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ABSTRACT

Objective: To evaluate the role of N-terminal pro-brain-type natriuretic peptide (NT-pro-BNP) and a cardiovascular (CV) risk score named FRESCO for predicting anthracycline-induced cardiotoxicity (AIC) in diffuse large B-cell lymphoma (DLBCL).

Methods: 130 consecutive DLBCL patients treated in first-line with anthracycline-containing immunochemotherapy. Competitive risk between NT-proBNP, FRESCO and time to AIC was considered.

Results: Cumulative incidence of AIC was 12.2% and 17.5% at 1 and 5 years, respectively. Median time to development cardiotoxicity was 6.4 months, with half of the cases showing heart failure and the other half silent AIC. Both NT-proBNP levels and FRESCO score were independently associated with higher risk of AIC ($p=0.001$ and $p=0.03$, respectively). Patients with NT-proBNP ≥ 600 pg/ml or those with FRESCO $\geq 4.5\%$ had 3.97 or 2.54 times higher risk of AIC than those with lower values ($p=0.001$ and $p=0.048$, respectively). According to the previous cut-offs, three groups of patients with a significantly different risk of AIC could be identified ($p<0.0001$).

Conclusions: Doxorubicin-containing chemotherapy is associated with increased risk of silent and overt AIC. Baseline NT-proBNP levels and FRESCO CV risk score are accurate predictors of AIC and can identify groups of patients at different risk, in which personalized cardiologic evaluation should be offered.

INTRODUCTION

Anthracycline-containing immunochemotherapy is the standard treatment for patients with diffuse large B-cell lymphoma (DLBCL).¹ Cardiotoxicity is not only a serious and common complication of doxorubicin, the most commonly used anthracycline, but also is a growing problem in the setting of lymphoma treatment, given the advanced age of many patients at presentation and the increasing number of long-term lymphoma survivors.² Several factors have been associated with anthracycline-induced cardiotoxicity (AIC).^{3,4} At the present time, AIC is considered a continuous phenomenon in which heart failure (HF) may be preceded by asymptomatic left ventricular systolic dysfunction (LVSD).⁵ Therefore, a rational strategy for minimizing AIC is early detection of subclinical cardiac dysfunction and its prompt treatment to prevent HF.^{6,7}

In clinical practice, regular cardiac function assessment is recommended by periodic measurement of left ventricular ejection fraction (LVEF).⁸ Unfortunately, this technique detects cardiotoxicity only when a functional impairment has already occurred. Measurement of cardiospecific biomarkers, such as troponins and natriuretic peptides (e.g. N-terminal pro-B natriuretic peptide –NT-proBNP–), has been recently proposed for the assessment of cardiac risk in these patients, but the utility of its routine monitoring for predicting AIC has not yet been established.^{9,10}

Other classical cardiovascular (CV) risk factors, such as hypertension or diabetes, can contribute to the subclinical cardiac damage induced by anthracyclines.^{3,4} Currently, CV risk functions –based on a series of characteristics (typically sex, age and risk factor profile)– are used to predict an individual risk of having a CV event.¹¹ The FRESCO function has recently proven to be an accurate and valid tool to estimate 10-year coronary and CV risk.¹²

The aims of our study were: i) to prospectively determine the incidence and time of onset of AIC in DLBCL patients treated with R-CHOP or R-CHOP-like regimens, ii) to assess the association between NT-proBNP and FRESCO risk score and AIC and mortality, and iii) to evaluate the value of adding NT-proBNP to the FRESCO risk score for predicting the incidence of AIC and death.

METHODS

Study Design, participant eligibility and lymphoma treatment

This is an observational study conducted at the Hospital del Mar, a tertiary center in the city of Barcelona, Spain. All patients with histologically confirmed DLBCL according to the WHO criteria and treated between May 2004 and May 2014 were consecutively selected. Eligibility criteria were as follows: previously untreated DLBCL, anthracycline containing-chemotherapy treatment, and age >18 years. The exclusion criteria were: chemotherapy without anthracyclines, palliative treatment, overt or unequivocal heart failure, human immunodeficiency virus positivity, Eastern Cooperative Oncology Group (ECOG) performance status ≥ 4 , and a life expectancy of <3 months. The Institutional Ethics Committee of Hospital del Mar approved the study, and all participants provided written informed consent. Treatment for DLBCL was administered according to institutional guidelines, taking into account biological characteristics of the lymphoma and age and/or functional status of patients. No pharmacological intervention was performed as cardio-protective strategy, however patients could maintain treatments for pre-existing heart conditions or CV risk factors.

FRESCO risk function, cardiac assessment and cardiotoxicity definition

A detailed review of the clinical records was performed to collect demographic data and medical history, emphasizing in presence of pre-existing cardiac disease, ischemic and

non-ischemic events, or stroke. Clinical CV risk factors and lifestyle risk factors were also recorded. CV risk was estimated using the FRESCO risk functions at baseline.¹² For this study, the non-laboratory FRESCO risk score (age, sex, smoking, body mass index) was used including variables at study entry (available at: <http://apps.datarus.eu/calculadorafresco/>).

LVEF evaluated by transthoracic echocardiography (General Electric, Vivid 7) using Simpson method and levels of NT-proBNP (measured in serum by ELISA on an Elecsys 2010 System [Roche Diagnostics®]) were performed prior to start of chemotherapy, post-therapy, every 12 months for 2 years, and then every 2 years or whenever required. AIC, the primary end point of the study, was defined using a modification of the Cardiac Review and Evaluation Committee supervising trastuzumab clinical trials¹³: LVEF <55%, a 15% decline or more of baseline LVEF if baseline LVEF was <55% or clinical evidence of heart failure. AIC diagnosis was blinded to FRESCO risk score and to NT-proBNP results. Vital status and mortality cause in fatal cases were also recorded. “Cardiac-related death” was defined as follows: unequivocally heart failure leading to death and in the absence of lymphoma progression, infection or other potential chemotherapy-related toxicity. The cause of death was adjudicated after the consensus of 2 clinicians (MF and AS).

Statistical analysis

Continuous variables were summarized as mean and standard deviation or median and quartiles if they did not follow a normal distribution, while categorical variables were described as proportions. To compare the means, medians or proportions between groups, Student-T, Mann-Witney U or chi-squared tests were performed for normal, non-normal distributed and categorical variables, respectively. When comparing more

than two groups, ANOVA and Kruskal-Wallis were used instead of Student-T and Mann-Whitney U tests, respectively.

To assess the association between NT-proBNP prior chemotherapy, FRESCO risk score at baseline and time to AIC or time to death, Cox proportional regression models that included the two predictive variables were used and competitive risk between the two outcomes were considered. All analyses involving time to AIC or time to death were conducted considering the presence of competing risk events using Fine-Gray subdistribution hazard regression models implemented in the *mstate* R package.¹⁴ NT-proBNP and FRESCO risk score were considered both as continuous and categorical variables. NT-proBNP was log2-transformed to achieve normality and a 2-base logarithm was selected in order to facilitate the interpretation. Linearity assumption of log2-transformed NT-proBNP effect was checked using a Cox proportional regression model using splines (Supplementary material: *Figure S1*). Two NT-proBNP and FRESCO risk score categories were defined, using the following cut-points >600 pg/ml and >4.5%, respectively. The cut-point value for NT-proBNP was chosen under the maximum likelihood criteria, while the FRESCO risk score >4.5% is the threshold used to separate low and intermediate risk categories. Possible interaction between NT-proBNP and FRESCO risk score was tested. Discrimination was assessed by area under the ROC curve (AUC) taking into account censoring with *pROC* R package.¹⁵ Reclassification, i.e. improvement in adding NT-proBNP to FRESCO risk scores to predict AIC or death, was estimated by categorical Net Reclassification Index taking tertiles of predicted risk as cut-off points and Integrated Discrimination Improvement.¹⁶ Finally, to compare AUC between models with and without NT-proBNP, the *pROC* package was also used. Both discrimination and reclassification measurements were computed for 1, 2, 4 and 7 years follow-up period.

Cumulative incidence curves were constructed using the Kaplan–Meier method and groups were compared using log-rank test. All analyses were repeated censoring at one year follow-up period on one hand, and taking the entire follow-up period among those one-year event free patients to assess how NT-proBNP and FRESCO risk score could influence on the short and long term cardiotoxicity or death. Significance level was set to 5% in all tests. All analyses were performed using R or STATA.

RESULTS

Patient population and follow-up

One hundred and sixty six patients with previously untreated DLBCL were diagnosed during the study period. Among these, 36 were excluded: ten patients had human immunodeficiency virus infection, 21 patients did not receive anthracyclines for different reasons, 2 patients had other concomitant solid cancer, 2 patients refused treatment and 1 patient moved to her country of origin after receiving the first cycle of chemotherapy (Supplementary material: *Figure S2*). Baseline characteristics of the 130 patients included in the study are shown in *Table 1*. Some patients were receiving concomitant cardioactive drugs: angiotensin converting enzyme inhibitors in 25 (19.2%), beta-blockers in 20 (15.4%), angiotensin II receptor blockers in 15 (11.5%), calcium channel blockers in 11 (8.5%) and statins in 27 (20.8%). Chemotherapy regimens were as follows: R-CHOP21 in 58 patients (44.6%), R-CHOP14 in 19 (14.6%), dose-adjusted R-EPOCH in 13 (10%), R-miniCHOP in 6 (4.6%) and R-CMyOP (containing liposomal doxorubicin) in 34 (26.2%). Cumulative dose of doxorubicin was significantly higher in patients receiving conventional doxorubicin than in those receiving liposomal doxorubicin (median cumulative dose and interquartile range (IQR): 248 mg (180-300 mg) vs 121 mg (60-180 mg); $p < 0.0001$)

(Supplementary material: *Table S1*). Only 7 patients received radiotherapy in the mediastinum (30-36 Gy). The median follow-up in survivors was 81.3 months (IQR: 40.1-108.2). At last contact, 39 (30%) patients had died: in 25 (19.2%) of them death was owing to tumor-related causes and in 14 (10.8%) to other causes, including cardiac-related death in 3 (2.3%) cases. Thirteen (10%) patients were lost to follow-up.

Cardiotoxicity

Cumulative incidence of AIC is shown in *Figure 1* and was 12.2% (95% CI 7.6-19.5) at 1 year, 14.1% (95% CI 9.0-21.8) at 2 years, 17.5% (95% CI 11.6-26.0) at 5 years, and 27.0% (95% CI 17.5-40.3) at 10 years. The median time that elapsed between the first dose of doxorubicin and the development of cardiotoxicity was 6.4 months (IQR: 2.1-32.2). Cardiotoxicity occurred within the first year in 15 cases, within the 2nd and 5th year in 5 cases and beyond the 5th year in 4 cases.

Cardiotoxicity and death, as competing events, occurred in 24 (18.5%) and 28 (21.5%) patients, respectively. The clinical characteristics of patients with or without cardiotoxicity and those who died are shown in *Table 1*. Median age and prior history of hypertension, dyslipidemia, chronic obstructive pulmonary disease or cardiac disease were statistically different among groups. Among cases with AIC, 12 patients developed clinical overt cardiotoxicity with signs of HF attributed to doxorubicin therapy after the exclusion of other potential causes (7 cases with LVEF <55% and 5 cases with normal LVEF), whereas subclinical cardiotoxicity developed in the remaining 12 patients (11 cases with decline of LVEF <55% and 1 case with decline >15% of LVEF who had a baseline LVEF <55%).

NT-proBNP levels and FRESCO risk score for prediction of cardiotoxicity and death

NT-proBNP did not follow a normal distribution and we therefore performed a logarithm transformation in base 2. Cox linearity for AIC and death were also better with the logarithm transformation (Supplementary material: *Figure S1*). Both NT-proBNP levels and FRESCO risk scale were independently associated with higher risk of AIC (*Table 2*). In addition, the Log_2 NT-proBNP at diagnosis was also associated with a higher risk of death (*Table 2*). The addition of doxorubicin type (conventional versus liposomal) to the model did not have impact on results. The inclusion of previous cardiac disease and comorbidities did not significantly change the association between NT-proBNP and cardiotoxicity. When the 4 variables included in the FRESCO risk score were analyzed individually, they showed a lower risk of AIC than the FRESCO risk score as a whole. The addition of NT-proBNP to the FRESCO risk score marginally improved the discrimination capacity of the predictive model (*Table 3*, Supplementary *Figure S3*).

In order to facilitate use to clinicians, we performed a second model using categorical variables and verified the absence of interaction between them for AIC and death (Supplementary material: *Table S2* and *Table S3*). In this model patients with NT-proBNP of 600 pg/ml or more had 3.97 times higher risk of cardiomyotoxicity than those with lower values ($p=0.001$). In addition, and independently, patients with FRESCO risk score of 4.5% or more did also have a significantly increased risk of cardiotoxicity (HR: 2.54; $p=0.048$) (*Table 2*). Three patterns of risk of AIC were identified according to FRESCO risk score and NT-proBNP ($p<0.0001$) (*Figure 2* and *Table 4*). Patients with NT-proBNP levels equal or higher than 600 pg/ml had the highest and earliest risk for developing AIC, independently of FRESCO score. An intermediate risk group included patients with FRESCO equal or higher than 4.5% and NT-proBNP levels lower than 600 pg/ml, showing a moderate but progressively rising risk of AIC. Finally, a third group including patients with FRESCO lower than 4.5%

and NT-proBNP lower than 600 pg/ml did have a very low risk of AIC, in both the short and long term.

DISCUSSION

To our knowledge, this study is the first to systematically examine the usefulness of NT-proBNP levels and a CV risk function called FRESCO for predicting AIC, including asymptomatic LVSD, in DLBCL after rituximab and doxorubicin-containing chemotherapy. We report that the incidence of AIC is around 12% at 1 year and 27% at 10 years after starting treatment. In this study, we observed an overall 4-fold cardiotoxicity risk increase for adult DLBCL patients with NT-proBNP levels of 600 pg/ml or more at diagnosis. In addition, a 2.5-fold increase in cardiotoxicity risk was also observed in those patients with FRESCO risk score of 4.5% or more. The addition of the NT-proBNP levels to the FRESCO risk score significantly improved the capacity of this risk function to predict the incidence of AIC events.

Our present work thus provides important insight on the incidence and risk factors predisposing to AIC in lymphoma patients. Although underestimated in the past, cardiotoxicity is common in adults with lymphoma receiving chemotherapy. Recent studies have reported that 20-30% of lymphoma patients treated with anthracycline containing regimens can develop cardiotoxicity.¹⁷⁻²⁰ Our results showing an AIC incidence of 18% at 5 years are in agreement with recently published series of adult patients with DLBCL. Interestingly, more than 60% of our patients who developed LVSD did so during the first year of treatment and also confirms recently published observations.¹⁷ This is in contrast with old series including childhood and breast cancers patients that mainly detected advanced and late chronic myocardial damage.²¹ In addition, we have also found that clinically overt cardiotoxicity occurred in 50% of

patients, whereas subclinical or silent cardiotoxicity developed in the other half. This observation is in line with a recent meta-analysis.²² Our results highlight that the physiologic reserve and normal compensatory responses are able to maintain normal myocardial function until a certain threshold is crossed. Then, while patients remain with asymptomatic cardiac damage, they might be susceptible to specialized cardiologic intervention to prevent further progression to overt congestive HF.

Multiple risk factors have been linked to the development of AIC.³⁻⁵ Since the incidence of lymphoma increases with age and the number of older people is rising, there is a growing population of elderly patients with lymphoma,²³ many of them have some of these aforementioned risk factors. A recent study from the SEER-Medicare has shown that patients with lymphoma aged 65 years or older have a high prevalence of diabetes, hypercholesterolemia and hypertension.²⁴ The use of risk scores as tools to predict CV disease, such as the Framingham function, has been widely advocated in primary prevention.¹¹ Investigators from our group have recently validated the FRESCO function for prediction of CV risk in a typical southern European population using easily implemented risk factor measurements.¹² Our study shows that half of our cases had FRESCO score of 4.5% or more, highlighting the advanced age of the population of our study as well as their co-morbidities. We have observed that FRESCO function is a useful tool to predict AIC and patients with score of 4.5% or more are at higher risk. Variables included in the non-laboratory FRESCO function are available in all oncologic patients, calculation of risk score is possible through an electronic tool on the Internet and we understand that its determination is feasible in a medical appointment by a physician or a nurse.

The role of natriuretic peptides in the diagnostic and risk stratification of chemotherapy-associated cardiotoxicity is still controversial.²⁵ NT-proBNP levels are

correlated with age, comorbidities and advanced disease but not with LVEF at diagnosis, as previously reported by our group.²⁶ In our patients with DLBCL, median level of NT-proBNP was 252 pg/ml, approximately twice the normal values, and 22% of them had NT-proBNP concentrations higher than 600 pg/ml at diagnosis. We have observed that the risk of suffering cardiotoxicity increases as the NT-proBNP rises (*Figure S1* in Supplementary material). Patients at the highest risk are those with NT-proBNP levels of 600 pg/ml or more, particularly during the first year after starting treatment. Although it is well-known that NT-proBNP indicates cardiac stress and not necessarily cardiomyocyte injury, we propose that DLBCL patients may benefit from screening NT-proBNP at diagnosis, when control of CV risk factors and specific early cardiac treatment can potentially reverse subclinical myocardial changes. Recently, Cardinale and collaborators have demonstrated in cancer patients developing AIC that LVEF recovery and cardiac event reduction may be achieved when cardiac dysfunction is detected early and a modern HF treatment is promptly initiated.²⁷⁻²⁹ In addition, a recent meta-analysis has shown that ACE inhibitors and beta-blockers were associated with better LVEF preservation in patients receiving anthracyclines.³⁰ Given the marked increased morbidity and mortality reported in the literature among patients who develop cardiotoxicity,²¹ there is an opportunity for improving patient's outcome through a personalized prompt cardiologic assessment according to NT-proBNP and FRESCO. In addition, optimizing CV risk factors and/or early cardiologic intervention according to these independent factors for developing AIC should be considered.

There are several limitations that should be considered when interpreting our results. LVEF calculations by two-dimensional echocardiography might be affected by geometric assumptions and inter-observer variability.³¹ At the time of study design in 2004, a consensus definition for cardiotoxicity or left ventricular dysfunction induced

by chemotherapy was lacking and we decided to use a modification of the definition proposed by the Cardiac Review and Evaluation Committee supervising trastuzumab clinical trials.¹³ Myocardial strain assessments that are able to detect early cardiac dysfunction are being studied in the last years by some cardiology teams but were not used in this cohort of patients who received treatment from 2004 to 2014, but it is being routinely determined in our patients since 2017.³¹ CV risk factors and FRESCO score were considered only at the time of lymphoma diagnosis and these factors can change over time. Although our median follow-up time was 7 years, even longer periods might be necessary to determine the real incidence of AIC.

In conclusion, anthracycline-containing chemotherapy is associated with a substantial risk of clinically silent or overt cardiotoxicity in adult patients with DLBCL, not only in the long term but also in the short term. Baseline NT-proBNP levels and FRESCO risk score are predictors of AIC and can identify groups of patients with significant different risk of AIC. We suggest that DLBCL patients at increased risk of AIC according to NT-proBNP/FRESCO should be evaluated and monitored by onco-cardiologist teams.

Supplemental material:

Additional Supporting Information may be found online.

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TABLE 1. History of cardiovascular risk factors and baseline characteristics in the whole cohort of patients and according to the presence of the competitive event (cardiotoxicity or death or none).

	All N=130	None N=78	Cardiotoxicity N=24	Death N=28	<i>p</i>
Age years, median (IQR)	68 (54-75)	62 (50-74)	73 (68-76)	72 (64-76)	0.001
Sex: female, n (%)	64 (49%)	41 (53%)	8 (33%)	15 (54%)	0.225
Hypertension, n (%)	54 (42%)	25 (32%)	16 (67%)	13 (46%)	0.009
Dyslipidemia, n (%)	34 (26%)	12 (15%)	15 (63%)	7 (25%)	<0.001
Diabetes, n (%)	24 (19%)	10 (13%)	6 (25%)	8 (29%)	0.105
Alcohol abuse, n (%)	7 (5%)	3 (4%)	3 (13%)	1 (4%)	0.206
Smoking, n (%)	46 (35%)	26 (33%)	10 (42%)	10 (36%)	0.756
Chronic obstructive pulmonary disease, n (%)	9 (7%)	1 (1%)	4 (17%)	4 (14%)	0.004
Cardiac disease, n (%)	25 (17%)	5 (6%)	12 (50%)	8 (32%)	<0.001

Body mass index ≥ 30 kg/m², n (%)	29 (27%)	15 (19%)	6 (25%)	7 (25%)	0.735
Liver disease, n (%)	15 (14%)	8 (10%)	2 (8%)	8 (29%)	0.057
FRESCO risk score (%), median (IQR)	4.5 (2.1-7.2)	3.7 (1.5-6.5)	7.1 (4.4-8.8)	5.3 (3.9-8.2)	<0.0001
NT-proBNP (pg/ml), median (IQR)	252 (76-560)	107 (50-342)	589 (290-1916)	329 (196-1117)	<0.0001
NT-proBNP ≥ 600 pg/ml, n (%)	28 (22%)	7 (10%)	12 (50%)	9 (33%)	<0.0001
LVEF %, median (IQR)	64 (60-69)	64 (60-70)	64 (58-70)	65 (60-68)	0.563
IPI Intermediate-high/High, n (%)	65 (50%)	28 (36%)	17 (71%)	20 (71%)	<0.0001

NT-proBNP: N-terminal pro-B-type natriuretic peptide, LVEF: Left ventricular ejection fraction; IPI: International Prognostic Index, IQR: interquartile range.

TABLE 2. Hazard ratio of Log₂ NT-proBNP and FRESCO risk score for anthracycline induced cardiotoxicity and death, using a competitive Cox regression model.

	Cardiotoxicity		Death	
	HR (95%CI)	<i>p</i>	HR (95%CI)	<i>p</i>
A) As continuous variables				
Log ₂ NT-proBNP	1.44 [1.17-1.77]	0.001	1.26 [1.04-1.52]	0.020
FRESCO risk score	1.15 [1.01-1.30]	0.030	1.01 [0.89-1.14]	0.888
B) As categorical variables				
NT-proBNP ≥600 pg/ml	3.97 [1.77-8.92]	0.001	1.78 [0.80-4.00]	0.160
FRESCO risk score ≥4.5%	2.54 [1.00-6.45]	0.048	1.71 [0.76-3.84]	0.193

NT-proBNP: N-terminal pro-B-type natriuretic peptide, HR: Hazard ratio; 95% CI: 95% confidence interval.

TABLE 3. Area under the curve (discrimination) for anthracycline-induced cardiotoxicity and death at several time-points for the FRESCO risk score and for the FRESCO risk score plus NT-proBNP (both as continuous variables).

	Cardiotoxicity			Death		
	FRESCO	FRESCO + NT-proBNP	<i>p</i>	FRESCO	FRESCO + NT-proBNP	<i>p</i>
At 1 year	0.714	0.817	0.103	0.564	0.702	0.075
At 2 years	0.676	0.784	0.060	0.559	0.713	0.032
At 4 years	0.679	0.760	0.158	0.586	0.672	0.226
At 7 years	0.759	0.831	0.115	0.616	0.665	0.538

NT-proBNP = N-terminal pro-B type natriuretic peptide

TABLE 4. Cumulative incidence (%) of cardiotoxicity according to FRESCO risk score and NT-proBNP levels at several time points.

NT-proBNP levels + FRESCO risk score	N (%)	1 year	2 years	4 years	7 years
≥600 pg/ml +/- <4.5%*	28 (23)	41.6	41.6	41.6	52.1
<600 pg/ml + ≥4.5%	47 (38.5)	11.7	14.2	16.7	28.3
<600 pg/ml + <4.5%	47 (38.5)	0	2.5	5.1	5.1

NT-proBNP: N-terminal pro-B type natriuretic peptide. *Patients with NT-proBNP of 600 pg/ml or more, regardless of the FRESCO risk score, had a significantly higher risk of AIC (p<0.0001).

FIGURE 1. Cumulative incidence curve of anthracycline-induced cardiotoxicity.

Whole cohort; shadows mean confident interval 95%.

FIGURE 2. Cumulative incidence curves of anthracycline-induced cardiotoxicity according to FRESCO risk score and NT-proBNP levels. Solid line: NT-proBNP levels equal or higher than 600 pg/ml independently of FRESCO score; Long dash-dot line: NT-proBNP levels lower than 600 pg/ml and FRESCO equal or higher than 4.5%; Long dash line: NT-proBNP lower than 600 pg/ml and FRESCO lower than 4.5%. The difference among groups reached statistical significance ($p < 0.0001$).