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Arginine-Nitric Oxide Metabolites and Cardiac Dysfunction in Patients With Breast Cancer

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ABSTRACT

BACKGROUND Oxidative/nitrosative stress and endothelial dysfunction are hypothesized to be central to cancer therapeutics–related cardiac dysfunction (CTRCD). However, the relationship between circulating arginine-nitric oxide (NO) metabolites and CTRCD remains unstudied.

OBJECTIVES This study sought to examine the relationship between arginine-NO metabolites and CTRCD in a prospective cohort of 170 breast cancer patients treated with doxorubicin with or without trastuzumab.

METHODS Plasma levels of arginine, citrulline, ornithine, asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), and *N*-monomethylarginine (MMA) were quantified at baseline, 1 month, and 2 months after doxorubicin initiation. Determinants of baseline biomarker levels were identified using multivariable linear regression, and Cox regression defined the association between baseline levels and 1- or 2-month biomarker changes and CTRCD rate in 139 participants with quantitated echocardiograms at all time points.

RESULTS Age, hypertension, body mass index, and African-American race were independently associated with ≥1 of baseline citrulline, ADMA, SDMA, and MMA levels. Decreases in arginine and citrulline and increases in ADMA were observed at 1 and 2 months (all $p < 0.05$). Overall, 32 participants experienced CTRCD over a maximum follow-up of 5.4 years. Hazard ratios for ADMA and MMA at 2 months were 3.33 (95% confidence interval [CI]: 1.12 to 9.96) and 2.70 (95% CI: 1.35 to 5.41), respectively, and 0.78 (95% CI: 0.64 to 0.97) for arginine at 1 month.

CONCLUSIONS In breast cancer patients undergoing doxorubicin therapy, early alterations in arginine-NO metabolite levels occurred, and early biomarker changes were associated with a greater CTRCD rate. Our findings highlight the potential mechanistic and translational relevance of this pathway to CTRCD. (J Am Coll Cardiol 2017;70:152–62)

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Anthracyclines and trastuzumab (Herceptin) are highly effective in the treatment of HER2-positive breast cancer but carry a significant risk of cancer therapeutic-related cardiac dysfunction (CTRCD) (1,2). Doxorubicin is associated with a dose-dependent risk of heart failure and cardiomyopathy, and doxorubicin and trastuzumab in combination result in an increased incidence of cardiotoxicity. Despite extensive research on CTRCD, there remains a critical need to develop new biomarkers that could yield a better understanding of the mechanistic pathways involved and, ultimately, more individualized prognosis and treatment strategies (3).

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Oxidative stress has been established as a primary mechanism of doxorubicin-induced toxicity. Doxorubicin forms a semiquinone moiety that reduces oxygen to superoxide, which then reacts with several molecules, including nitric oxide (NO). This results in the production of reactive oxygen and nitrogen species, disruption of cellular redox balance, and increased oxidative/nitrosative stress (4–6). Indeed, previous studies by our group have suggested that higher circulating levels of oxidative stress, as quantified by myeloperoxidase, are associated with increased CTRCD risk in breast cancer patients (7). However, other studies, such as those showing that the oxidative effects of doxorubicin can be mediated through topoisomerase-IIβ (8), suggest that the pathways involved are likely to be multiple and complex.

Endothelial dysfunction further promotes CTRCD by disrupting normal paracrine interactions between endothelial cells and cardiomyocytes through signaling factors such as NO, vascular endothelial growth factor (VEGF), and neuregulin-1 (9,10) and by leading to increases in blood pressure and afterload (11). ErbB2 inhibition from trastuzumab can exacerbate these effects, resulting in worse oxidative stress and cardiomyocyte and endothelial cell dysfunction (10,12,13).

The arginine-NO metabolism pathway, which has been implicated in a variety of cardiovascular disease states and specifically in anthracycline-induced cardiotoxicity (14), plays a central role in both cellular oxidative/nitrosative stress and endothelial dysfunction (**Central Illustration**). NO is an essential molecular mediator of normally functioning endothelium, and reductions in NO bioavailability lead to DNA damage, lipid peroxidation, cardiomyocyte apoptosis, endothelial cell dysfunction, and reduced cardiac contractility (15,16). L-arginine, a key substrate in NO production, can be alternatively catabolized to urea or converted to *N*-monomethylarginine (MMA), which results in nitric oxide synthase (NOS) inhibition and less NO bioavailability. MMA is a precursor to 2 amino acid derivatives: asymmetric dimethylarginine (ADMA), which potently inhibits NOS, and symmetrical dimethylarginine (SDMA), which is inactive against NOS. Prior studies have demonstrated that increased levels of ADMA, as an inhibitor of endothelial NOS, is a predictor of cardiovascular mortality in individuals with coronary artery disease (17), myocardial infarction (18), and heart failure (19), and is an independent risk factor for heart failure, hypertension, coronary artery disease, diabetes, and renal dysfunction (20).

However, despite the potential relevance of this pathway to CTRCD, to the best of our knowledge there have been no studies to date of circulating arginine-NO metabolite biomarkers in cardio-oncology. Thus, we sought to determine whether arginine-NO metabolites have the potential to function as informative biomarkers in breast cancer patients treated with doxorubicin. In this study, we examined the clinical determinants of baseline arginine-NO metabolite levels, characterized early changes in these metabolites with doxorubicin therapy, and determined whether these early changes were associated with CTRCD in a longitudinal prospective cohort of breast cancer patients.

ABBREVIATIONS AND ACRONYMS

ADMA = asymmetric dimethylarginine
BMI = body mass index
CI = confidence interval
CTRCD = cancer therapeutics–related cardiac dysfunction
IQR = interquartile range
LVEF = left ventricular ejection fraction
MMA = *N*-monomethylarginine
NO = nitric oxide
NOS = nitric oxide synthase
SDMA = symmetric dimethylarginine
VEGF = vascular endothelial growth factor

METHODS

STUDY COHORT. The study cohort was a subset of the Cardiotoxicity of Cancer Therapy study, an ongoing, prospective longitudinal cohort study of women with breast cancer recruited from the Rena Rowan Breast Cancer Center of the Abramson Cancer Center at the University of Pennsylvania (Philadelphia, Pennsylvania). Inclusion and exclusion criteria were minimal and have been described previously (21). Treatment regimens were determined by the treating oncologist and consisted of either: 1) doxorubicin (240 mg/m^2) and cyclophosphamide for a total of 4 cycles every 2 weeks, followed by paclitaxel either 4 cycles every 2 weeks or weekly for 12 weeks; or 2) doxorubicin (240 mg/m^2) and cyclophosphamide for a total of 4 cycles every 2 weeks, followed by paclitaxel and trastuzumab (**Figure 1**). Trastuzumab was prescribed for a total of 1 year at dosages per standard guidelines.

Each participant completed standardized questionnaires at baseline (prior to initiation of doxorubicin) and at each subsequent follow-up visit. Clinical data were verified via review of medical records. The