Alcohol and *HER2* Polymorphisms as Risk Factor for Cardiotoxicity in Breast Cancer Treated with Trastuzumab

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Abstract. *Background: Trastuzumab has no major side-effects except the potential for cardiac toxicity. The main objective of this study was to evaluate the association between trastuzumab-associated cardiac toxicity and two potential risk factors: alcohol intake and HER2 polymorphisms. Patients and Methods: In a retrospective cohort study of 237 women with non-metastatic HER2-positive breast cancer treated with trastuzumab, traditional risk factors were assessed by review of medical records, alcohol use by an administered questionnaire to women (n=132), and HER2 polymorphisms (Ile655Val and Ala1170Pro) using TaqMan assays (n=73). Results: Association was observed between alcohol intake (10 drinks and more per week) during the trastuzumab treatment and cardiac toxicity (p=0.04). For polymorphisms, compared to Ile/Ile carriers, HER2 Ile/Val was associated with a higher risk of cardiac toxicity (p=0.02). Conclusion: Heavy alcohol use during the course of trastuzumab treatment and the HER2 Ile/Val genotype may constitute risk factors for cardiac toxicity.*

Patients with human epidermal growth factor receptor type-2 (HER2)-positive breast cancer [10.9% of nuclear grade 2 and 27.8% of those grade 3 (1)] have a worse prognosis (2) compared to those with HER2-negative disease. A 2012 meta-analysis including eight trials (n=11,991) found that regimens containing trastuzumab significantly improve overall survival [hazard ratio (HR)=0.66, $p<0.00001$] and disease-free survival (HR=0.60, $p<0.00001$) (3).

The treatment is well tolerated but a decrease in the left ventricular ejection fraction (LVEF) can occur. The meta-analysis showed that administration of trastuzumab increases the risk of congestive heart failure (CHF) [relative risk (RR)=5.11] and of LVEF decline (RR=1.83) (3). The incidence of severe cardiac toxicity (cardiac death or symptomatic heart failure) with trastuzumab use in five large adjuvant setting trials was shown to be between 2.2% (4) and 4.1% (5). A higher proportion of patients (4.3-18.9%) had to stop trastuzumab due to an asymptomatic drop of the LVEF, CHF or other adverse cardiac effect (6). Given the magnitude of the clinical benefits from trastuzumab for patients with HER2-positive breast cancer, the cardiac function has to be monitored (6).

In the general population, the common risk factors for heart failure are: coronary artery disease (CAD) (RR=8.1), diabetes (RR=1.9), cigarette smoking (RR=1.6), valvular heart disease (RR=1.5), hypertension (RR=1.4) and obesity (RR=1.3) (7). The following risk factors of cardiac toxicity have been observed in data from trials comparing trastuzumab to no trastuzumab in the adjuvant setting: low baseline LVEF (4, 8), increased age (8, 9), use of anti-hypertensive medication (8, 9), cumulative dose of anthracyclines (4) and a higher body mass index (BMI >25 kg/m^2) (4). On occasion, these risk factors failed to predict cardiac toxicity related to trastuzumab. Therefore, the identification of other risk factors related to trastuzumab-induced cardiac toxicity would help identify patients most likely to experience a decrease of their LVEF, helping physicians in their choice of adjuvant chemotherapy regimens.

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Among the possible new risk factors, there is alcohol intake (10). While low to moderate alcohol use reduces the risk of heart failure, heavy drinking is associated with an increase of that risk (11, 12). Women are more sensitive to alcohol (13, 14).

Another hypothesized risk factor is the *HER2* polymorphism (15). The *HER2* gene is important for normal cardiac function. Mice that are *HER2*-deficient develop dilated cardiomyopathy with structural changes (increase in mitochondria and vacuoles) and have an increased sensitivity to anthracyclines (16). Two of the best characterized single-nucleotide polymorphisms (SNPs) of *HER2* are Ile655Val and Ala1170Pro (17, 18). Beauclair *et al*. studied the relationship between Ile655Val polymorphism and cardiac toxicity (15). They reported that the Ile/Val genotype is associated with cardiac toxicity (p=0.0058). However, two recent studies published as abstracts reported no association between cardiac toxicity and the Ile655Val SNP (19, 20). Considering these conflicting results, further studies are needed to clarify the role of *HER2* Ile655Val SNP as a potential risk factor for cardiac toxicity.

Thus, we hypothesized that the risk of developing cardiac toxicity among women treated with trastuzumab is increased by the alcohol use and affected by the *HER2* polymorphism. The principal objective of our study was to evaluate the association of alcohol use and *HER2* polymorphisms with cardiac toxicity in women with non-metastatic breast cancer receiving trastuzumab in the neo-adjuvant/adjuvant setting. The secondary objective was to evaluate the association of cardiac toxicity with previously recorded risk factors.

Patients and Methods

Study population and data collection. This was a retrospective cohort study of women (n=299) with non-metastatic *HER2*-positive invasive breast cancer diagnosed (or chemotherapy completed) between July 1st 2005 and January 1st 2010. The study took place at the Centre des Maladies du Sein Deschênes-Fabia, a specialized breast center in Quebec City, Canada. Its database provided information on tumor characteristics and treatment received. Data on traditional risk factors were collected from medical records. All patients gave informed consent. The study and substudies were all approved by a Research Ethics Board (#DR-002-1227 and DR-002-1265).

Cardiac toxicity. Cardiac toxicity was defined as follows: a decrease of at least 10% from baseline with a resulting LVEF <50% at follow-up or any decrease resulting in LVEF <45%. Since not all patients had LVEF measured prior to the first dose of chemotherapy, the baseline value used for analysis was the first LVEF available in this order of priority: LVEF before chemotherapy, LVEF before trastuzumab or first other LVEF measured.

Alcohol sub-study. Women who were part of the HER2-positive breast cancer cohort (n=139) between July 1st 2005 and February 15th 2008 were invited to participate. A total of 132 patients completed a questionnaire, with a participation rate of 95%. The questionnaire used included questions about the type and quantity of alcohol intake per week (21) at three time periods: before cancer diagnosis, during chemotherapy, and during trastuzumab maintenance. A graduated beverage-specific frequency questionnaire was used (22).

Table I. *Reasons for not receiving trastuzumab*.

Main reasons	Number of patients (%)
Tumor smaller than 1 cm	22 (35.5)
Older age	8 (12.9)
Decision not to give chemotherapy	8 (12.9)
Patient refusal	6 (9.8)
No reason specified	5 (8.1)
Comorbidity (except cardiac)	3 (4.8)
Cardiac comorbidity	3 (4.8)
Cardiac toxicity with chemotherapy	3 (4.8)
Heterogeneous tumor	3 (4.8)
No indication: FISH borderline	1 (1.6)
Total	62 (100.0)

FISH, Fluorescent *in situ* hybridization.

Polymorphism sub-study. A total of 73 patients were included in this sub-study. Normal breast tissues and blood were obtained following written informed consent from participants at the center as part of the tissue banking activities. DNA was extracted from normal breast tissue (formalin-fixed paraffin-embedded tissues) located more than 1 cm from any lesion as selected by the study pathologist (SJ), and from available blood (n=19) using the Qiagen DNA Mini Kit (Qiagen, Mississauga, On, Canada) (23). DNA samples were then blindly genotyped for two SNPs [Ile655Val (rs1136201) and Ala1170Pro (rs1058808)] located in *HER2* gene by TaqMan SNP Genotyping Assays (Life Technologies, Burlington, On, Canada). Each batch also included negative (no DNA) and positive controls to ensure accuracy of genotyping. Protocols can be provided upon request. In this study, concordance of genotyping between normal breast tissue and blood was 100% for both SNP. Deviation from Hardy-Weinberg equilibrium was assessed for each SNP and the p-value was 0.39 for Ile655Val SNP and 0.03 for Ala1170Pro SNP. The linkage disequilibrium strength was evaluated with Lewontin's D' statistic for pair-wise SNPs and was 0.02 which is similar to that of another other study (24).

Statistical analysis. Descriptive statistics were used for baseline characteristics. The outcome of cardiac toxicity was dichotomous ("yes"/"no"). Logistic regressions were used to evaluate the associations between risk factors and cardiac toxicity. Traditional risk factors identified in univariate analyses to be associated with cardiac toxicity with a global p-value <0.20 were included in multivariate analyses to account for potential confounders.

Results

A total of 299 patients with HER2-positive, non-metastatic breast cancer diagnosed between July 1st 2005 and January 1st 2010 were identified. Among the 299 patients, 62

Table II. *Baseline characteristics.*

N (%)	Total population 237 (100%)
Patient's characteristics	
Age (years, median)[1]	55
Weight (kg, median)[1]	66
BMI (kg/m^2, median)[1]	25.8
Smoking, n (%)	
Yes	38 (16.0)
No	199 (84.0)
Hypertension, n (%)	
Yes	56 (23.6)
No	181 (76.4)
CAD, n (%)	
Yes	14 (5.9)
No	223 (94.1)
Diabetes, n (%)	
Yes	10 (4.2)
No	227 (95.8)
Tumor's characteristics	
Stage[2], n (%)	
I	60 (25.3)
II	106 (44.7)
III	71 (30.0)
Grade, n (%)	
I	6 (2.5)
II	80 (33.8)
III	149 (62.9)
Unknown	2 (0.8)
Lymphovascular invasion, n (%)	
Yes	99 (41.8)
No	113 (47.7)
Unknown	25 (10.5)
Hormone receptor status[3], n (%)	
Positive	149 (62.9)
Negative	88 (37.1)
Treatment's characteristics	
Type of surgery	
Partial mastectomy	141 (59.5)
Total mastectomy	94 (39.7)
No surgery	1 (0.4)
Unknown	1 (0.4)
Radiation therapy	
Yes	202 (85.2)
No	34 (14.4)
Unknown	1 (0.4)
Neoadjuvant chemotherapy type	
AC	0 (0.0)
CEF, CE, FEC	10 (4.2)
Anthracycline + taxane	17 (7.1)
Taxanes-based	7 (3.0)
Other[4]	4 (1.7)
No neoadjuvant chemotherapy	199 (84.0)
Adjuvant chemotherapy type	
AC	55 (23.3)
CEF, CE, FEC	33 (13.9)
Anthracycline + taxane	66 (27.8)
Taxane-based	52 (21.9)
Other[5]	8 (3.4)
No adjuvant chemotherapy	23 (9.7)

BMI, Body mass index; CAD, coronary artery disease; AC, adriamycin and cyclophosphamide; CEF or FEC. cyclophosphamide, epirubicin and 5-fluorouracil; CE, cyclophosphamide and epirubicin; [1]One missing for age, six missing for weight and six missing for BMI; [2]for neoadjuvant treatment, stage is clinical; [3]positive if estrogen and/or progesterone receptors are positive else negative; [4]trastuzumab + pertuzumab on a clinical trial; [5]trastuzumab + pertuzumab on a clinical trial (1 patient) and BACH (liposomal doxorubicin (Caelyx) + cyclophosphamide + trastuzumab OR doxorubicin + cyclophosphamide, each followed by paclitaxel + trastuzumab) clinical trial (7 patients).

Table III. *Characteristics of patients with cardiac toxicity.*

N (%)	32 (100%)
Symptoms associated with LVEF decrease (dyspnea, cough, edema)	
Symptomatic decrease	16 (50.0)
Asymptomatic decrease	16 (50.0)
Effect of cardiac toxicity on treatment	
Dose reported	9 (28.1)
Treatment stopped	18 (56.3)
No change	5 (15.6)

LVEF, Left ventricular ejection fraction

(20.7%) did not receive trastuzumab for various reasons (Table I). Almost one-third of those (n=22) did not receive trastuzumab since the tumor was smaller than 1 cm. Three patients did not receive trastuzumab because they developed cardiac toxicity during the administration of chemotherapy prior to trastuzumab treatment. As a result, the final study population included 237 patients.

Baseline characteristics of the 237 patients who received trastuzumab are reported in Table II. The vast majority of patients had surgery (99.2%) and radiotherapy (85.2%). A total of 16.0% received neoadjuvant chemotherapy treatment and 90.3% received adjuvant chemotherapy. Anthracyclines were included in the majority of chemotherapy regimens: 71% (27/38) in the neoadjuvant setting and 76% (162/214) in the adjuvant setting. More than 89% of women received trastuzumab on an every 3-week schedule.

Of the 237 patients who received trastuzumab, 32 (13.5%) developed cardiac toxicity. The baseline LVEF used for analysis was: the one before chemotherapy for 64.1% of patients, the one before trastuzumab therapy for 19.4% and the first LVEF evaluated after start of trastuzumab for 16.5%. A sensitivity analysis was conducted in the 152 patients with an available LVEF before start of chemotherapy and the proportion of cardiac toxicity was similar at 14.5% compared to 13.5% using the entire population.

Table III describes the clinical characteristics of patients with cardiac toxicity. Out of the 32 patients who developed cardiac toxicity, 50.0% were symptomatic. Following cardiac toxicity, trastuzumab treatment was delayed for 28.1% of patients and stopped definitively for 56.3%. For 15.6%, the trastuzumab treatment was continued. In this subgroup, 4/5 had an asymptomatic drop of LVEF.

Analysis of traditional risk factors for developing cardiac toxicity is shown in Table IV. Among risk factors studied, the following were associated with an increase in the odds ratio: BMI of 25-29, baseline LVEF 50-60%, smoking, diabetes, CAD, hypertension, use of anthracyclines, and five or more cycles of anthracyclines. However, none of these factors

Table IV. *Odds ratio of risk factors associated with cardiac disease in the population of patients with and without cardiac toxicity.*

Risk factors	Without cardiac toxicity	With cardiac toxicity	Odds ratio (95%CI)	*p*-Value	Adjusted[1] odds ratio (95% CI)	Adjusted[1] *p*-Value
Traditionnal (N=237)	(N=205)	(N=32)				
Age (years)[2]						
26-55	101 (49.5)	19 (59.4)	1.00 (Reference)		1.00 (Reference)	
56-80	103 (50.5)	13 (40.6)	0.67 (0.32-1.43)	0.30	0.77 (0.35-1.69)	0.52
BMI (kg/m^2)[2]						
15-19	17 (8.5)	1 (3.1)	0.34 (0.04-2.77)	0.31	0.23 (0.03-2.00)	0.18
20-24	75 (37.7)	13 (40.6)	1.00 (Reference)		1.00 (Reference)	
25-29	65 (32.7)	15 (46.9)	1.33 (0.59-3.00)	0.49	1.20 (0.52-2.78)	0.67
30 +	42 (21.1)	3 (9.4)	0.41 (0.11-1.53)	0.19	0.35 (0.09-1.36)	0.13
Baseline LVEF (%)						
<50	2 (1.0)	0 (0.0)	N/A	N/A	N/A	N/A
50-55	23 (11.2)	5 (15.6)	1.89 (0.59-6.08)	0.28	1.87 (0.58-6.04)	0.30
56-60	56 (27.3)	16 (50.0)	2.49 (1.05-5.87)	0.04	2.44 (1.03-5.78)	0.04
61-70	87 (42.4)	10 (31.3)	1.00 (Reference)		1.00 (Reference)	
>70	37 (18.1)	1 (3.1)	0.24 (0.03-1.90)	0.18	0.25 (0.03-2.00)	0.19
Smoking						
Yes	30 (14.6)	8 (25.0)	1.94 (0.80-4.73)	0.14	1.69 (0.68-4.22)	0.25
No	175 (85.4)	24 (75.0)	1.00 (reference)		1.00 (Reference)	
Diabetes						
Yes	8 (3.9)	2 (6.3)	1.64 (0.33-8.10)	0.83	1.74 (0.34-8.96)	0.51
No	197 (96.1)	30 (93.7)	1.00 (Reference)		1.00 (Reference)	
CAD						
Yes	11 (5.4)	3 (9.4)	1.82 (0.48-6.93)	0.38	1.93 (0.49-7.60)	0.35
No	194 (94.6)	29 (90.6)	1.00 (Reference)		1.00 (Reference)	
Hypertension						
Yes	46 (22.4)	10 (31.3)	1.57 (0.70-3.55)	0.28	1.81 (0.78-4.21)	0.17
No	159 (77.6)	22 (68.7)	1.00 (Reference)		1.00 (Reference)	
Anthracycline use						
Yes	148 (72.2)	24 (75.0)	1.16 (0.49-2.72)	0.74	1.41 (0.58-3.44)	0.45
No	57 (27.8)	8 (25.0)	1.00 (Reference)		1.00 (Reference)	
Doses of anthracyclines[3]						
0 dose[4]	59 (28.8)	8 (25.0)	1.00 (Reference)		1.00 (Reference)	
3 doses	65 (31.7)	7 (21.9)	0.79 (0.27-2.32)	0.67	1.00 (0.33-3.02)	0.99
4 doses	58 (28.3)	9 (28.1)	1.14 (0.41-3.17)	0.80	1.38 (0.48-3.95)	0.55
5 doses and more	23 (11.2)	8 (25.0)	2.56 (0.86-7.65)	0.09	2.34 (0.77-7.15)	0.13
Alcohol use per week						
Alcohol use by week[5] (N=131)	(N=113)	(N=18)				
Before cancer diagnosis (N=131)						
0-2 drinks	68 (60.2)	9 (50.0)	1.00 (Reference)		1.00 (Reference)	
3-9 drinks	34 (30.1)	6 (33.3)	1.33 (0.44-4.05)	0.61	1.50 (0.47-4.73)	0.49
10 drinks and more	11 (9.7)	3 (16.7)	2.06 (0.48-8.82)	0.33	2.00 (0.42-9.52)	0.38
During chemotherapy (N=130)	(N=112)	(N=18)				
0-2 drinks	96 (85.7)	15 (83.3)	1.00 (Reference)		1.00 (Reference)	
3-9 drinks	14 (12.5)	3 (16.7)	1.37 (0.35-5.35)	0.65	1.04 (0.26-4.26)	0.95
10 drinks and more	2 (1.8)	0 (0.0)	N/A	N/A	N/A	N/A
During trastuzumab (N=129)	(N=111)	(N=18)				
0-2 drinks	79 (71.2)	9 (50.0)	1.00 (Reference)		1.00 (Reference)	
3-9 drinks	29 (26.1)	6 (33.3)	1.82 (0.59-5.55)	0.30	1.74 (0.56-5.48)	0.34
10 drinks and more	3 (2.7)	3 (16.7)	8.77 (1.54-50.14)	0.01	7.42 (1.09-50.38)	0.04
HER2 polymorphism						
Genotype (N=73)	(N=63)	(N=10)				
Ile655Val[6]						
Ile/Ile	48 (76.2)	4 (40.0)	1.00 (Reference)		1.00 (Reference)	
Ile/Val	12 (19.1)	6 (60.0)	6.00 (1.46-24.69)	0.01	5.87 (1.33-25.82)	0.02
Val/Val	3 (4.8)	0 (0.0)	N/A	N/A	N/A	N/A
Ile/Val + Val/Val	15 (23.9)	6 (60.0)	4.80 (1.19-19.30)	0.03	5.33 (1.20-23.61)	0.03

Table IV. *continued*

Table IV. *continued*

Risk factors	Without cardiac toxicity	With cardiac toxicity	Odds ratio (95%CI)	*p*-Value	Adjusted[1] odds ratio (95% CI)	Adjusted[1] *p*-Value
Ala1170Pro[6]						
Ala/Ala	28 (44.4)	4 (40.0)	1.00 (Reference)		1.00 (Reference)	
Pro/Ala	22 (34.9)	3 (30.0)	0.96 (0.19-4.71)	0.95	0.79 (0.14-4.30)	0.78
Pro/Pro	13 (20.6)	3 (30.0)	1.62 (0.32-8.29)	0.57	1.40 (0.26-7.50)	0.70

CI, Confidence interval; N/A, non-applicable; BMI, body mass index; LVEF, left ventricular ejection fraction; CAD, coronary artery disease. [1]Adjusted for baseline LVEF (in continue) and smoking (yes/no) when possible; [2]one missing for Age and six missing for BMI not included in odds ratio determination; [3]number of anthracycline doses has been calculated from a standard dose anthracycline *e.g.*: one dose of epirubicin is equal to 100 mg/m^2 and one dose of doxorubicin is equal to 60 mg/m^2. Each dose received by patients was calculated with this new standard dose. For example, for six cycles of epirubicin the patient is considered to have received six standard doses of epirubicin, and a patient who received four cycles of doxorubicin is considered to have received four standard doses of doxorubicin; [4]One patient received 0.83 dose and one patient received 2 doses; [5]One drink is equal to one glass of 112 ml of wine, or 341 ml of beer, or 42 ml of alcohol 40%; [6]One missing for Ile655Val and one missing for Ala1170Pro.

reached statistical significance, except for baseline LVEF of 56-60% (p=0.04 for crude model and adjusted model).

Table IV also shows the results of alcohol consumption analyzed at three different periods. The proportion of cardiac toxicity in this substudy was 13.7% (18/131). The distribution of heavy drinkers was 14/131 (10.7%) before cancer diagnosis, 2/130 (1.5%) during chemotherapy and 6/129 (4.7%) during the trastuzumab treatment. A significant increase in the odds ratio was noted for heavy drinkers during trastuzumab therapy (OR=8.77, p=0.01). This association remains after adjustment for potential confounders (OR=7.42, p=0.04).

Among the 73 patients in the polymorphism sub-study, the distribution of the Ile655Val SNP was 52 with Ile/Ile (71%), 18 with Ile/Val (25%) and three with Val/Val (4%), and of the Ala1170Pro SNP was 32 with Ala/Ala (44%), 25 with Pro/Ala (34%) and 16 with Pro/Pro (22%) (Table IV). In this sample from the French Canadian population, the frequencies of genotypes of both polymorphisms were similar to that for other Caucasian populations (25, 26). In this sub-study, 10/73 (13.7%) developed cardiac toxicity. Compared to Ile/Ile carriers, Ile/Val carriers were more likely to experience cardiac toxicity (OR=6.00, p=0.01). This association persisted after adjustment for potential confounders (OR=5.87, p=0.02). For the Ala1170Pro polymorphism, no association with cardiac toxicity was observed.

Discussion

This single-institution retrospective study indicates that heavy alcohol intake, especially during trastuzumab treatment, is a risk factor of cardiac toxicity compared to low alcohol intake. The *HER2* Ile/Val genotype is also a risk factor of trastuzumab-induced cardiac toxicity. The *HER2* Ala1170Pro polymorphism is not a risk factor.

In this study, a significant effect was observed of the increase of the risk of cardiac toxicity with alcohol consumption of 10 drinks and more per week during trastuzumab treatment. The World Cancer Research Fund (WCRF) recommends that women limit alcohol to seven drinks a week or fewer (27). Up to 14 drinks a week for a woman can increase blood pressure and reduce cardiac function (28).

In regard to *HER2* Ile655Val polymorphism, cardiac toxicity was present in 33% of those with the Ile/Val genotype compared to 8% of the Ile/Ile carriers. This difference of 25% was statistically significant (p=0.02) and similar to that reported by one of the two other studies conducted on Caucasian patients (15, 20). Indeed, the Ile655Val SNP has been significantly associated with cardiac toxicity in a population of 61 patients with metastatic breast cancer among whom 24% of Ile/Val carriers developed cardiac toxicity compared to none of the Ile/Ile carriers (15). The other study included 48 patients treated with trastuzumab (either as neo-adjuvant, adjuvant or metastatic treatment) (20). In this study, a difference of 11% of cardiac toxicity was observed between those with the Ile/Val genotype compared to Ile/Ile carriers, but this difference was not statistically significant. However, another study among 101 Caucasian patients with early breast cancer treated with trastuzumab concluded that Ile655Val and Ala1170Pro SNPs were not associated with cardiac events (19). Unfortunately, no trend can be drawn from this study published as an abstract.

In our study, the use of five doses or more of anthracyclines shows a tendency to increase the risk of developing trastuzumab-induced cardiac toxicity. This is in accordance with literature showing that use of anthracyclines followed by trastuzumab increases the cumulative incidence of cardiac dysfunction (4, 6, 8, 9). The concomitant use of anthracyclines and trastuzumab in the adjuvant setting has

been discouraged due to the greater risk of asymptomatic and symptomatic cardiac events (29, 30). Even with sequential administration in the adjuvant setting, anthracyclines are associated with increased cardiac toxicity compared to non-anthracyline-based regimen such as docetaxel, carboplatin and trastuzumab. The Breast Cancer International Research Group (BCIRG) 006 trial demonstrated a significant difference in the proportion of patients with LVEF decrease (18.6% to 9.4%, $p<0.001$) in the anthracycline *vs*. non-anthracycline regimen (31).

Besides anthracycline use, some traditional risk factors for heart failure have been reported to increase the risk of cardiotoxicity during trastuzumab treatment. In the present study, significant effects were observed with low baseline LVEF (56-60%) and a tendency with LVEF of 50-55%. Tendencies were also observed for BMI of 25-29Kg/m^2, smoking habit, diabetes, CAD and hypertension, but age did not appear to be a risk factor. In the Herceptin Adjuvant (HERA) trial, positive associations were observed for a higher BMI (>25 kg/m^2), lower LVEF, smoking and diabetes, but no association was found for older age, CAD and hypertension (4). The NSABP-B-31 five-year update reported age, baseline LVEF value of 50-54%, and use of hypertensive medication as risk factor of heart failure (8). The NCCTG N9831 study found the same association for these three risk factors, but added that BMI was not a risk factor (9).

Our study reports that 13.5% of the patients develop cardiac toxicity. This proportion is higher compared to large randomized trials (5, 32-34) but comparisons between studies are difficult. In fact, patients were excluded from those studies if there was a pre-existing history of clinical heart failure or the presence of a risk factor as CAD, valvular disease, and many others. The reported incidence of cardiac toxicity in studies also varies due to differences in definitions of cardiac dysfunction. Although the prevalence of trastuzumab-mediated cardiac dysfunction appears to be low in clinical trials, it seems not to always be the case in routine clinical practice. In this context, different studies reported incidences of cardiac events in their cohorts from 12% to 31.1% (35-39).

The use of trastuzumab is associated with both symptomatic and asymptomatic decrease in LVEF. In this study, 50.0% of patients with cardiac toxicity reported symptoms. In other clinical studies, the proportion of symptomatic patients with cardiac toxicity varied widely from 14% to 63.2% (35, 37, 38, 40, 41).

The primary limitations of this study are its small sample size and its retrospective design. In addition, a mix of multiple gated acquisition (MUGA) and echocardiography was used to measure the LVEF. Furthermore, baseline LVEF prior to chemotherapy was not available in 35.9% of patients because HER2 status was not always available at cycle 1 of chemotherapy and LVEF evaluation in healthy patients was not a routine practice at that time. Population stratification is a confounding source in SNP studies (42, 43), although this problem might not be as important in North American Caucasian population (44-46). In our study, the majority of women were Caucasians.

Conclusion

In conclusion, alcohol use (10 drinks or more) during trastuzumab administration and *HER2* Ile/Val genotype are risk factors for cardiac toxicity related to trastuzumab use. Women should be advised to limit their alcohol intake to no more than seven drinks per week in accordance with WCRF recommendations (27). Since a recent study showed that consuming three to four alcoholic drinks or more per week after breast cancer diagnosis may increase the risk of breast cancer recurrence, women should also be advised to limit their alcohol use (47). In regard to alcohol intake and *HER2* polymorphism, further studies with a larger sample size are required to strengthen the conclusion. For now, clinical history and traditional risk factors such as low baseline LVEF, must be carefully weighed in the decision to use an anthracycline or non-anthracycline-based regimens in patient with HER2-positive disease. The benefits of treatment with trastuzumab is so high in the HER2-positive breast cancer cohort that knowing factors which might increase toxicity can open the way to optimize cardioprotection for those patients.

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