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Background—Diminished serum arylesterase activity, catalyzed by the high-density lipoprotein–associated paraoxonase-1, is associated with heightened systemic oxidative stress and atherosclerosis risk. In the present study, we sought to determine the prognostic role of serum arylesterase activity in subjects with systolic heart failure, particularly in relation to established cardiac biomarkers.

Methods and Results—We measured serum arylesterase activity in 760 subjects with impaired left ventricular systolic function (left ventricular ejection fraction <50%), and prospectively followed major adverse cardiac events (including death, nonfatal myocardial infarction, and stroke) for 3 years. In our study cohort (mean age, 64±11 years; 74% men; median left ventricular ejection fraction, 35%; median creatinine clearance, 96 mg/dL), mean serum arylesterase activity (98±25 μmol/L/min/mL) was lower compared with that in healthy control subjects (mean, 115±26 μmol/L/min/mL, $P<0.01$) but higher compared with advanced decompensated heart failure subjects (mean, 69±22 μmol/L/min/mL, $P<0.01$). Within our cohort, there was modest correlation between serum arylesterase activity and high-density lipoprotein cholesterol ($r=0.33$, $P<0.01$) as well as B-type natriuretic peptide ($r=-0.23$, $P<0.01$). Lower serum arylesterase activity was a strong predictor of poorer outcomes (hazard ratio, 2.94; 95% confidence interval, 1.54, 5.62; $P<0.001$). After adjusting for traditional risk factors, medication use, B-type natriuretic peptide, and creatinine clearance, lower serum arylesterase still conferred an increased risk of major adverse cardiac events at 3 years (hazard ratio, 2.69; 95% confidence interval, 1.37 to 5.28; $P=0.004$).

Conclusions—In patients with systolic heart failure, decreased serum arylesterase activity, a measure of diminished antioxidant properties of high-density lipoprotein, predicts higher risk of incident long-term adverse cardiac event independent of established clinical and biochemical risk factors.

Oxidative stress plays an important role in the pathogenesis and progression of heart failure.[1,2] Measures of stable oxidative byproducts, including oxidized low-density lipoproteins (LDL),[1] malondialdehyde,[2] isoprostanes,[3] and urinary biopyrrins,[4] are increased in the setting of heart failure. Increased oxidative stress results from an imbalance between reactive oxygen, nitrogen, and halogenating species and endogenous antioxidant defense mechanisms to scavenge free radicals and their byproducts.[5,6] Therefore, an imbalance between oxidative and antioxidative mechanisms may lead to deleterious consequences.[7]

Paraoxonase-1 (PON-1) is a high-density lipoprotein (HDL)-associated glycoprotein believed to play a key role in facilitating systemic antioxidant activities of HDL, including the remodeling of oxidized phospholipids.[8,9] PON-1 is by far the most abundant of all paraoxonases within the vascular compartment. Numerous studies have shown that PON-1 serves as a primary contributor to systemic (serum) arylesterase activity (hydrolase activity on carboxylic ester bonds such as phenyl acetate).[10] Serum arylesterase activity has been shown to have strong correlations with multiple systemic measures of oxidant stress, including multiple distinct fatty acid oxidation products quantified by liquid chromatography with on-line stable isotope dilution tandem mass spectrometry.[10] Thus, both human clinical investigations and studies using PON-1 knockout mice[11,12] are consistent with PON-1 serving a major antioxidant function in vivo. Herein, we examine the potential role of HDL antioxidant activity, as monitored by serum arylesterase activity measurements of PON-1, as a predictor of adverse disease progression among patients with systolic heart failure.

Methods

Study Population

The Cleveland Clinic GeneBank study is a large, prospective cohort study conducted from 2001 to 2006 that established a well-characterized clinical repository with data of clinical and longitudinal outcomes comprised from consenting subjects undergoing elective diagnostic cardiac catheterization procedure not in the setting of acute coronary syndrome. All GeneBank participants gave written informed consent approved by the Cleveland Clinic Institutional Review Board. Clinical outcomes were prospectively ascertained over the ensuing 3 years for all subjects after enrollment. Major adverse cardiovascular event (MACE) was defined as all-cause mortality, nonfatal myocardial infarction, or nonfatal cerebrovascular accident after enrollment.

The present analysis included 760 consecutive subjects with stable systolic heart failure (left ventricular ejection fraction [LVEF] $<50\%$ as determined by echocardiography, radionuclide, or contrast ventriculography) enrolled in GeneBank with serum samples available for analysis. An estimate of creatinine clearance (CrCl) was calculated using the Cockcroft-Gault equation. B-type natriuretic peptide (BNP), creatinine, and fasting blood glucose and lipid profiles were measured on the Abbott Architect platform (Abbott Laboratories, Abbott Park, Ill).

To examine the range of serum arylesterase activity using this assay, we performed a cross-sectional comparison between our cohort of stable systolic heart failure with 2 independent sets of subjects, all prospectively enrolled, with written informed consent approved by the Cleveland Clinic Institutional Review Board. The first set is a cohort of 300 prospectively recruited, apparently healthy individuals without known cardiac diseases from a health screening program at various locations across Cleveland, Ohio. The second set is a cohort of 73 consecutive patients with advanced decompensated heart failure admitted to the heart failure intensive care unit for hemodynamically guided therapy including intravenous diuretic therapy.

Serum Arylesterase Activity Assay

Serum arylesterase activity was measured by UV spectrophotometry in a 96-well plate format (Spectramax 384 Plus, Molecular Devices, Sunnyvale, Calif) using phenyl acetate (Sigma-Aldrich, St Louis, Mo) as substrate. Briefly, initial hydrolysis rates were determined at 270 nm in 50-fold diluted serum (final) in reaction mixtures composed of 3.4 mmol/L phenylacetate, 9 mmol/L Tris hydrocholoride, pH 8, and 0.9 mmol/L calcium chloride at 24°C. An extinction coefficient (at 270 nm) of 1310 $mol/L^{-1} \cdot cm^{-1}$ was used for calculating units of arylesterase activity, which are expressed as the amount of phenyl acetate hydrolyzed (μM/min/mL) of serum. The intra-assay and interassay coefficients of variation for performance of arylesterase were 1.2% and 3.9%, respectively, on 20 replicates performed on 10 different days.

Statistical Analyses

We compared baseline characteristics between subjects with high versus low serum arylesterase activity levels by means of the Student *t* test (normally distributed) or Wilcoxon rank sum test (nonnormally distributed) for continuous variables and χ^2 test for categorical variables. One-way ANOVA was used to compare serum arylesterase activity levels between healthy, left ventricular systolic dysfunction (LVSD), and advanced decompensated heart failure cohorts. The Spearman correlation was performed to determine the relationship between serum arylesterase activity levels and other biochemical parameters. For each continuous variable, we investigated the log-linearity assumption of Cox models by introducing a cubic spline component. Receiver operator characteristic curve analyses in the context of the time to event were performed to determine the optimal cutoff at 121 μmol/L/min/mL, with risk of event estimated using 5-fold cross-validation by a Cox model. Kaplan–Meier analysis with log-rank test was used to compare the survival curves of the 2 groups (serum arylesterase activity <121 versus ≥ 121 μmol/L/min/mL). Cox proportional hazards regression was used for time-to-event analysis to determine hazard ratios (HR) and 95% confidence intervals (95% CI) for MACE. Adjustments were made for individual traditional cardiac risk factors including age, sex, systolic blood pressure, cigarette smoking, fasting cholesterol values (including LDL and HDL cholesterol levels). Additional adjustments included ischemic etiology, as well as medication use (including angiotensin-converting enzyme [ACE] inhibitors, β-blockers, and statin therapy), BNP, and CrCl (both logarithmic-transformed) to predict incident 3-year MACE risks. In the case of non–log-linearity such as serum arylesterase activity, the continuous variable was transformed into a binary variable, the optimal cutoff value for dichotomization being the value minimizing the prediction error in MACE event. All analyses were performed using SAS version 8.2 (Cary, NC) and R 2.8.0 (Vienna, Austria). A probability value <0.05 was considered statistically significant.

Results

Study Population

Table 1 describes the baseline characteristics of the subjects, which is a relatively well-compensated patient cohort with median LVEF of 35%, normal mean CrCl of 100 ± 41 mL/min/1.73 m^2, 63% ACE inhibitor use, and 39% treated with standing diuretic therapy. Serum arylesterase activity levels were normally distributed, with a mean of 98 ± 25 μmol/L/min/mL, which was lower than levels in the apparently healthy control population (mean, 115 ± 26 μmol/L/min/mL, $P<0.01$, Figure 1) but higher than levels of hospitalized patients with advanced systolic heart failure (mean, 69 ± 22 μmol/L/min/mL, $P<0.01$). In general, male subjects tended to have lower serum arylesterase activity levels than female subjects (97 ± 25 versus 102 ± 25 μmol/L/min/mL; $P<0.01$). Compared with the GeneBank cohort, the apparently healthy control population was younger (64 ± 11 versus 42 ± 14 years, $P<0.01$) and had less hypertension (72% versus 13%, $P<0.01$) and diabetes mellitus (39% versus 2%, $P<0.01$). In contrast, subjects with advanced decompensated heart failure were more likely to be male and had lower CrCl (68 ± 35 versus 100 ± 41 mL/min/1.73 m^2 $P<0.01$) and LVEF (26% versus 35%, $P<0.01$) than that in the GeneBank cohort.

Serum Arylesterase Activity, Fasting Lipid Profile, and Cardiac Biomarkers

There was modest correlation between serum arylesterase activity and HDL cholesterol ($r=0.33$, $P<0.001$), LDL cholesterol ($r=0.20$, $P<0.001$), and triglyceride ($r=0.18$, $P<0.001$) levels. Lower serum arylesterase activity levels were associated with higher plasma BNP levels ($r=-0.23$, $P<0.01$; Kruskal-Wallis test across BNP quartile groups, $P<0.001$). In contrast, weaker correlations were observed between serum arylesterase activity and LVEF ($r=0.12$, $P<0.01$). As a result, subjects with lower serum arylesterase activity at baseline demonstrated greater abnormalities in fasting lipid profiles as well as higher plasma BNP levels compared with that of subjects with higher serum arylesterase activity (Table 1).

Serum Arylesterase Levels and Major Adverse Cardiac Outcomes

A total of 134 events (nonfatal myocardial infarction, stroke, or death) were recorded within the 3-year period of follow-up. As continuous variables, lower serum arylesterase activity

Table 1. Baseline Subject Characteristics

Variable	Total	Serum Arylesterase Activity		*P* Value
		<121 μmol/L/min/mL (n=622)	≥121 μmol/L/min/mL (n=138)	
Age, y	64±11	64±11	64±11	0.346
Male, %	74	74	73	0.800
Diabetes mellitus, %	39	40	35	0.289
Hypertension, %	72	71	76	0.270
Ischemic etiology, %	68	67	73	0.192
LDL cholesterol, mg/dL	94 (75, 116)	93 (74, 113)	108 (84, 129)	<0.001
HDL cholesterol, mg/dL	32 (26, 38)	31 (26, 36)	36 (30, 45)	<0.001
BNP, mg/dL	193 (86, 481)	212 (94, 505)	133 (67, 351)	<0.001
LVEF, %	35 (30, 45)	35 (30, 45)	40 (30, 45)	0.110
CrCl, mL/min/1.73 m^2	100±41	100±42	101±36	0.652
Baseline medications				
Aspirin, %	73	72	80	0.075
Statins, %	62	61	68	0.149
Diuretics, %	39	40	38	0.704
ACE inhibitors, %	63	64	61	0.579
β-blockers, %	67	67	67	0.983

Values are expressed as mean±standard deviation or median (interquartile range).

was associated with poorer long-term outcomes (HR for lowering serum arylesterase activity per standard deviation decrease, 1.32; 95% CI, 1.12 to 1.54; $P<0.001$). This was observed in the setting of both ischemic (HR, 1.28; 95% CI, 1.05 to 1.56, $P=0.013$) and nonischemic (HR for lowering serum arylesterase activity, 1.52; 95% CI, 1.11 to 2.04, $P=0.008$) heart failure. When stratified according to serum arylesterase activity quartiles, subjects within the lowest 3 quartiles (<116 μmol/L/min/mL) demonstrated increased risk compared with the highest quartile (HR, 1.98; 95% CI, 1.23 to 3.18; $P=0.005$). In Kaplan–Meier analysis, serum arylesterase activity levels below the receiver operator characteristic–determined optimal cutoff level of 121 μmol/L/min/mL were associated with increased risk for development of MACE at 3 years (HR, 2.94; 95% CI, 1.54 to 5.62; $P=0.003$; Figure 2). After adjusting for traditional risk factors, medication use, BNP, and CrCl, serum arylesterase levels below this cutoff value still maintained a 2.7-fold increased risk in the development of future MACE (HR, 2.69; 95% CI, 1.37 to 5.28; $P=0.004$; Table 2). To test whether associations differed by heart failure etiology, we included an interaction term in our Cox models between serum arylesterase activity and ischemic/nonischemic etiology. This was no significant interaction, suggesting that a higher serum arylesterase activity maintains a 3-fold increased risk in the development of future MACE regardless in both groups (HR, 3.03; 95% CI, 1.35 to 6.79; $P<0.001$).

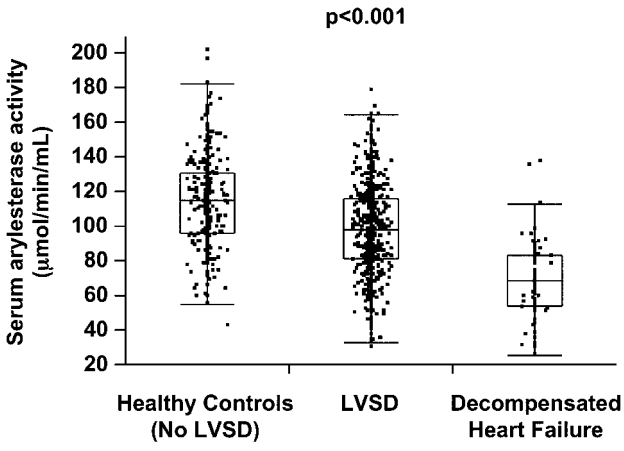

Figure 1. Comparison of serum arylesterase activity between healthy control subjects, patients with stable systolic heart failure, and hospitalized patients with advanced decompensated heart failure. Probability value is for 1-way ANOVA comparison across the 3 subgroups.

Discussion

We report for the first time there is a notable reduction in HDL-associated antioxidant PON-1 activity (as monitored by

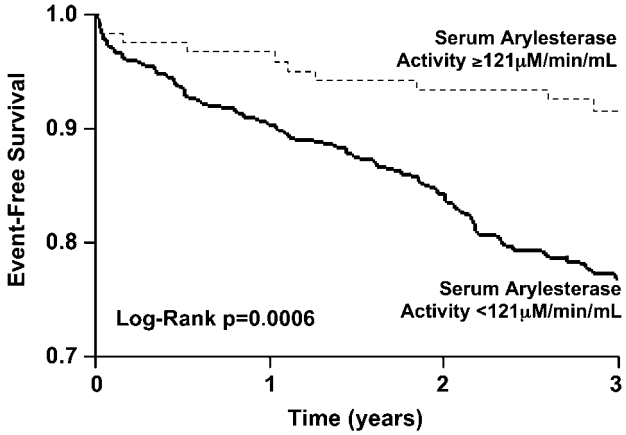

Figure 2. Kaplan–Meier analysis for long-term MACE. Probability value is for log-rank test.

Table 2. Cox Proportional Hazard Analysis for Diminished Serum Arylesterase Activity on Major Adverse Cardiac Events at 3 Years

Analysis	HR (95% CI)	*P* Value
Univariate analysis*	2.94 (1.54–5.62)	0.001
Multivariate analysis*		
Model 1 (traditional risk factors+ischemic etiology)†	3.14 (1.57–6.30)	0.001
Model 2 (traditional risk factors+medications+CrCl+BNP)‡	2.69 (1.37–5.28)	0.004

*Serum arylesterase activity levels were dichotomized at 121 μmol/L/min/mL.

†Traditional risk factors include age, sex, systolic blood pressure, diabetes mellitus, LDL cholesterol, HDL cholesterol, smoking, and ischemic etiology.

‡Medication use includes ACE inhibitors, b-blockers, and statins. CrCl and BNP were log-transformed.

serum arylesterase activity) in stable patients with impaired LV systolic function, as well as those admitted with advanced decompensated systolic heart failure, when compared with healthy control subjects. We further demonstrated a robust association between low serum arylesterase activities and poor long-term prognosis independent of ischemic etiology and traditional cardiac risk factors in patients with impaired LV systolic function undergoing cardiac evaluation. These observations are in parallel with reports of the prognostic value of other oxidative stress markers in patients with heart failure but were performed on a large scale. Our findings imply a potential importance of the HDL-associated protein PON-1, which has established links with multiple measures of systemic oxidant stress, as an important protective pathway that is diminished in the setting of cardiac dysfunction.

Human studies of oxidative stress in heart failure have focused on end-products rather than mediators of (or in this case protectors against) the oxidative process. The potential contribution of low HDL and low apolipoprotein A1 levels to increased risk of incident heart failure has been demonstrated in large epidemiology studies,[13,14] although there has been some dispute noting that coronary ischemia may be an important confounder.[15] These findings are supported by animal studies of human apolipoprotein A1 gene transfer that prevents the development of diabetic cardiomyopathy.[16] In patients with established heart failure, the role of low HDL and/or low apolipoprotein A1 in predicting heart failure events as well as mortality has been demonstrated,[17–20] even in those with nonischemic etiologies.[21] Although most have speculated the primary contribution of HDL's favorable effects is via its reverse cholesterol transport properties, other anti-inflammatory, antiapoptotic, and antithrombotic effects of HDL can also play important roles as evidenced by with its inverse relationship with inflammatory mediators.[22,23]

PON-1 activity monitored via serum arylesterase activity is a readily detectable and quantifiable process in human serum. Much of the HDL attributed antioxidant effects reported are now believed to be mediated by PON-1, which in the plasma compartment exists essentially only as an HDL-associated protein owing to the high binding affinity of PON-1 to HDL and the ability of HDL to stabilize PON-1 activity. Low levels of serum arylesterase activity are associated with increased systemic measures of oxidative stress, heightened cardiovascular disease risk,[13] and increased disease severity in other end-organ dysfunction.[16,17] Instead of indirectly measuring byproducts of oxidative stress, which is often unstable and tedious, quantifying serum arylesterase activity levels facilitates a direct measurement of the antioxidative process that has been previously described in protecting against organophosphate toxicity[24] and reducing atherosclerotic cardiovascular risk in both humans[10,25] and animal models.[12] The broad overlap between serum arylesterase activity levels in normal control subjects versus those with underlying systolic heart failure may preclude the potential for its use as a diagnostic marker for the presence of cardiac dysfunction or heart failure. However, the ability of arylesterase levels to predict long-term outcomes in patients with heart failure is intriguing, especially when the strength of risk prediction for serum arylesterase activity appears robust, even after adjustments for BNP and other cardiometabolic risk factors and renal function. The strong prognostic value of serum arylesterase activity is not linear because the lowest 3 quartiles of subjects had poorer outcomes compared with the highest quartile. This finding may imply that there exists a "threshold" of antioxidant activity needed. The cutoff level of activity suggested by receiver operator characteristic curve analyses for defining a high-risk cohort suggests a broad systolic heart failure population may be identified as being at risk using this biomarker. Of note, the present results also suggest that those with relatively preserved (high) serum arylesterase activity may also be reassured that they are in a relatively lower risk category for long-term adverse cardiac events.

The precise pathophysiologic mechanism of PON-1 activity in human heart failure has not been previously examined. The expression and variability of systemic arylesterase activity over the natural history of heart failure is largely unknown, although it is conceivable that reduced serum arylesterase activity can adversely contribute to disease progression in a number of ways. The best-described mechanism is the association between PON-1 activity and protection against lipoprotein oxidation, thereby antagonizing progression of atherosclerotic coronary artery disease, a major cause of heart failure in Western societies. Another potential protective mechanism for arylesterase activity may include the possibility for limiting microvascular dysfunction as a result of endothelial dysfunction.[26,27] Indeed, lower serum paraoxonase/arylesterase activity has been reported in patients with cardiac syndrome X,[28] and genetic polymorphisms favoring preserved paraoxonase/arylesterase are protective of microvascular diseases in patients with diabetes mellitus.[29] Regardless, the possibility that a specific antioxidative pathway, such as catalyzed by PON-1, modulates systemic oxidative stress in protecting heart failure progression warrants further mechanistic investigations.

Study Limitations

Because serum arylesterase levels were only measured at a single time-point, we were unable to examine the variability and prognostic value of level changes over time or the impact of different therapeutic strategies (such as ACE inhibitors or

β-blockers) on serum arylesterase activity. The heterogeneity of LVEF determination (either by echocardiography, contrast ventriculography, or radionuclide ventriculography) may arise because not all patients in the GeneBank study had simultaneous echocardiographic evaluation at the time of blood sample collection. Even though the traditional "cutoff" of LVEF for determining systolic heart failure is ≤40%, our results were essentially identical when only analyzed within the subgroup of patients defined by this criterion. The original GeneBank protocol also did not include prospective collection of heart failure outcomes including heart failure–related hospitalizations, cardiac transplantation, or mechanical circulatory assist devices. Selection bias may also be present for those undergoing cardiac catheterization for evaluation and management of heart failure at a tertiary care setting despite being a common practice to rule out underlying ischemia (as we observed a relatively large majority of patients had ischemic etiology) and for those potentially earlier in the disease course (evident from comparison with an advanced decompensated heart failure cohort). The relative risks might be smaller if heart failure–specific covariates and end points are applied. Nevertheless, the large sample size of our patient population together with careful phenotypic evaluation at the time of enrollment provided a well-characterized population of stable patients with impaired LV systolic function. Further understanding of such antioxidative pathways is warranted and may provide opportunities to modulate or enhance such pathways in the setting of systolic heart failure.

Conclusions

In patients with systolic heart failure, diminished activity levels of the antioxidant HDL-associated enzyme PON-1, as monitored by serum arylesterase levels, predict shorter time to develop incident long-term adverse cardiac events independent of established clinical and biochemical risk factors. These intriguing results provide further support for a role for oxidative processes in the disease progression of heart failure and for a potential antioxidant compensatory role of HDL. Further investigations on the relationship of serum arylesterase activity and heart failure are warranted, as are studies aimed at modulating PON-1 activity as a means of potentially protecting the failing heart from disease progression.

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Disclosures

Dr Tang received research grant support from Abbott Laboratories, Inc, and serves as a consultant for Medtronic, Inc, and St Jude Medical. Dr Hazen reports being listed as coinventor on pending and issued patents held by the Cleveland Clinic relating to cardiovascular diagnostics. Dr Hazen was paid as a consultant for the following companies: Abbott, AstraZeneca Pharmaceuticals LP, BG Medicine, Inc, Merck & Co, Inc, Pfizer, Inc, Cleveland Heart Laboratory, Inc, Esperion, Liposcience, and Takeda. Dr Hazen received research funds from Abbott, Esperion, Liposcience, and Cleveland Heart Laboratory, Inc. Dr Hazen received royalty payments for inventions or discoveries related to cardiovascular diagnostics and from Cleveland Heart Laboratory, Inc, Abbott Laboratories, Inc, Biosite, Inc, Frantz Biomarkers, LLC, and Siemens.

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