of LVEF was 60% (±7.9). The minimum registered was 25%, while the maximum was 85%. Likewise, the mean level (±SD) of NT-proBNP was 320.6 pg/ml (±1256.8), with a minimum of 13 pg/ml and a maximum of 13 913 pg/ml. Table 3 shows the values of both variables considering two age groups (women <50 years vs women ≥50 years). 18 patients (27.3%) suffered from trastuzumab-induced cardiotoxicity. Among them, 6 patients (9.1%) had subclinical cardiotoxicity, and they were diagnosed by a drop in LVEF ≥10% by echocardiography with a resulting LVEF <50%, and 12 patients (18.2%) presented clinically symptomatic cardiotoxicity at some point. The remaining 48 patients (72.7%) did not develop trastuzumab-induced cardiotoxicity at any time during the follow-up.

3.2. Association between NT-proBNP and LVEF

The correlation test between LVEF and NT-proBNP values showed a significant correlation between both variables (Spearman's coefficient −0.156, $p = 0.040$). Considering the referred two age groups, we found that women under 50 years presented a weak correlation (−0.119) but not significant ($p = 0.362$). However, in women ≥50 years old the correlation was significant (−0.194, $p = 0.039$).

3.3. Association among NT-proBNP, cardiovascular risk factors and cardiotoxicity

To assess the effect of cardiovascular risk factors (hypertension, smoking, hypercholesterolemia, diabetes mellitus and BMI) and pathological NT-proBNP levels on the appearance of trastuzumab-induced cardiotoxicity, we performed a binary logistic regression analysis. The model was statistically significant with $\chi^2 = 27.059$ and $p < 0.0001$. From the 6 predictor variables of cardiotoxicity, only diabetes and pathological levels of NT-proBNP, were statistically significant with a $p < 0.05$ and a 95% confidence interval (CI) that excluded the null value of odds ratio (OR). Thus, for diabetes, we obtained an adjusted OR = 5.861 (95% CI: 1.206–28.496) and a $p = 0.028$. For NT-proBNP levels we found an adjusted OR = 22.039 (95% CI: 5.689–85.385) and a $p < 0.0001$. Table 4 summarizes the information extracted from the regression analysis of these two variables.

3.4. ROC curve for the diagnosis of cardiotoxicity

The diagnostic validity of NT-proBNP as a cardiotoxicity biomarker was evaluated using the non-parametric ROC curve, in which the Y-axis corresponds to sensitivity and the X-axis represents the false positive rate (1 − specificity). The area under the ROC curve (AUC) was 0.879 (95% CI: 0.793–0.965), with a standard error of 0.044. This AUC was statistically significant, with a $p < 0.0001$. Besides that, we obtained an optimal cut-off point of NT-proBNP of 206.5 pg/ml, considering as maximum values a sensitivity = 75% and a specificity = 88% (Youden index = 0.63) (Fig. 1A).

Considering two age groups, only the ROC curve for patients ≥50 years old was statistically significant ($p < 0.0001$). The AUC was 0.874 (95% CI: 0.771–0.977), with a standard error of 0.053. The optimal cut-off point of NT-proBNP found in this group was 239.5 pg/ml, taking into account as maximum achievable values a sensitivity = 79% and a specificity = 87% (Youden index = 0.65) (Fig. 1B). On the contrary, in women <50 years of age, we could not

Table 1
Baseline patient characteristics and treatment.[a].

Characteristic	Cardiotoxicity (n = 18)	No cardiotoxicity (n = 48)	Total (n = 66)
Age at diagnosis (years)			
Mean [±SD]	53.22 [11.23]	49.79 [11.88]	50.73 [11.72]
Range	31–71	25–76	25–76
<50 years old	6 (9.1)	24 (36.4)	30 (45.5)
≥50 years old	12 (18.2)	24 (36.4)	36 (54.5)
Menopausal status			
Premenopause	6 (9.1)	28 (42.4)	34 (51.5)
Postmenopause	12 (18.2)	20 (30.3)	32 (48.5)
Estrogen receptor status			
Negative (<1%)	10 (15.2)	17 (25.8)	27 (40.9)
Positive (≥1%)	8 (12.1)	31 (47.0)	39 (59.1)
Progesterone receptor status			
Negative (<1%)	8 (12.1)	18 (27.3)	26 (39.4)
Positive (≥1%)	10 (15.2)	30 (45.5)	40 (60.6)
Ki-67			
Negative (<20%)	4 (6.0)	5 (7.6)	9 (13.6)
Positive (≥20%)	12 (18.2)	25 (37.9)	37 (56.1)
Unknown			20 (30.3)
HER2 Ile655Val polymorphism			
Ile/Ile genotype	4 (6.1)	20 (30.3)	24 (36.4)
Ile/Val genotype	8 (12.1)	9 (13.6)	17 (25.8)
Val/Val genotype	0	1 (1.5)	1 (1.5)
Unknown			24 (36.4)
Histological type			
Invasive ductal	17 (25.8)	45 (68.2)	62 (93.9)
Invasive lobular	0	1 (1.5)	1 (1.5)
Others	1 (1.5)	2 (3.0)	3 (4.5)
Tumor grade			
G-I	0	0	0
G-II	13 (19.7)	30 (45.5)	43 (65.2)
G-III	4 (6.0)	12 (18.2)	16 (24.2)
Unknown			7 (10.6)
Stage			
Stage I	3 (4.5)	16 (24.2)	19 (28.8)
Stage II	11 (16.7)	14 (21.2)	25 (37.9)
Stage III	2 (3.0)	5 (7.6)	7 (10.6)
Stage IV	1 (1.5)	4 (6.1)	5 (7.6)
Unknown			10 (15.2)
BMI (kg/m^2)			
Normal weight (18.50–24.99)	5 (7.6)	17 (25.8)	22 (33.3)
Overweight (25.00–29.99)	4 (6.1)	21 (31.8)	25 (37.9)
Obesity (≥30.00)	9 (13.6)	10 (15.2)	19 (28.8)
HBP			
Yes	8 (12.1)	13 (19.7)	21 (31.8)
No	10 (15.2)	35 (53.0)	45 (68.2)
DM			
Yes	4 (6.1)	4 (6.1)	8 (12.1)
No	14 (21.2)	44 (66.7)	58 (87.9)
Hypercholesterolemia			
Yes	10 (15.2)	19 (28.8)	29 (43.9)
No	8 (12.1)	29 (43.9)	37 (56.1)
Tobacco use			
Non-smoker	11 (16.7)	26 (39.4)	37 (56.1)
Smoker	4 (6.1)	14 (21.2)	18 (27.3)
Former smoker	3 (4.5)	8 (12.1)	11 (16.7)
Treatment			
Neoadjuvant	4 (6.1)	14 (21.2)	18 (27.3)
Adjuvant	11 (16.7)	23 (34.8)	34 (51.5)
Both	2 (3.0)	8 (12.1)	10 (15.2)
None	1 (1.5)	3 (4.5)	4 (6.1)
Radiotherapy			
Yes	17 (25.8)	43 (65.2)	60 (90.9)
No	1 (1.5)	5 (7.6)	6 (9.1)
Chemotherapy			
Anthracyclines	3 (4.5)	15 (22.7)	18 (27.3)
Taxanes	0	3 (4.5)	3 (4.5)
Both	14 (21.2)	26 (39.4)	40 (60.6)
Other drugs	0	1 (1.5)	1 (1.5)
None	1 (1.5)	3 (4.5)	4 (6.1)
Trastuzumab emtansine			
Yes	1 (1.5)	3 (4.5)	4 (6.1)
No	17 (25.8)	45 (68.2)	62 (93.9)

BMI: body mass index; HBP: high blood pressure; DM: diabetes mellitus; SD: standard deviation.
[a] Values are n (%) or mean [SD].

Table 2
LVEF and NT-proBNP value pairs obtained from breast cancer patients treated with trastuzumab.

LVEF and NT-proBNP pairs	n (%)
1	29 (43.9%)
2	12 (18.2%)
3	8 (12.1%)
4	6 (9.1%)
5	5 (7.6%)
6	2 (3%)
7	1 (1.5%)
9	2 (3%)
11	1 (1.5%)

Table 3
Distribution of LVEF and NT-proBNP according to age.

		<50 years	≥50 years
LVEF	Frequency	61	113
	Mean	60.6%	59.7%
	SD	6.3	8.7
NT-proBNP	Frequency	61	113
	Mean	99 pg/ml	440.2 pg/ml
	SD	77.4	1547.7

prove statistical significance ($p = 0.093$).

4. Discussion

Cardiac side effects associated with trastuzumab treatment may seriously compromise the prognosis and well-being of patients with HER2-positive breast cancer. The incidence of trastuzumab-induced cardiotoxicity varies by series but is estimated to range from 1.7% when used alone to approximately 20% when used in combination with other chemotherapy drugs such as anthracyclines or cyclophosphamide. In clinical practice, however, these percentages rise even higher, as seen in numerous clinical trials in which the number of cardiac side effects after adjuvant treatment with trastuzumab was prospectively evaluated, such as the rate of left ventricular dysfunction ranged from 7 to 34%, with heart failure rates between 0 and 4% [21].

There are currently two types of cardiotoxicity secondary to the chemotherapy administration. Type I, characteristic of anthracyclines, appears to be due to the direct oxidative stress-induced death of cardiomyocytes. It is an irreversible, dose-dependent and easily identifiable process at myocardial biopsy [22]. Conversely, type II corresponds to trastuzumab-induced cardiotoxicity, characterized by a transient deterioration of myocardial function without resulting in cell death. It is not a dose-dependent process and, in most cases, reversible so that the drug can be readministered once the myocardial function has been recovered [9].

The etiopathogenesis of trastuzumab-induced cardiotoxicity is not fully clarified. Some authors advocate a “multiple hit” hypothesis, in which the chemotherapy drug would cause direct damage to the cardiomyocyte, while the involvement of other risk factors would have an indirect and synergistic effect, increasing the risk of cardiotoxicity [23]. Following this theory, several risk factors have been identified that are related to an increased risk of trastuzumab-induced cardiotoxicity. One of the most important is the concurrent administration of anthracyclines. It has been widely demonstrated that there is a clear relationship between the cumulative dose of anthracyclines and trastuzumab-induced cardiotoxicity. A meta-analysis of 11 882 patients showed an increased

Table 4
Logistic regression analysis of the risk of cardiotoxicity during trastuzumab treatment in breast cancer patients. From all the factors considered, only diabetes and abnormal NT-proBNP values adjusted to age were statistically significant.

	β	S.E.	Wald statistic	*p*-value	Exp (β)	95% CI for Exp (β)	
						Lower	Upper
Diabetes	1.768	0.807	4.803	0.028	5.861	1.206	28.496
NT-proBNP > ULN	3.093	0.691	20.033	<0.0001	22.039	5.689	85.385

ULN: Upper limit of the normal range of NT-proBNP values adjusted to age.

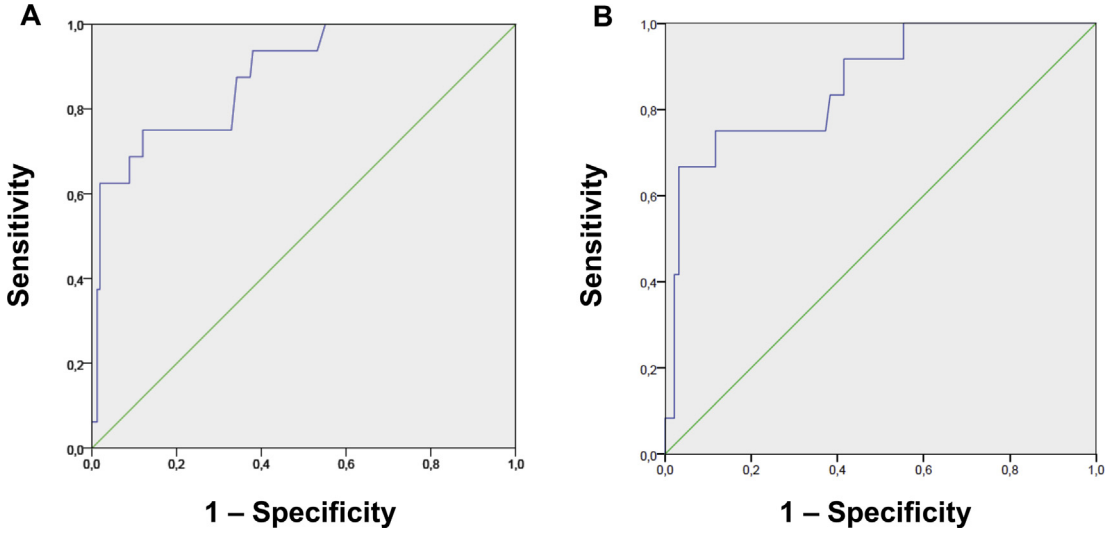

Fig. 1. ROC curves of plasma NT-proBNP levels for the diagnosis of cardiotoxicity during trastuzumab treatment in breast cancer patients. ROC curves considering all the patients in the study (A) or those patients aged 50–75 years old (B).

risk of heart failure in those who received trastuzumab and anthracyclines together compared to those who only received trastuzumab [24]. In the study carried out by Breast Cancer International Research Group, the clinical heart failure rate was 5 times lower in the group of patients treated with trastuzumab without anthracyclines [25]. This association between anthracyclines and trastuzumab leads to the conclusion that when both therapies are administered concomitantly or separated by a maximum time interval of 90 days, the incidence of left ventricular dysfunction and congestive heart failure rises to 28% and 27% respectively [26]. In fact, the risk decreases as the time between the administration of both drugs increases, with an incidence of 4.3% if the period time is longer than 90 days [27]. Other factors influencing trastuzumab-induced cardiotoxicity are advanced age, previous radiation therapy and cardiovascular risk factors. In a retrospective analysis, women > 65 years of age had an increased risk of cardiotoxicity when treated with trastuzumab, with a hazard ratio (HR) = 2.08 [28]. The risk of cardiotoxicity is higher in patients treated with cardiotoxic chemotherapy (including trastuzumab) and left breast radiotherapy, suggesting a synergistic effect of the latter one on cardiac risk [29]. Cardiovascular risk factors such as hypertension, smoking, obesity, diabetes mellitus, coronary artery disease, atrial fibrillation or renal failure, which lead to an increased risk of developing heart failure 3 years after initiating treatment with trastuzumab, especially in older women, with a maximum incidence of 45% [30].

Given the magnitude of the problem coupled with the limitations of echocardiography, multiple studies have been conducted to identify other biomarkers as possible early predictors of trastuzumab-induced cardiotoxicity. Plasmatic biomarkers could complement echocardiographic screening techniques due to their easy obtaining (a simple blood draw) and interpretation, cost-effectiveness, and most of all, their relationship with myocardial injury in patients with heart failure [31]. Two biomarkers have been widely studied as predictive factors for the early diagnosis of trastuzumab-induced cardiotoxicity: cardiac troponins and natriuretic peptides.

Troponin I elevation has been shown in several studies to be associated with a 17.6-fold increase in the risk of trastuzumab-induced cardiotoxicity, as well as a lower probability of LVEF recovery after treatment interruption [32]. This has led several organizations to consider the use of troponin I as an early marker of cardiotoxicity secondary to the treatment with trastuzumab, as is the case with the consensus established by the American Society of Echocardiography and the European Association of Cardiovascular Imaging [12].

Studies on natriuretic peptides are contradictory. Brain natriuretic peptide (BNP) and its precursor, N-terminal pro-brain natriuretic peptide (NT-proBNP), are both secreted in response to ventricular wall distension and associated with the appearance of CHF in patients with left ventricular overload [33]. Some studies indicate that variations in NT-proBNP concentrations may correlate with the risk of cardiotoxicity caused by anthracyclines. De Iuliis et al. developed a prospective study that recruited HER2-positive breast cancer patients treated with trastuzumab in combination with anthracyclines and taxanes. Echocardiographic measurements, as well as NT-proBNP determinations, were performed before starting treatment (T0), at 3 months (T1), at 6 months (T2), and one year after starting treatment (T3). A significant increase in NT-proBNP concentrations was observed in T1, T2 and T3 ($p < 0.0001$) with values 4 times higher than those measured in T0, even in absence of clear changes in LVEF measured by echocardiography. In addition, NT-proBNP was the only prognostic biomarker related to mortality after 1 year from the start of chemotherapy, being the most significant values those measured at 6 months (T2) and one year after (T3): NT-proBNP T2 (AUC = 0.788; 95% CI: 0.631–0.901; $p = 0.0003$) and NT-proBNP T3 (AUC = 0.859; 95% CI: 0.712–0.948; $p < 0.0001$) [34]. Another example is the research conducted by Urun et al., which managed to establish a correlation between NT-proBNP levels above 300 pg/ml and an increased risk of symptomatic heart failure and a lower probability of later recovery in patients treated with trastuzumab [35]. Additionally, Zardavas et al. observed an increase in baseline NT-proBNP levels in patients with cardiotoxicity associated with trastuzumab administration compared to those who did not. Besides, a Cox regression model was designed to assess the influence of increases in NT-proBNP values on time to significant LVEF drop. The results of the multivariate analysis were as follows: HR = 1.03 (95% CI: 1.02–1.03) with a value of $p < 0.001$. This resulted in an increase in the risk of cardiotoxicity > 30% for each 10 ng/dl increase in NT-proBNP concentration [36].

All these studies along with other authors' conclusions [37,38] contribute to support the results found in the present study. We have found that there is an inverse relationship between LVEF and NT-proBNP levels in patients with HER2-positive breast cancer. Also, we have found that those patients with NT-proBNP levels above the upper limit of normal range adjusted to age or who present diabetes mellitus as associated comorbidity have a higher risk of developing cardiotoxicity after administration of trastuzumab than those without any of these conditions (OR = 22.039 and OR = 5.861, respectively). These findings, if confirmed in prospective studies, could lead to an important change in the prognosis of patients treated with trastuzumab, as those factors would allow detecting those patients with an increased risk of cardiotoxicity. In this way, we could interrupt the treatment before potentially lethal structural changes in the myocardium may occur.

Still, there are many other studies whose results are opposed to them, rejecting NT-proBNP as a diagnostic biomarker of trastuzumab-induced cardiotoxicity [39–43]. To dissolve these discrepancies, we require additional studies about the role of NT-proBNP in the early diagnosis of trastuzumab-related acute cardiac dysfunction in breast cancer patients, although our data suggest that NT-proBNP levels above the upper limit of the normal range adjusted to age may correlate with the occurrence of cardiotoxicity.

Concerning the role of diabetes mellitus in trastuzumab-induced cardiotoxicity, there appears to be a clear relationship between this comorbidity and an increased risk of adverse cardiac events, as our results are endorsed by extensive scientific evidence. The study conducted by Serrano et al. on women ≥70 years of age showed that those patients who were on treatment with trastuzumab and had diabetes presented a higher risk of developing cardiotoxicity with or without symptoms ($p = 0.010$) [44]. Another cohort study conducted by Ezaz et al. with 1664 patients aged from 67 to 94 years showed that both previous history of coronary artery disease (HR = 2.16; 95% CI: 1.21–3.86; $p = 0.009$) and diabetes (HR = 1.50; 95% CI: 1.03–2.18; $p = 0.034$) were associated with a higher rate of heart failure and cardiomyopathy 3 years after treatment with trastuzumab [30]. Urun et al. also correlated diabetes and a high BMI with an increased risk of cardiotoxicity ($p = 0.002$ and $p = 0.004$, respectively) [35]. Finally, we highlight a meta-analysis of 6527 patients who were treated with trastuzumab, which showed an association between adverse cardiac effects derived from its use with risk factors such as hypertension (OR = 1.61; 95% CI: 1.14–2.26; $p < 0.01$), diabetes (OR = 1.62; 95% CI: 1.10–2.38; $p < 0.02$), previous use of anthracyclines (OR = 2.14; 95% CI: 1.17–3.92; $p < 0.02$) and advanced age ($p = 0.013$) [45]. All this supports the hypothesis that diabetes is an important risk factor for trastuzumab-induced cardiotoxicity, so special care should be taken into account in these patients. In that regard, it

would be useful to perform cardiac function controls before chemotherapy administration, to schedule serial echocardiography in a shorter time frame than in the rest of the population, and of course, to achieve a strict glycemic control to avoid disease progression and thus, the deterioration of myocardial function. Regarding other cardiovascular risk factors, although our study has not been able to establish statistically significant associations with trastuzumab-induced cardiotoxicity, other studies have demonstrated a correlation between major cardiovascular risk factors such as hypertension, obesity or smoking with an increased risk of cardiotoxicity [46–48].

Apart from the above, our study has some limitations. We must advise the current lack in the scientific literature of a universal consensus on the definition of trastuzumab-induced cardiotoxicity. The criteria used by the oncologists in our study to discontinue treatment with trastuzumab were based on the definition of cardiotoxicity used in the Herceptin Adjuvant (HERA) trial carried out by Suter et al., according to which cardiotoxicity is considered any decrease in LVEF accompanied by symptoms of CHF, or a decrease in baseline LVEF $\geq$ 10% with a resultant value < 50% in asymptomatic patients [18]. However, not all studies conducted to date follow this definition. For example, the occurrence of cardiotoxicity according to the Cardiac Review and Evaluation Committee supervising trastuzumab clinical trials is given by one or more of the following facts: (1) reduction of LVEF, either global or more specific and severe in the interventricular septum; (2) symptoms or signs associated with CHF; (3) associated signs of CHF, including but not limited to S3 gallop, tachycardia, or both; and (4) decline in LVEF of at least 5% to < 55% in the presence of signs or symptoms of CHF, or a reduction in LVEF $\geq$ 10% to < 55% without signs or symptoms of CHF [49]. Another definition is the one established by the consensus of the American Society of Echocardiography together with the European Association of Cardiovascular Imaging, based on a decrease in LVEF > 10% with a resulting value < 53%, and being confirmed 2–3 weeks after initial diagnosis by a new imaging technique [12]. This lack of agreement makes it difficult to compare the results of our study with those of others who were guided by a different definition of cardiotoxicity.

Moreover, the study population analyzed included only 66 patients. This limitation is even more relevant in certain subgroups, such as the number of patients treated with anthracyclines or taxanes (18 and 3, respectively), or those affected by diabetes (8), a factor that showed statistically significant association with cardiotoxicity. The small sample size may bias the results of our study due to a possible heterogeneous distribution of prognostic variables. Furthermore, the patient inclusion period lasted from 1995 to 2015, and since then, there may be differences in diagnostic methods, especially concerning the sensitivity of echocardiography and NT-proBNP plasma level determination.

Another limitation is the fact that in 43.9% of patients it was only possible to obtain a single pair of LVEF and NT-proBNP values, which precludes the analysis of the intra-individual variability in these patients. Moreover, baseline NT-proBNP values before drug administration were not available, and the change of LVEF values may have been preceded by NT-proBNP increments within a maximum interval of 30 days or vice versa.

Finally, we must also emphasize the role of additional adjuvant treatments as possible effect modifiers. In our study sample, more than 90% of patients were treated with chemotherapy drugs before or in combination with trastuzumab (27.3% anthracyclines, 4.5% taxanes, and 60.6% both). Moreover, 90.9% received adjuvant radiotherapy after surgery. As we mentioned before, both treatments have a synergistic effect on trastuzumab-induced cardiotoxicity.

The limitations exposed above make it difficult to ensure the role of NT-proBNP as an early marker of cardiotoxicity during trastuzumab treatment in breast cancer, raising the possibility that NT-proBNP elevation may be a consequence of cardiotoxicity or trastuzumab administration rather than being a predictive factor of cardiotoxicity.

5. Conclusion

Evaluating the association of trastuzumab-induced cardiotoxicity during breast cancer treatment with different factors, we have found that there is an inverse relationship between LVEF and NT-proBNP plasma levels. Also, we have found that NT-proBNP levels above the upper limit of the normal range adjusted to age or diabetes mellitus may be associated with a higher risk of developing cardiotoxicity during trastuzumab treatment. However, some limitations of the present study regarding sample size and amount of data, among others, make necessary further studies aimed to clarify whether NT-proBNP and diabetes-associated markers determinations can be useful in the monitoring of cardiotoxicity risk in breast cancer patients undergoing trastuzumab therapy.

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Declaration of competing interest

The authors have stated that they have no conflict of interest.

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