Statins Do Not Significantly Affect Oxidative Nitrosative Stress Biomarkers in the PREVENT Randomized Clinical Trial

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ABSTRACT

Purpose: Preventing Anthracycline Cardiovascular Toxicity with Statins (PREVENT; NCT01988571) randomized patients with breast cancer or lymphoma receiving anthracyclines to atorvastatin 40 mg daily or placebo. We evaluated the effects of atorvastatin on oxidative and nitrosative stress biomarkers, and explored whether these biomarkers could explain the lack of effect of atorvastatin on LVEF (left ventricular ejection fraction) in PREVENT.

Patients and Methods: Blood samples were collected and cardiac MRI was performed before doxorubicin initiation and at 6 and 24 months. Thirteen biomarkers [arginine–nitric oxide metabolites, paraoxonase-1 (PON-1) activity, and myeloperoxidase] were measured. Dimensionality reduction using principal component analysis was used to define biomarker clusters. Linear mixed-effects models determined the changes in biomarkers over time according to treatment group. Mediation analysis determined whether biomarker clusters explained the lack of effect of atorvastatin on LVEF.

Results: Among 202 participants with available biomarkers, median age was 53 years; 86.6% had breast cancer; median LVEF was 62%. Cluster 1 levels, reflecting arginine methylation metabolites, were lower over time with atorvastatin, although this was not statistically significant ($P = 0.081$); Cluster 2 levels, reflecting PON-1 activity, were significantly lower with atorvastatin ($P = 0.024$). There were no significant changes in other biomarker clusters ($P > 0.05$). Biomarker clusters did not mediate an effect of atorvastatin on LVEF ($P > 0.05$).

Conclusions: Atorvastatin demonstrated very modest effects on oxidative/nitrosative stress biomarkers in this low cardiovascular risk population. Our findings provide potential mechanistic insight into the lack of effect of atorvastatin on LVEF in the PREVENT trial.

Introduction

Although significant progress has been made in cancer therapeutics over the past decades, conventional chemotherapeutics such as anthracyclines remain a cornerstone of treatment in several solid and hematologic malignancies. Despite their proven efficacy, anthracyclines can cause a range of dose-dependent cardiotoxic effects from asymptomatic left ventricular ejection fraction (LVEF) declines to overt cardiomyopathy and heart failure (1).

A widely accepted mechanism of anthracycline cardiotoxicity focuses on increased oxidative and nitrosative stress; this pathway has been interrogated as a means to both predict and mitigate cardiotoxicity (2–4). The semiquinone moiety of doxorubicin reduces oxygen to superoxide, resulting in toxic reactive oxygen species and peroxynitrite generation, the latter via interactions with nitric oxide (NO). Quantitation of NO substrates, including L-arginine, monomethylarginine (MMA) and asymmetric dimethylarginine (ADMA), has been used to understand NO synthase uncoupling, NO inhibition, and bioavailability. Moreover, oxidative stress may lead to endothelial damage and lipid peroxidation, and antioxidant enzymes such as paraoxonase-1 (PON-1) have a potentially protective effect. Studies in patients with breast cancer treated with anthracyclines indicate that changes in circulating levels of oxidative and nitrosative stress, quantified by myeloperoxidase (MPO), MMA, ADMA, and PON-1 activity, are associated with an increased cardiotoxicity risk and LVEF declines (3–5).

Similarly, strategies that mitigate oxidative and nitrosative stress may prevent anthracycline cardiotoxicity. Statins decrease oxidative and nitrosative stress markers, including MPO and ADMA levels, raising the question of whether these biomarkers can be used to understand the potential effect (or lack of effect) of statins in anthracycline cardiotoxicity (6). To investigate whether biomarkers of oxidative and nitrosative stress are informative in patients with cancer treated with anthracyclines, we used prospectively collected blood samples and cardiac MRI (CMR) data from the Preventing Anthracycline Cardiovascular Toxicity with Statins (PREVENT) trial (NCT01988571). PREVENT was a multi-center, double-blind, randomized controlled trial of atorvastatin in patients with breast

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Translational Relevance

The effects of atorvastatin on biomarkers of oxidative/nitrosative stress, key pathways implicated in the development of anthracycline cardiotoxicity, were modest in the Preventing Anthracycline Cardiovascular Toxicity with Statins (PREVENT) trial. Biomarker clusters did not mediate an effect of atorvastatin on LVEF. Our findings provide potential mechanistic insight into the lack of association between atorvastatin and LVEF in this low cardiovascular risk, anthracycline-treated population of patients with breast cancer or lymphoma. Future studies in higher cardiovascular risk populations are needed.

cancer or lymphoma initiating treatment with anthracyclines that did not demonstrate a statistically significant difference in LVEF in the atorvastatin arm compared with placebo at 6 or 24 months after anthracycline initiation (7). Our objectives were to: (i) determine the longitudinal effects of statin therapy on circulating measures of oxidative and nitrosative stress, and (ii) explore whether these biomarkers could provide insight into the lack of atorvastatin effect on LVEF through mediation analysis, an approach used to understand the hypothesized mechanisms of an effect (or lack of effect) of an intervention (8).

Patients and Methods

Study design and procedures

This was a pre-planned analysis of the PREVENT study that enrolled 279 participants through the Wake Forest NCI Community Oncology Research Program, Eastern Cooperative Oncology Group–American College of Radiology Imaging Network (ECOG-ACRIN), and Alliance for Clinical Trials networks. Details of the study design and main findings of the trial have been described previously (7). Briefly, the PREVENT trial included participants older than 21 years with stage I–III breast cancer or stage I–IV lymphoma with an expected survival of >2 years who were initiating anthracycline-based chemotherapy. Exclusion criteria included an indication to receive a statin for primary or secondary cardiovascular disease prevention, concurrent use of a CYP 3A4 inhibitor, a pre-cancer treatment LVEF <55%, pregnancy or breast feeding, a contraindication to receipt of a statin or an inability to undergo CMR imaging. The study was registered on ClinicalTrials.gov (NCT01988571), and approved by the NCI Division of Cancer Prevention and Control, the National Heart, Lung, and Blood Institute (NHLBI), and the Wake Forest and the University of Pennsylvania institutional review boards. The study was conducted in accordance with the Declaration of Helsinki and US Common Rule. All participants provided witnessed, written informed consent.

Participants were randomized in a 1:1 double-blind fashion to receive 40 mg of atorvastatin or placebo daily for at least 24 to 27 months beginning 48 hours before receipt of the first dose of doxorubicin. Randomization was stratified according to cancer type (breast cancer or lymphoma) and doxorubicin equivalent dosing (≤240 or >240 mg/m^2). Blood samples were collected at baseline (T0, before doxorubicin), and during prespecified follow-up visits at 6 months (T1) and 24 months (T2) after doxorubicin initiation. Cardiovascular function was assessed at these time points with CMR imaging. Clinical data were also collected.

Biomarker measurements

Thirteen biomarkers of oxidative and nitrosative stress were measured from blood samples collected at baseline and during the 6- and 24-month follow-up visits. Measurements of plasma levels of these biomarkers were performed by personnel blinded to study assessments using stable-isotope dilution high-performance liquid chromatography (HPLC) with online tandem mass spectrometry using the API 365 triple quadrupole mass spectrometer (Applied Biosystems) with an Ionics EP 10+ upgrade (Ionics Mass Spectrometry Group, Concord) and the Aria LX series HPLC multiplexing system (Cohesive Technologies, Inc.). The biomarkers include arginine, ornithine, citrulline, symmetric dimethylarginine (SDMA), ADMA, MMA, and homoarginine. Global arginine bioavailability ratio (GABR) was calculated as the ratio of arginine to the sum of ornithine and citrulline. Methylation index for arginine (ArgMI) was calculated as the ratio of the sum of SDMA and ADMA to MMA. PON-1 enzymatic activity was quantified on the basis of its paraoxonase (Pon), arylesterase (Aryl), and lactonase (Lac) activities. Serum Pon and Lac activities were measured in an open channel on a Roche Cobas 6000 platform (Roche Diagnostics). Serum Aryl activity was measured in a 96-well plate format (Spectramax 384 Plus; Molecular Devices). Serum Lac activity assay was performed using y-thiobutyrolactone as a substrate. The rate of generation of free thiols in serum were determined at 412 nm using DTNB. The final reaction mixtures were composed of 5 mmol/L y-thiobutyrolactone, 10 mmol/L Tris hydrocholoride, pH 8, 1 mol/L sodium chloride, and 2 mmol/L calcium chloride at 37°C. An extinction coefficient (at 412 nm) of 150,000 $(mol/L)^{-1}\cdot cm^{-1}$ was used for calculating units of thiolactonase activity, which is expressed as the amount of free thiol produced in micromoles per minute per milliliter of serum. Plasma MPO was quantified by ELISA (Cleveland HeartLab Inc.) on the Roche Cobas 6000 platform with a c501 module. Assay details have been described previously (4, 5, 9).

CMR imaging

Participants underwent CMR imaging with a standard set of acquisition parameters across a range of 1.5 and 3.0 Tesla scanners (General Electric of Wisconsin; Philips from Amsterdam, the Netherlands; and Siemens of Erlangen, Germany). Left ventricular end-diastolic and end-systolic volumes were obtained via previously published methods using cine white blood steady-state free precession techniques with a 256×128 matrix, a 40 cm field of view, a 10 ms repetition time, a 4 ms echo time, a 20-degree flip angle, an 8-mm-thick slice with a 2-mm gap, and a 40 ms temporal resolution (10). All images were analyzed by readers blinded to patient characteristics in an unpaired read.

Statistical analysis

The primary analysis was performed in the intention-to-treat population. A sensitivity analysis was performed, limiting the analysis to the subgroup of participants who were compliant with the study drug, which was defined on the basis of a compliance rate of >90% (7). Baseline characteristics were summarized according to treatment randomization using count (percentages) for categorical variables and median (25th, 75th percentiles; Q1, Q3) for continuous variables.

Principal component analysis

Principal component analysis (PCA) is a widely used dimensionality reduction technique that allows the transformation of high-dimensional biomarker data into a smaller number of principal components (referred to as biomarker clusters in the subsequent

sections) while retaining most of the original variability (11, 12). Baseline biomarker values were standardized using z-transformation. The optimal number of biomarker clusters was determined after evaluating a scree plot of principal component eigenvalues. Orthogonal rotation was implemented to facilitate interpretation of the identified biomarker clusters. A biomarker was considered to have a predominant influence in a cluster if its factor loading was >0.5 and biological interpretation was guided by the predominant biomarkers in each cluster (11). These biomarker clusters were considered to represent distinct underlying pathophysiologic domains in the oxidative/nitrosative stress pathway. Individual participant scores were calculated for each biomarker cluster based on the linear combination of the biomarker factor loadings on the cluster and z-transformed biomarker values. Biomarker cluster scores at the 6- (T1) and 24-month (T2) follow-up visits were calculated using the baseline PCA model after biomarker values were standardized using the mean and standard deviation of baseline biomarker values (12).

Effects of atorvastatin on longitudinal biomarkers

We first determined the effects of atorvastatin on biomarker levels over time using longitudinal linear mixed-effects models. Follow-up biomarker cluster score was included as the dependent variable, whereas treatment arm, visit number, and baseline biomarker cluster score were included as fixed effects. Individual participants were considered as random effects. The effect of timing of biomarker measurements was allowed to differ according to treatment randomization by including an interaction term. Marginal mean [95% confidence interval (CI)] biomarker cluster scores were estimated at 6 and 24 months in each treatment arm.

Causal mediation analysis evaluating relationships between biomarkers, atorvastatin, and LVEF

Causal mediation analysis was performed to determine whether biomarkers of oxidative and nitrosative stress explained the lack of association between atorvastatin and LVEF. In mediation analysis, the association between an exposure (e.g., atorvastatin) and outcome (e.g., LVEF) was decomposed into an indirect effect mediated through an intermediate variable (e.g., biomarker cluster) and a direct effect that is independent of the intermediate variable. The presence of a statistically significant ($P < 0.05$) average causally mediated effect suggests mechanistic relevance of the intermediate variable in the causal pathway between exposure and outcome. An indirect effect can still exist despite the absence of overall effect of treatment on outcome (13). Linear mixed effects models were used for both the mediator and outcome models. The mediator models included follow-up biomarker cluster score as the dependent variable and treatment arm, baseline biomarker cluster score and visit number as fixed effects. The outcome models included follow-up LVEF as the dependent variable and baseline LVEF, baseline biomarker cluster score, follow-up biomarker cluster score and visit number as fixed effects. The outcome models

Table 1. Baseline characteristics—overall and stratified according to treatment randomization.

Variable	Overall ($n = 202$)	Placebo ($n = 103$)	Statin ($n = 99$)
Age, y	53 (45–63)	53 (45–61)	55 (46–63)
Female sex	185 (91.6)	93 (90.3)	92 (92.9)
Race			
White	173 (85.6)	87 (84.5)	86 (86.9)
Black/African American	22 (10.9)	15 (14.6)	7 (7.1)
Other	7 (3.5)	1 (1)	6 (6.1)
Disease type			
Breast cancer	175 (86.6)	88 (85.4)	87 (87.9)
Lymphoma	27 (13.4)	15 (14.6)	12 (12.1)
Disease stage			
1	31 (17.7)	13 (14.8)	18 (20.7)
2	95 (54.3)	48 (54.5)	47 (54)
3	49 (28)	27 (30.7)	22 (25.3)
4[a]	1 (0.5)	0	1 (1)
Radiotherapy	147 (72.8)	73(70.9)	74 (74.8)
$HER2^+$ Therapy	6(3)	5(4.9)	1(1)
Planned cumulative anthracycline dose			
Doxorubicin dose <240 mg/m^2	64 (32.0)	30 (29.0)	34 (34.0)
Doxorubicin dose ≥240 mg/m^2	138 (68.0)	73 (71.0)	65 (66.0)
SBP, mmHg	124 (116–136)	126 (118–137)	121 (115–133)
DBP, mmHg	76 (69–84)	76 (69–83)	75 (70–84)
BMI, kg/m^2	29 (25–35)	30 (25–36)	29 (25–33)
Current or past smoking	23 (11.4)	9 (8.7)	14 (14.1)
LVEF (%)	62 (58–67)	62 (58–66)	63 (59–67)
Total cholesterol, mg/dL	190 (168–213)	190 (168–206)	190 (169–217)
Triglycerides, mg/dL	109 (75–146)	105 (74–136)	113 (79–147)
LDL, mg/dL	111 (93–127)	110 (89–126)	111 (95–129)
HDL, mg/dL	55 (48–65)	55 (49–64)	55 (47–66)
ACEI or ARB	17 (8.5)	8 (7.8)	9 (9.3)
Beta-blocker	6 (3)	2 (1.9)	4 (4.1)

Note: Categorical variables are summarized using count (%) and continuous variables are summarized using median (interquartile range).
Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; SBP, systolic blood pressure.
[a]Stage 4 applies to Lymphoma only.

additionally adjusted for potential confounders, including age, race, body mass index (BMI), high density lipoprotein (HDL), and cancer type. Individual participants were considered as random effects both in the mediator and outcome models.

Missing LVEF data were assumed to be missing at random and multiple imputation was performed by chained equations to impute missing LVEF values, as in the primary study analysis (7). Pooled estimates are presented for all the analyses using LVEF as an outcome. Missing biomarker data were also assumed to be missing at random, and not imputed. A two-sided alpha level of 0.05 was used to assess statistical significance. Analyses were conducted using R 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria).

Data availability

De-identified data will be publicly available as part of the NCORP/NCTN Data Archive https://nctn-data-archive.nci.nih.gov/ after NCI review and approval. In the interim, requests for data can be made to NCORP@wakehealth.edu.

Results

Baseline characteristics

Of the 279 participants initially enrolled in PREVENT, a total of 202 participants with available biomarker measurements were included; 103 were randomized to placebo and 99 to atorvastatin (**Table 1**). Reasons for exclusion included lack of baseline blood sample ($n = 51$), lack of consent for future blood sample use ($n = 9$), or missing biomarker measurements ($n = 17$). The baseline characteristics between the participants included in this analysis and those excluded were balanced. The median age for the overall study population was 53 years (Q1, Q3: 45, 63). Females comprised 91.6% of the cohort, and the percentage of female participants (i.e., consideration of sex as a biologic factor) was similar across the two groups. The majority (86.6%) of participants had breast cancer and were White (85.6%), with 10.9% identified as Black/African American. Supplementary Table S1 depicts the representativeness of our study population.

PCA and biomarker clusters

The correlations among the baseline levels of the 13 biomarkers are shown in **Fig. 1**. A six-component PCA model capturing approximately 80% of the variance in the baseline biomarkers was identified as optimal. The contributions of biomarkers to each cluster quantified by factor loadings are summarized in Supplementary Table S2. Briefly, Cluster 1 denoted arginine methylation metabolites (ADMA, SDMA); Cluster 2, PON-1 activity (Pon, Aryl, Lac); Cluster 3, arginine bioavailability (arginine, homoarginine, GABR); Cluster 4, arginine methylation index (MMA, ArgMI); Cluster 5, oxidative stress (MPO); and Cluster 6, metabolites of non-methylation arginine pathways (citrulline, ornithine).

Longitudinal changes in biomarker clusters according to atorvastatin therapy

To determine the effects of atorvastatin on longitudinal biomarker changes, baseline-adjusted mean (95% CI) estimates of the biomarker clusters at the 6- (T1) and 24-month (T2) time points were compared by treatment arm (**Fig. 2**; **Table 2**). Baseline-adjusted follow-up Cluster 1 (ADMA, SDMA) levels were numerically lower in the atorvastatin arm compared with placebo, although this difference did not reach statistical significance (overall $P = 0.081$). Cluster 2 (PON-1 activity) was significantly lower at the 24-month time point (T2) in the atorvastatin arm compared

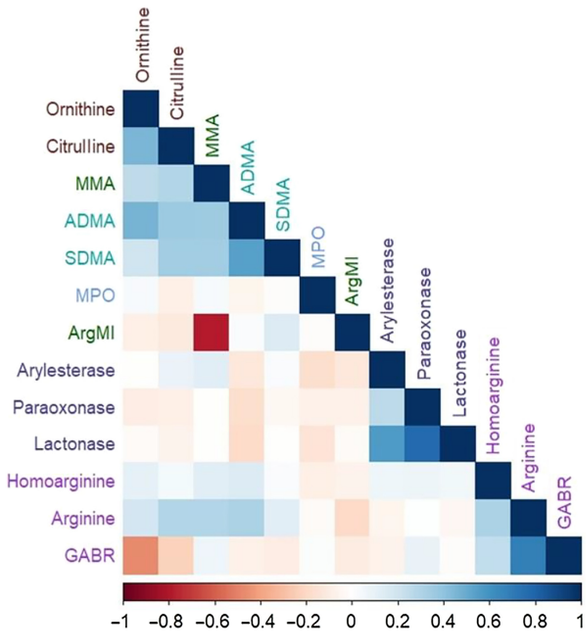

Figure 1.

Biomarker correlations for markers used in principal component analysis. Correlations between the baseline levels of each biomarker. The darker the color on the blue spectrum, the stronger the positive correlation; the darker the color on the red spectrum, the stronger the negative correlation. ADMA, asymmetric dimethylarginine; ArgMI, arginine methylation index; GABR, global arginine bioavailability ratio; MMA, monomethylarginine; MPO, myeloperoxidase; SDMA, symmetric dimethylarginine.

with placebo [atorvastatin arm, 0.19 (95% CI, −0.02–0.40) vs. placebo arm, 0.55 (95% CI, 0.34–0.75), mean difference, −0.36 (95% CI, −0.64 to −0.07), $P = 0.016$], and the overall P value across both follow-up visits was significant ($P = 0.024$). There was no significant difference in the remaining clusters between the treatment arms. Sensitivity analysis in participants who were >90% compliant with study treatment demonstrated similar findings.

Causal mediation analysis evaluating relationships between biomarkers, atorvastatin, and LVEF

Causal mediation analysis was performed with the goal of determining whether atorvastatin might have an indirect effect on LVEF through its effect on biomarker clusters. Our findings did not demonstrate a significant average causally mediated effect for any of the biomarker clusters ($P > 0.05$). Furthermore, our findings did not show a significant direct non-mediated effect of atorvastatin on LVEF ($P > 0.05$). However, the causally mediated effect of atorvastatin observed with biomarker Cluster 1 was numerically positive (mean difference, 0.17; 95% CI, −0.10 to 0.44; $P = 0.23$). The estimate of average causally mediated effect of atorvastatin on follow-up LVEF associated with Cluster 1 was also numerically greater in secondary analysis limited to participants who were compliant with the study treatment, although again not statistically significant (mean difference, 0.26; 95% CI, −0.12–0.63; $P = 0.18$).

Discussion

We report this pre-planned analysis of longitudinal measures of biomarkers of oxidative and nitrosative stress in the PREVENT trial,

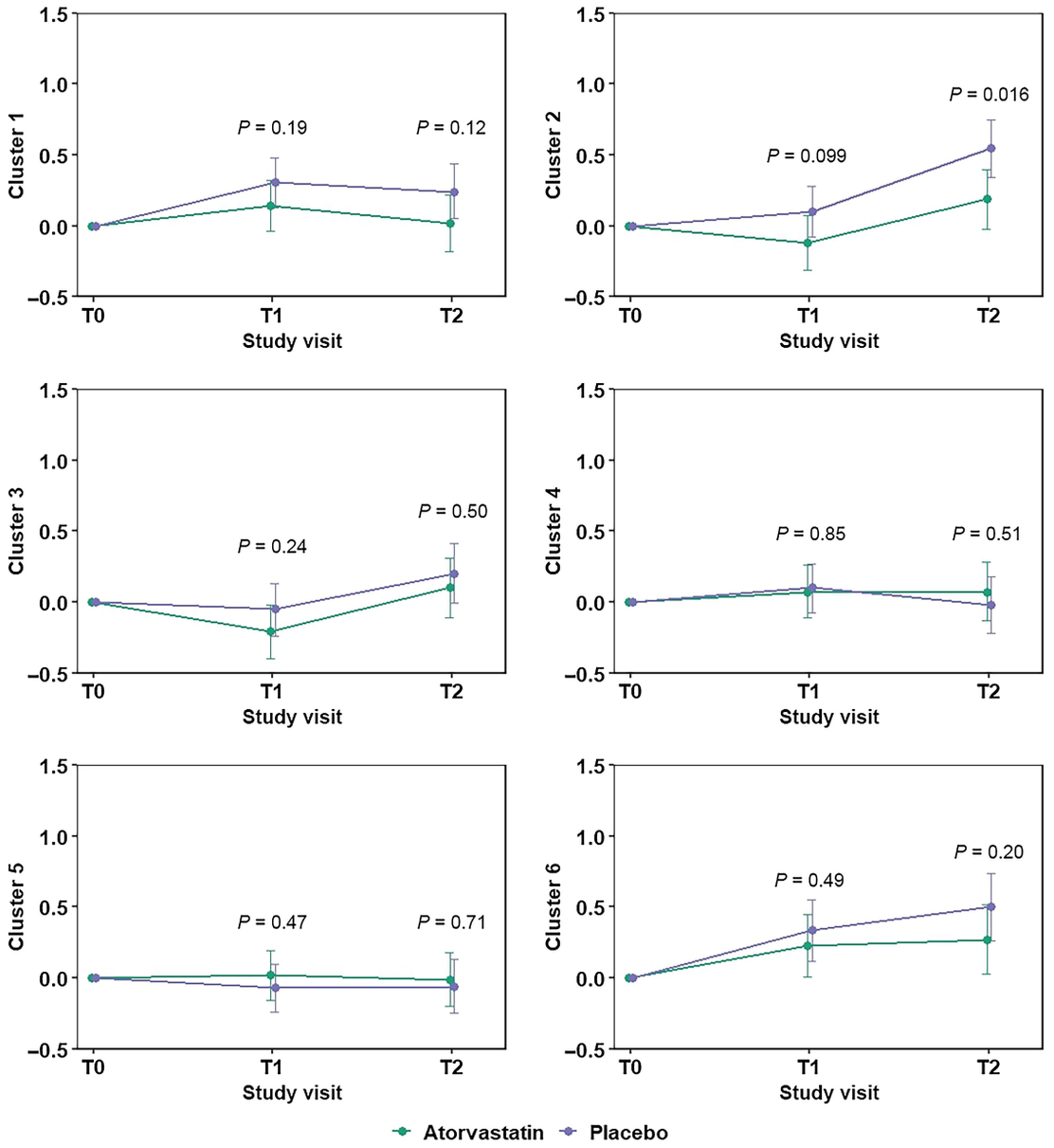

Figure 2.

Longitudinal changes in biomarker clusters by treatment arm. Comparison of baseline-adjusted mean (95% CI) estimates of the biomarker clusters at the 6- (T1) and 24-month (T2) time points by treatment arm.

which randomized participants undergoing treatment for breast cancer or lymphoma with anthracyclines to placebo versus atorvastatin. Our results are as follows: (i) atorvastatin modestly attenuated changes in biomarker clusters representing PON-1 enzymatic activity (Pon, Aryl and Lac), and arginine methylation metabolites (ADMA and SDMA), although the latter was not statistically significant; (ii) biomarkers did not influence the association (or lack thereof) between atorvastatin and LVEF change in our mediation analysis. Our findings suggest minimal attenuation of markers of oxidative and nitrosative stress by atorvastatin. As changes in these markers have previously been shown to be associated with LVEF (3, 4, 5), we believe these findings provide mechanistic insight and basis for the lack of significant association between atorvastatin and LVEF change in this population.

The current study is built upon a foundation of earlier studies investigating the role of biomarkers of oxidative/nitrosative stress in patients undergoing treatment with anthracyclines. In a cohort study of patients with breast cancer receiving doxorubicin±trastuzumab, we previously reported that early increases in ADMA and MMA were associated with increased cardiotoxicity risk and LVEF declines (4). We also demonstrated that increases in PON-1 activity were associated with development of cardiotoxicity and LVEF declines in patients with breast cancer treated with doxorubicin ± trastuzumab (5). Our prior work also supports a role for MPO and associations with cardiotoxicity

Table 2. Follow-up biomarker cluster scores according to treatment randomization using an intention-to-treat analysis.

Biomarker cluster	T1 mean (95% CI)		Mean difference (95% CI)	*P*	T2 mean (95% CI)		Mean difference (95% CI)	*P*	Overall *P* value[a]
	Placebo	Statin			Placebo	Statin			
Cluster 1	0.31 (0.13–0.48)	0.14 (−0.04–0.32)	−0.17 (−0.42–0.08)	0.19	0.24 (0.05–0.44)	0.02 (−0.18–0.22)	−0.22 (−0.50–0.06)	0.12	0.081
Cluster 2	0.10 (−0.08–0.28)	−0.12 (−0.31–0.07)	−0.22 (−0.48–0.04)	0.099	0.55 (0.34–0.75)	0.19 (−0.02–0.40)	−0.36 (−0.64 to −0.07)	0.016	0.024
Cluster 3	−0.05 (−0.24–0.13)	−0.21 (−0.40 to −0.02)	−0.16 (−0.42–0.10)	0.24	0.20 (−0.01–0.41)	0.10 (−0.11–0.31)	−0.10 (−0.39–0.19)	0.50	0.26
Cluster 4	0.10 (−0.08–0.27)	0.07 (−0.11–0.26)	−0.02 (−0.27–0.23)	0.85	−0.02 (−0.22–0.18)	0.07 (−0.13–0.28)	0.09 (−0.19–0.37)	0.51	0.82
Cluster 5	−0.07 (−0.24–0.10)	0.02 (−0.16–0.19)	0.09 (−0.15–0.33)	0.47	−0.06 (−0.25–0.13)	−0.01 (−0.20–0.18)	0.05 (−0.22–0.32)	0.71	0.48
Cluster 6	0.34 (0.12–0.55)	0.23 (0.01–0.45)	−0.11 (−0.41–0.20)	0.49	0.50 (0.26–0.74)	0.27 (0.03–0.52)	−0.22 (−0.56–0.11)	0.20	0.25

Note: These analyses were performed in 194 participants with at least one biomarker available at baseline and during at least one follow-up visit. Marginal mean (95% CI) estimates were determined using linear mixed effects models, including follow-up biomarker cluster score as dependent variable; treatment, visit number, treatment visit number and baseline biomarker score values as fixed effects; individual participants were considered as random effects. T1 represents 6 months after anthracycline initiation; T2 represents 24 months after anthracycline initiation.

Cluster 1, arginine methylation metabolites (ADMA, SDMA); Cluster 2, PON-1 activity (Pon, Aryl, Lac); Cluster 3, arginine bioavailability (arginine, homoarginine, GABR); Cluster 4, arginine methylation index (MMA, ArgMI); Cluster 5, oxidative stress (MPO); Cluster 6, metabolites of non-methylation arginine pathways (citrulline, ornithine).

[a]Overall *P* values indicate the statistical significance for the comparison of baseline-adjusted follow-up biomarker cluster scores between the placebo and statin treatment groups.

risk (3). Taken together, our previous findings suggest that these biomarkers may be useful in identifying patients at high risk for the development of cardiotoxicity, and provide rationale for studying interventions that attenuate oxidative stress to mitigate LVEF declines with cancer therapy.

The evaluation of atorvastatin in the PREVENT trial was also informed by prior studies demonstrating that statins exert pleiotropic, cardioprotective effects independent of their activity on LDL cholesterol (14). Statins have also been shown to modulate biomarkers of these same pathways such as decreasing MPO and ADMA levels through reductions in NO and inflammatory signaling pathways, suggesting that these markers may serve as a potential link between statin cardioprotection and anthracycline cardiotoxicity, providing motivation for this work (6).

The negative results of our study are interpreted in the context of the PREVENT population, which was by design a group of cancer patients at lower risk for cardiotoxicity as they did not meet guideline recommendations to receive a statin, and received relatively lower doses of doxorubicin (approximately one-third received <240 mg/m^2). Only 3.9% of the overall PREVENT trial participants experienced LVEF declines to absolute values <50%. We hypothesize that this low cardiovascular risk population had less baseline risk, experienced less substantive perturbations in the key pathways of oxidative/nitrosative stress that we comprehensively evaluated, and also a low risk of cardiotoxicity from anthracyclines. As evidence of this, in addition to a lower burden of cardiovascular risk factors, comparison of baseline biomarker levels in PREVENT with our prior cohort studies (3–5) suggests a lower level of oxidative/nitrosative stress in PREVENT (comparing arylesterase, ADMA, SDMA, MPO, $P < 0.001$ for all; paraoxonase $P < 0.05$).

Given the low-risk subgroup, we postulate that the effects of statins on these biomarkers of oxidative and nitrosative stress were minimal, and that these effects were not substantive enough to mitigate any potential cardiotoxic effects, nor were the cardiotoxic effects as substantial as observed in higher risk populations. An alternative interpretation of our findings could be that oxidative stress is not causal in anthracycline cardiotoxicity and that atorvastatin does not exert cardioprotection. However, we believe this to be a less likely explanation for our findings given our prior body of work supporting the associations between oxidative and nitrosative stress biomarkers and LVEF declines (3–5), and the recently reported STOP-CA trial (15). STOP-CA was a phase 3 trial randomizing patients with lymphoma undergoing anthracycline-based chemotherapy to atorvastatin or placebo that showed a significant attenuation in incident LVEF declines among those receiving atorvastatin. The positive results of the STOP-CA trial are hypothesized to be due to differences in cardiovascular risk factors of the study population, including higher cumulative anthracycline dose, especially in comparison with PREVENT. Given these differences in study population characteristics and cardiotoxicity risk, and discordant trial results, the differential changes in markers of oxidative and nitrosative stress in the STOP-CA trial compared with that observed in PREVENT deserve further study.

There are some limitations to note. First, some participants withdrew or were noncompliant with study drug; however, we also evaluated those participants with >90% compliance in sensitivity analyses. A proportion of participants had missing biomarker data and were not included in this analysis, although the patient characteristics were largely similar. With circulating biomarkers, we do not have direct insight into the correlation between circulating levels and intracellular levels of these measures of oxidative-nitrosative stress, and further research is needed to better understand this relationship.

Our study also has several strengths: *a priori* hypotheses, an innovative design and execution of a comprehensive ancillary study within a rigorously conducted randomized clinical trial in cardio-oncology; detailed longitudinal analysis of novel, mechanistic biomarkers; blinded CMR and biomarker quantitation; application of statistical methodology, including PCA to handle high-dimensional data. Importantly, our findings may provide a potential biologic explanation for the lack of effect of statin therapy in this low-risk population.

In summary, our analysis of markers of oxidative and nitrosative stress was largely concordant with the overall clinical results of the PREVENT study. We believe these findings provide a potential mechanistic basis for the lack of significant association between atorvastatin and LVEF. Given the ongoing use of anthracyclines in cancer and their known cardiotoxicity risk, further studies in higher risk populations, along with correlative biomarker studies aimed at identifying those most likely to benefit clinically from cardioprotection, are important translational studies motivated by this work.

Authors' Disclosures

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Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Authors' Contributions

I. Makhlin: Investigation, visualization, writing–original draft, writing–review and editing. **B.G. Demissei:** Software, formal analysis, validation, investigation, visualization, methodology, writing–original draft, writing–review and editing. **R. D'Agostino:** Data curation, software, formal analysis, supervision, investigation, visualization, methodology, writing–review and editing. **W.G. Hundley:** Resources, data curation, formal analysis, supervision, funding acquisition, investigation, visualization, methodology, project administration, writing–review and editing. **C. Baleanu-Gogonea:** Data curation, investigation, methodology, writing–review and editing. **N.S. Wilcox:** Writing–review and editing. **A. Chen:** Investigation, writing–review and editing. **A.M. Smith:** Investigation, project administration, writing–review and editing. **N.S. O'Connell:** Data curation, software, formal analysis, investigation, writing–review and editing. **J. Januzzi:** Formal analysis, investigation, visualization, writing–review and editing. **G.J. Lesser:** Data curation, funding acquisition, investigation, project administration, writing–review and editing. **M. Scherrer-Crosbie:** Writing–review and editing. **B. Ibáñez:** Writing–review and editing. **W.H.W. Tang:** Data curation, formal analysis, supervision, funding acquisition, investigation, visualization, project administration, writing–review and editing. **B. Ky:** Conceptualization, resources, data curation, software, formal analysis, supervision, funding acquisition, validation, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing.

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Note

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