Published in final edited form as:

J Card Fail. 2013 January ; 19(1): 10–15. doi:10.1016/j.cardfail.2012.11.006.

Circulating Neuregulin During the Transition from Stage A to Stage B/C Heart Failure – A Breast Cancer Cohort

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Abstract

Background—Breast cancer (BC) treatments can cause heart failure (HF) in a subset of patients. ACC/AHA guidelines classify patients receiving cardiotoxic medications as Stage A, a high-risk population for the development of HF. Circulating neuregulin (NRG) correlates with outcomes in Stage C and D HF. We examined the levels of NRG in a BC cohort receiving cardiotoxic chemotherapy and its relationship with adverse cardiac effects during the transition from Stage A to Stage B/C HF.

Methods—In an ongoing prospective study, a planned interim analysis of 78 BC women receiving either anthracycline (AC) or trastuzumab (Tsz) was performed. Biometric data, cardiac risk factors, and NRG levels, were collected pre-chemotherapy and after completion of AC therapy or three months into Tsz therapy. Cardiac function was measured by left ventricular ejection fraction (LVEF) by echocardiography at the above time points and longitudinally as standard of care.

Results—The interim cohort was predominately Caucasian with Stage II BC and a median age of 50 years. A reduction of > 10 absolute percentage points in LVEF was observed in 21.4% of the cohort, representing a transition from Stage A to Stage B or C HF. A statistically significant drop in plasma NRG was observed in women treated with either AC and/or Tsz ($p < 0.001$). Additionally, baseline NRG correlated with the maximal change in LVEF.

Conclusions—More than 20% of women experienced cardiac dysfunction, detected by decline in LVEF, and are reclassified as Stage B or C HF. Plasma NRG levels are reduced after exposure to cardiotoxic chemotherapy, suggesting a loss in a cardioprotective growth factor. Higher

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Disclosures:

The authors have no potential conflicts or relevant financial disclosures related to this study.

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baseline NRG levels were observed in those with the greatest decline in LVEF, supporting the continued investigation of NRG as a potential prognostic marker in early Stage HF.

Keywords

cardiac function; cardiotoxicity; heregulin; cardio-oncology

Introduction

Breast Cancer (BC) patients receiving cardiotoxic chemotherapy such as anthracycline-based regimens and/or HER-2 antagonists are classified by the ACC/AHA grading system for heart failure (HF) as Stage A for the development of HF(1). Stage B HF represents the development of cardiac dysfunction without overt HF symptoms (subclinical cardiotoxicity) and has occurred in up to 57% of childhood cancer survivors who received cardiotoxic chemotherapy(2). In contrast, Stage C represents structural heart disease and clinical HF symptoms, while Stage D represents end-stage HF requiring specialized interventions(3). The purpose of the staging system is to identify high risk (Stage A) and asymptomatic (Stage B) HF patients so that proactive strategies can be developed to manage incipient HF before it becomes refractory to treatment. Currently, oncologists and cardiologists have difficulty predicting which cancer patients exposed to cardiotoxic chemotherapy (Stage A) will transition to Stage B or higher HF Stage.

Neuregulin (NRG) is a paracrine growth factor required for myocardial homeostasis and cardiac repair. Circulating levels of NRG correlate with outcomes in patients with stage C and D HF(4). Additionally, patients with stress-induced ischemia have higher circulating levels of NRG, suggesting that NRG may be a potential biomarker of ongoing myocardial stress and compensatory repair(5).

Anthracycline-based chemotherapeutic agents as well as HER-2 targeting therapies, like trastuzumab, can cause cardiac dysfunction. There is also a growing body of evidence that patients with comorbid cardiovascular risk factors are at a higher risk of developing chemotherapy-related cardiotoxicity(6). While identifying and managing cardiac risk factors remain important during BC treatment, the cardiovascular risk profile alone does not determine who will transition from Stage A to Stage B or higher HF Stage. The biology of chemotherapy-mediated cardiotoxicity and cardiac dysfunction remains complicated, and thus biomarkers of cardiac homeostasis and repair may help prognosticate patients at highest risk.

The aim of our current interim analysis is to prospectively describe the cardiovascular risk profile of BC patients undergoing treatment with AC and/or Tsz-based regimens and to characterize the effects on cardiac function by measuring changes in LVEF. Additionally, we explore whether NRG, a cardiac growth factor involved in modulating cardiac repair, may serve as a biomarker to identify those Stage A (high risk) patients at risk for progressing to a higher Stage of HF.

Methods

Study Participants

The interim study population consisted of a prospective cohort of 78 women with newly diagnosed BC undergoing treatment with either an anthracycline and/or a HER-2 antagonist (trastuzumab) from August 2008 – April 2012 from an urban, academic medical center. Patients underwent two study visits with yearly phone follow-up interviews and electronic medical record review to ascertain change in cardiac function or the development of

symptomatic HF. The current study was a planned interim analysis to determine total sample size needed to develop a predictive statistical model for risk stratification of cardiotoxicity. The purpose of the interim analysis was to characterize the cardiovascular risk profile, cardiac function and neuregulin levels in the ongoing prospective cohort. Women were excluded if they had distant metastasis or prior anthracycline exposure. The Institutional Review Board approved the study (IRB #070927) and all participants signed an informed consent prior to any study procedures.

Cardiac Risk Factors

Baseline data on age, body mass index (BMI), personal history of hypertension, hyperlipidemia, diabetes mellitus, tobacco use, coronary artery disease, family history of HF and physical activity were collected at enrollment. Definitions for optimal blood pressure and BMI were derived from JNC-7 and World Health Organization guidelines (7). Physical activity scores were calculated from previously validated self-reported activity questionnaires (8). Physical activity > 21 MET-hrs/wk was defined as a “physically active” group. Previously published literature shows that > 21 MET-hrs/wk is needed to maintain a healthy weight (9). Data was analyzed to examine the rates of cardiac risk factors within this population.

Cardiac Function

Cardiac function was measured by 2-dimensional echocardiography (2D-echo) pre-chemotherapy, at the completion of anthracycline therapy, and/or after three months of trastuzumab treatment. Quantitative biplane Simpson’s method was used to monitor changes in LVEF and to improve intra-observer variation. Additional longitudinal cardiac function data was collected from the electronic medical record as standard of care echocardiograms were performed for routine surveillance for chemotherapy cardiotoxicity. Cardiac dysfunction was defined as an absolute reduction of 10 units in LVEF from baseline or a drop below 55% [lower limits of normal for the accredited ICAEL (Intersocietal Commission for the Accreditation of Echocardiography Laboratories) core lab].

Collection of Samples

Blood samples were collected in vacuum tubes containing EDTA from an implantable venous access site (Port-A-Cath) to avoid unnecessary peripheral blood draws and to standardize processing of blood samples. After collection, plasma samples were centrifuged at 3,000 rpm for 10 min at room temperature. Next, the plasma was separated and stored in aliquots at -80°C until measurement. Frozen plasma samples were thawed for NRG in a single run.

Description of NRG ELISA

Plasma NRG was measured using DuoSet ELISA development system (R&D cat# DY377) as previously described(10). Briefly a 96 well plate (Costar #9017) was coated with the capture antibody overnight at room temperature on a plate shaker. Capture antibody was washed and the plate was blocked with blocking buffer as described in the package insert. A standard curve was generated using the lyophilized NRG according to manufacturer’s instructions with 2% normal goat serum (Santa Cruz #sc-2028). The standards and diluted samples (1:3 in 1% BSA/PBS) were added to each well and incubated for 2 hours. After serial washing, the detection antibody was added, incubated at room temperature and then washed. Streptavidin-HRP was added and incubated on the plate shaker for 30 minutes at room temperature. The plate was washed and then the substrate solution (Thermo Scientific 1-Step Ultra TMB-ELISA cat # 34028) was added for 10 minutes protected from light.

Sulfuric acid stop solution was added and the absorbance was read at 450nm using a spectrophotometric plate reader.

Aliquoted samples were run in duplicates and the average of the two values was used in the analysis. Plasma NRG detection limit ranged from 0.3 to 30 ng/ml. Eight (9.7%) of the samples were above 30 ng/ml and values were estimated by extrapolating from standard curve. None of the samples were below the detection limit. The average intra-assay coefficient of variation for plasma NRG was less than 10%, and the inter-assay coefficient of variation was 5.1%.

Statistical analysis

Continuous variables were summarized as mean $\pm$ SD. Plasma NRG values were non-normally distributed based on Shapiro-Wilks test and therefore the median values and ranges were reported ($\pm$ SEM). Wilcoxon signed-rank test was used to test for a difference in paired samples of NRG pre/post anthracycline exposure and/or pre and post three months of trastuzumab. Categorical variables were summarized as count and percentages. Spearman correlation coefficients were calculated between plasma NRG, change in LVEF and self-reported physical activity. A p-value < 0.05 was considered statistically significant. Statistical analysis was performed with statistical software SPSS Statistics version 17.0 (SPSS Inc, Chicago Illinois).

Results

Characteristics of study patients

Baseline characteristics demonstrate that the cohort is predominately Caucasian women, median age of 50 years, and with early stage breast cancer (average stage II) (Table 1). HER-2 breast cancer patients comprise 37% of the cohort. Average number of cardiac risk factors in the cohort was 2.7. The percentage of patients at the initiation of the study who would qualify as Stage A HF prior to chemotherapy administration was 84.6%. Thirty-two percent have a history of hypertension, 24% have a history of hyperlipidemia, 10% are diabetic, 22% have a family history of heart failure, 1% have a history of coronary artery disease, and 26% have some history of tobacco use (Figure 1). More significantly, 64% of the women enrolled were overweight (defined by a BMI > 25 , by the World Health Organization) (Figure 1). Likewise, of the 56 patients within this cohort for whom physical activity questionnaires were collected, only 37.5% of patients reported a physical activity amount of greater than 21 MET-hrs/week, a level recently delineated as the amount of activity required to maintain a healthy weight (9), while 61% of patients reported less than 21 MET-hrs/week of activity (defined as “inactive”).

Cardiac Function

Cardiac function was measured during the follow-up period of four years. Cardiac dysfunction, defined as the absolute maximal decline in LVEF greater than 10 absolute percentage points, was observed in 21.4% (15 of the 70) of participants (Figure 2). The mean change in LVEF for the entire cohort was -5.1 percentage points. Thus in this cohort of cancer patients receiving standard therapy, the rate of progression from Stage A to Stage B HF was 12.9% (9 out of 70 Stage B) and 8.6% progressed to Stage C HF (6 out of 70 Stage C). There was no correlation between the number of cardiac risk factors and the change in LVEF ($\rho = 0.14$, $p = \text{NS}$).

Plasma NRG

There was a statistically significant decline in plasma NRG levels in patients treated with either AC and/or Tsz (median baseline plasma NRG = 4.0 ng/ml, post chemotherapy = 2.9

ng/ml, $p < 0.001$ $n=62$ [reflecting the number of patients with paired samples]) (Figure 3). Additionally there was a small but statistically significant correlation between baseline plasma NRG and the maximal change in cardiac function (LVEF) (NRG $\rho = 0.26$, $p = 0.036$). There was no statistically significant association with baseline plasma NRG and self-reported fitness (NRG $\rho = 0.17$, $p = \text{NS}$).

Discussion

Women treated for BC in the United States are expected to survive their disease, adding to the growing number of cancer survivors (11, 12). Health-care providers are now confronted with the potential complications from BC treatment including cardiac dysfunction and accelerated cardiovascular disease. In attempts to reduce cardiac complications, the ACC/AHA guidelines have identified all patients receiving cardiotoxic chemotherapy as Stage A for the development of HF. Unfortunately physicians are not able to stratify which patients are likely to progress to subclinical cardiac dysfunction (Stage B), symptomatic HF (Stage C), or end-stage HF (Stage D). In the following discussion, we highlight the prevalence of cardiac risk factors in a typical BC cohort, describe the percentage of patients that progress to Stage B/C HF, and introduce NRG as a potential prognostic biomarker of cardiac dysfunction.

Recent adjuvant chemotherapy trials in BC using classes of anthracyclines, cyclophosphamide, taxanes, and/or trastuzumab have observed Stage C (symptomatic) HF in 4.1% (NSABP-31 for Operable HER-2 positive BC) (13, 14). However many of the clinical trials excluded participants with underlying cardiac disease or significant cardiovascular risk factors (i.e. LVEF at the lower limits of normal, uncontrolled hypertension, history of arrhythmias, valvular heart disease, history of myocardial infarction, ventricular hypertrophy, or pericardial effusion) and often have limited follow-up on cardiac outcomes.

One of the main findings from this interim analysis highlights the prevalence of cardiac risk factors in BC patients who are classified as Stage A (high risk for the development of HF). More than 20% of women experienced cardiac dysfunction detected by reduction in LVEF and are reclassified as Stage B or C HF. Hypertension, overweight, and physical inactivity were the most common cardiac risk factors in the cohort (58%, 68% and 63% respectively). This overlap between cardiovascular risk factors and exposure to cardiotoxic chemotherapy has led Jones and colleagues to propose a “multiple-hit” hypothesis (6). The authors have advocated that women with early BC have baseline risk factors predisposing them to cardiovascular disease and with exposure to cardiotoxic agents, this leads to a decrease in cardiovascular reserve with resultant subclinical and clinical cardiac dysfunction. Our current prospective study adds support to the multiple-hit hypothesis by demonstrating that approximately one in five BC patients with Stage A HF in a typical oncology practice will progress to a higher stage of HF. This work is similar to where the risk of cardiovascular disease was estimated to be higher than the risk of breast cancer recurrence in post-menopausal women. Therefore there is growing evidence that physicians should weigh competing risks and offer early screening and cardiac prevention strategies for women at high risk for progressing from Stage A to higher Stages of HF.

We observed a relatively high number BC patients who experienced a progression to Stage B or higher stage of HF than what is generally cited by oncology clinical trials. One of the earliest studies documenting anthracycline-induced toxicity reported a 2.2% incidence of HF which was dose-dependent (15). More recent studies have reported an incidence of HF between 1% and up to 27% which is directly proportional to cumulative doxorubicin dosing (16–18) and timing of trastuzumab therapy (19). One of the reasons for the wide variation in

HF risk after chemotherapy is the lack of a consensus definition of cardiac dysfunction which may or may not include asymptomatic decline in LVEF. Our cohort is representative of a typical oncology practice demonstrating that many oncology patients have underlying cardiovascular risk factors. Longer-term follow-up is ongoing to determine the significance of clinical and subclinical HF and if a statistical modeling system can predict the high-risk patients.

We were unable to demonstrate a correlation between the number of cardiovascular risk factors and the development of cardiac dysfunction (Stage B or C HF). We suspect that baseline cardiovascular risk factors influence the development of cardiotoxicity; however cardiac homeostasis is more complex than additive effects of traditional risk factors. This has prompted further work to utilize biomarkers as an additional marker of cardiac stress and homeostasis. Identifying novel biomarkers as predictors of cardiotoxicity could facilitate screening and earlier detection of these patients. Investigators Cardinale et al. have utilized troponin I as a tool to identify cardiac injury that predicted changes in cardiac function (including HF and asymptomatic reduction in LVEF) during treatment of BC with anthracycline-based regimens (20, 21). Other studies have identified BNP and NT-proBNP levels as potential biomarkers for cardiac toxicity (22, 23).

While BNP is a measure of cardiac stretch/overload and troponin identifies cardiac injury, neither of these markers have been useful at baseline to stratify Stage A HF patients undergoing potentially cardiotoxic chemotherapy for the treatment of cancer. In contrast, a biomarker of cardiac stress, myocyte turnover, and repair may shed insight into a patient's ability to maintain normal myocardial homeostasis. We have been interested in the role of neuregulin (NRG) in modulating and maintaining cardiac function. NRG is a paracrine cardiac growth factor which acts through the epidermal growth factor receptor family (ErbB or HER receptors). Promising preclinical studies show that treatment with recombinant NRG improves cardiac function in ischemic, viral and anthracycline-induced HF (24). Since anthracycline exposure is known to cause myocyte disarray and death, and in vivo work suggests a cardioprotective role of NRG signaling, we postulated that circulating plasma NRG levels would relate to changes in cardiac function induced by exposure to cardiotoxic chemotherapy as well as ErbB2-targeted therapies.

Circulating levels of NRG have been shown to correlate with outcomes in Stage C and D HF patients and are elevated in stress-mediated ischemia, suggesting that NRG is a biomarker of cardiac homeostasis required for cardiac repair and maintenance (25, 26). One interpretation of the current finding is that higher levels of NRG represents underlying cardiac stress and repair and therefore may identify those Stage A patients at higher risk of progressing to a higher stage of HF. It is also interesting that circulating plasma NRG levels fall in BC patients treated with either AC or Tsz, suggesting a loss of a paracrine factor important in cardiac repair. The study's relatively small sample size at the interim analysis precludes us from applying a statistical modeling system to determine if plasma NRG predicts cardiac dysfunction, however it does suggest that NRG signaling plays a role in cardiac homeostasis. Given the continued risk of late decline in LVEF after AC exposure, further investigation in a larger cohort over a longer duration seems warranted.

Our study highlights that cardiac risk factors are prevalent in a BC population. Although patients should undergo scrutiny of their cardiovascular profile, including optimizing blood pressure, weight loss and dietary counseling, and hyperlipidemia management, these risk factors are not the sole predictors for chemotherapy-induced cardiac dysfunction. Therefore identifying novel cardiac biomarkers such as NRG may potentially increase our specificity when screening these patients. Cardiovascular disease remains the leading cause of death in breast cancer survivors (27). Therefore it is even more paramount that we optimally manage

the cardiovascular risk factors in BC patients at Stage A HF and work to identify those at risk for progression of HF and development of late cardiovascular disease.

Limitations

The exclusion criteria of newly diagnosed, metastatic BC patients limits the generalizability of our findings to other cohorts. Additionally, our cohort is predominately Caucasian and therefore may not adequately reflect the demographic profile of other centers. The study was performed at an urban academic medical center in the state of Tennessee, and therefore is subject to aggregation bias.

Acknowledgments

Support

This project was supported in part by American Heart Association Established Investigator Award, Dallas, Texas, USA (DBS), HL068144, Heart Failure Society of America Research Fellowship Grant, Saint Paul, Minnesota, USA (CAG), and Vanderbilt CTSA grant 1 UL1 RR024975 from NCRR/NIH, Nashville, TN, USA (CAG).

We want to acknowledge and thank all the women who participated in this voluntary study. We want to acknowledge Yan Ru Su, Jared LeBoeuf, Kelsey Tomasek in the Vanderbilt Cardiovascular Translational and Clinical Research Lab for coordinating the specimen bio-repository. Lastly, we want to thank the staff at Vanderbilt's Clinical Research Center and JoAnn Gottlieb for her assistance with performing the echocardiograms.

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Figure 1. Cardiac Risk Factors in Treated BC Patients

Women treated for breast cancer (BC) have significant cardiac risk factors. A personal history of hypertension was reported in 58%, 24% had hyperlipidemia (HL), 10% had diabetes (DM), 1% had coronary artery disease (CAD), 30% had former or ongoing tobacco use, 23% had a family history of heart failure (family Hx HF), 68% were overweight (BMI > 25), and 63% reported low levels of physical activity.

Figure 2. Change in Cardiac Function During Follow-up

Absolute maximum change in left ventricular ejection fraction (LVEF) from baseline in 70 individuals who were treated with either anthracycline chemotherapy or trastuzumab for breast cancer (BC). Seven patients had no change in LVEF from baseline and are represented without bars. Fifteen women (24.1%) experienced a reduction in LVEF of greater than 10 units during the follow-up period as defined by the dotted line.

Figure 3. Change in Plasma NRG Due to Chemotherapy

AC and/or Tsz therapy caused a statistically significant drop in circulating levels of plasma neuregulin (NRG), a cardiac growth factor (baseline NRG median 4.0 ng/ml, Post Chemo NRG median 2.9 ng/ml, n = 62, p = < 0.001).

Table 1

Breast cancer patient demographics (n = 78)

Median Age (years)	50.1 (range 31– 67)
Average BMI	29.1 (range 17.7– 45.9)
Race (%)	
Caucasian	89.7
African American	9.0
Pacific Islander	1.2
Hispanic	2.6
Stage (%)	
1	24.3
2	40.0
3	35.7
T (%)	
1	44.1
2	33.8
3	17.6
4	4.4
N (%)	
0	31.9
1	49.3
2	13.0
3	5.8
M (%)	
0	100.0
Chemotherapy & Medications	
AC+T (%)	64.9
AC+Tsz (%)	6.5
TCH (%)	15.6
Tsz (%)	31.2
Aromatase inhibitor (%)	54.5
Beta-blocker* (%)	13.0
ACE-inhibitor* (%)	15.6

AC = Anthracycline, Tsz = Trastuzumab T = taxol, TCH = taxol, carboplatin, and trastuzumab.

* Therapies patients were receiving prior to any chemotherapy treatments.