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High-density Lipoprotein Associated Paraoxonase-1 Activity for Prediction of Adverse Outcomes in Outpatients with Chronic Heart Failure

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Abstract

Aims—Decreased arylesterase (AryLE) activity of paraoxonase-1, a HDL-associated protein with anti-inflammatory and antioxidant properties, has been associated with increased risk of cardiac events in patients with ischemic heart failure (HF). We aim to investigate the prognostic significance of changes in serum AryLE activity over time.

Methods and results—We examined the association between baseline and follow up serum AryLE activity and HF outcomes (death, cardiac transplantation or ventricular assist device implantation) in 299 patients with HF enrolled in a prospective cohort study from 1/2008 to 7/2009, with 145 patients have available follow up levels at 1 year. A significant drop in AryLE activity on follow up was defined as a drop of $\geq 25\%$ vs. baseline levels. Mean baseline and follow up AryLE activity levels were $110.5 \pm 29.8 \mu\text{mol}/\text{min}/\text{ml}$ and $106.2 \pm 29.9 \mu\text{mol}/\text{min}/\text{ml}$, respectively. After mean follow up of 2.8 ± 1.1 years, low baseline AryLE activity was associated with increased risk of adverse HF events [HR (lowest vs highest tertile)=2.6(95% CI 1.3–5.5), $p=0.01$] and HF related hospitalization [incidence rate ratio (lowest vs highest tertile)=2.1(95%CI 1.2–4.1), $p=0.016$], which remained significant after adjustment for age, male, systolic blood pressure, diabetes, creatinine clearance, coronary artery disease, and HDL Cholesterol levels. Patients who had a significant drop in AryLE activity on follow up ($\geq 25\%$ of baseline levels, $n=18$), had significantly increased risk of HF events [HR=4.9(95%CI 1.6–14.6), $p=0.005$], even after adjustment for baseline levels of AryLE activity.

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DISCLOSURE

All other authors have no relationships to disclose.

Conclusions—Reduced baseline AryLE activity and decreased levels on follow up are associated with adverse outcomes in stable outpatients with HF.

Keywords

Paraoxonase; Heart failure; High density lipoprotein; Prognosis

INTRODUCTION

Oxidative stress has been implicated in the pathophysiology of heart failure (HF) as well as other disease states.(1, 2) While there are many contributing factors leading to heightened oxidative stress, the downstream consequences may lead to disease progression and adverse outcomes.(1) As one of the most abundant circulating anti-oxidants in the body, high-density lipoprotein (HDL) serves as an important regulator of oxidative stress but the major focus has been its role in reverse cholesterol transport as well as its effects on countering the pathogenesis of atherosclerosis.(3) The clinical relevance of HDL cholesterol in HF has only been recently appreciated with data from epidemiological studies recognizing low HDL cholesterol as an important predictor of incident HF.(4, 5)

Circulating paraoxonase-1 (PON-1) has long been associated with anti-oxidant functions of HDL, being the major “detoxification” protein that is bound to apolipoprotein A1 in the HDL particle.(6) PON-1 catalytic activity within crude serum mixtures has traditionally been measured by quantifying enzymatic hydrolysis rates of 2 known in vitro substrates, with functional activities named paraoxonase (hydrolyzes organophosphates, like paraoxon) and arylesterase (hydrolyzes aromatic esters, like phenyl acetate) activities.(3, 7) We have recently demonstrated that in a cohort of stable cardiac patients with HF undergoing cardiac catheterization, diminished serum arylesterase (AryLE) activity, a functional measure of PON-1 activity corresponds to higher rates of long-term major adverse cardiac events including death, myocardial infarction, and stroke. (8) Herein, we examined the impact of HDL anti-oxidant function on disease progression of chronic HF by measuring serum AryLE and paraoxonase activities levels in ambulatory patients with chronic HF.

METHODS

Study Population

A total of 299 subjects with HF were enrolled from the Atlanta Cardiomyopathy Consortium, a prospective cohort study conducted between 2007–13 enrolling outpatients with HF from 3 university-affiliated hospitals in the greater metropolitan Atlanta area. The inclusion criteria included age older than 18 years, ability to understand and sign written informed consent to participate, and a diagnosis of HF with either reduced or preserved ejection fraction (EF). The diagnosis of HF with preserved EF required, in addition to the clinical diagnosis of HF, elevated B-type natriuretic peptide level >200 pg /dL and / or echocardiographic evidence of diastolic dysfunction. Exclusion criteria included congenital heart disease, previous heart transplant, known cardiac infiltrative disease (e.g., amyloidosis), previous other solid organ transplantation, and end-stage HF requiring outpatient continuous inotrope infusion. Creatinine clearance was calculated using

Cockcroft-Gault formula. Patients were followed up prospectively for HF events [death, transplant and left ventricular assist device implantation (LVAD)] and HF related hospitalizations. The Emory University Institutional Review Board approved the research protocol, and all participants provided written informed consent.

Serum AryIE activity and paraoxonase activity measurements

Blood samples were collected on enrollment for all patients and in 145 patients who had clinic visit after one year. Serum AryIE activity and paraoxonase activity assays were measured by spectrometry in an open channel on Architect ci8200 platform, and in a 96-well plate format (Spectramax 384 Plus, Molecular Devices, Sunnyvale, California), respectively, as previously described.(3, 8) Briefly, for serum AryIE activity measurement, initial hydrolysis rates were determined at 270 nm in 50-fold diluted serum (final) in reactions mixtures composed of 3.4mM phenyl acetate (Sigma-Aldrich, St Louis, Missouri), 9mM Tris hydrochloride, pH 8, and 0.9mM calcium chloride at 24°C. An extinction coefficient (at 270 nm) of $1310\text{M}^{-1}\cdot\text{cm}^{-1}$ was used for calculating units of AryIE activity, which are expressed as micromoles of phenyl acetate hydrolyzed per minute per milliliter of serum. The intra-assay and inter-assay coefficients of variance for AryIE activity assay were 3.4% and 3.9%, respectively, on 20 replicates performed on 10 different days. For serum paraoxonase activity, the rate of generation of para-nitrophenol was determined at 405 nm in 40-fold diluted serum (final) in reaction mixtures compose of 1.5mM paraoxon (Sigma-Aldrich, St Louis, Missouri), 10mM Tris hydrochloride, pH 8, 1M sodium chloride, and 2mM calcium chloride at 24°C. An extinction coefficient (at 405 nm) of $17\ 000\ \text{M}^{-1}\cdot\text{cm}^{-1}$ was used for calculating units of paraoxonase activity, which is expressed as nanomoles of para-nitrophenol produced per minute per milliliter of serum. The intra-assay and inter-assay coefficients of variance for the high throughput paraoxonase activity assay were 1.9% and 3.3%, respectively, on 30 replicates performed on 15 different days.

Statistical analysis

The Student *t* test or Analysis of variant (ANOVA) test, for continuous variables, and Chi-square test for categorical variables were used to examine differences between the groups. Linear regression was used to study factors correlation with AryIE activity. Survival and event rates were described with the Kaplan Meier method. Cox proportional hazards regression was used to determine hazard ratios (HR) and 95% confidence intervals (CI) for events. In multivariable models, we adjusted for age, gender, diabetes mellitus, systolic blood pressure, calculated creatinine clearance, coronary artery disease and HDL-cholesterol levels. The negative binomial regression model was used to obtain an estimate of the effect of AryIE activity on the rate of HF hospitalizations giving the skewed distribution in the frequency of hospitalizations.(9, 10) The negative binomial naturally accommodates the different probabilities for events across members of the population. General linear models with repeated measures were used to study the change of AryIE overtime. A Significant drop in AryIE activity over time was defined as a drop of 25% of the baseline activity.

RESULTS

Table 1 describes the baseline characteristics of the study population. Baseline AryIE activity was normally distributed with a mean of 110.6 ± 29.9 $\mu\text{mol}/\text{min}/\text{mL}$. No significant difference in baseline characteristics was observed between groups with different AryIE activity. No difference was observed in AryIE activity between patients with preserved or reduced EF.

Serum AryIE activity and clinical variables

On univariate linear regression analysis, increased HDL-cholesterol levels ($\beta=0.61$, $p<0.001$), female gender ($\beta=8.2$, $p=0.024$) and ACEi/ARBs use ($\beta=10.8$, $p=0.01$) were associated with increased activity of baseline AryIE activity, while log transformed BNP ($\beta=-3.1$, $p=0.01$) was inversely correlating to AryIE activity. However, on multivariate linear regression including basic demographics, medication use and EF, only HDL-cholesterol ($\beta=0.63$, $p<0.001$) and ACEi/ARBs use ($\beta=13.3$, $p=0.01$) were independently associated with increased baseline AryIE activity.

Baseline AryIE activity and HF adverse outcomes

After mean follow up of 2.8 ± 1.1 years, 53 patients had HF events (42 deaths, 7 transplants, 4 ventricular assist devices), while 95 patients experienced a total of 283 HF related hospitalizations. Distribution of HF related hospitalizations is shown in Online Figure 1. Patients with low HDL-cholesterol (<40 mg/dL for men and <50 mg/dL for women) had higher risk of HF events (log rank = 0.034) but not HF related hospitalizations ($p=0.298$), (Figure 1A&B).

Patients, who had HF events, had significantly lower AryIE activity (102.4 ± 27.8 vs 112.3 ± 30.2 $\mu\text{mol}/\text{ml}/\text{min}$, $p=0.028$). Patients with low AryIE activity had a higher incidence of HF events (23.5% vs 9.9%, for lowest vs highest tertile, $p=0.031$) and HF related hospitalization (49 vs 20.2 per 100-patient-years for lowest vs highest tertile, respectively). Patients with lower levels of AryIE activity, have a significantly increased risk of HF events [HR(95%CI) for lowest vs highest tertile of 2.6 (1.3–5.5), $p=0.01$] and HF related hospitalization [incidence rate ratio (95%CI) for lower vs higher tertile of 2.1 (1.2–4.1), $p=0.016$] (Figure 1 C & D, respectively) (Table 2). AryIE activity remained significant even after adjustment for age, gender, diabetes, systolic blood pressure, creatinine clearance and CAD (Table 2). In a subset of patients with available baseline HDL-cholesterol levels ($n=253$), low AryIE activity was independently associated with adverse HF events and hospitalization, even after adjustment for HDL-cholesterol levels.

Follow up AryIE activity and HF outcomes

Total of 145 patients had 1 year follow up clinic visit and AryIE activity was reassessed. No significant difference was found in patients with follow up levels and those without in regard to basic demographics and HF severity (EF, BNP)(Online Table 1). However, HF events were lower (9.7% vs 25.3%, $p=0.001$) in the group with available follow up samples for AryIE activity measurement.

Significant decrease in AryIE activity was observed on follow up (-5.9 ± 28.6 $\mu\text{mol}/\text{min}/\text{mL}$, $p=0.015$) (Figure 2A). No significant correlation was found between basic demographics, medications, BNP or EF and change in AryIE activity overtime. When the change in AryIE activity overtime was stratified by HF events, those who developed HF events had a trend of higher drop in AryIE activity overtime (mean of -19.2 ± 37.3 vs -4.4 ± 27.3 $\mu\text{mol}/\text{min}/\text{mL}$, for events and non-events, respectively, $p=0.066$) (Figure 2B).

After adjustment for baseline level, low follow up AryIE activity was also independently associated with increased HF events [lowest tertile vs highest tertile HR(95%CI) of 6 (1.04–34.7), adjusted $p=0.046$] (Table 2). Drop in AryIE activity was associated with increased risk of HF events [40% (95%CI of 8–80%) increased risk for every 10% drop; or HR(95%CI) of 4.9 (1.6–14.6), for a drop 25%, adjusted p value for baseline levels of 0.01 and 0.005, respectively] (Figure 3). No correlation between follow up AryIE activity and HF related hospitalization was observed (Table 2).

Serum paraoxonase activity and HF outcomes

We also assessed paraoxonase activity (hydrolyzes organophosphates, like paraoxon) of PON-1 on baseline and on follow up. Paraoxonase activity was positively skewed with a median (interquartile range; IQR) baseline of 829 (350 – 1233) $\text{nmol}/\text{min}/\text{mL}$, which increased significantly on follow up to 883 (351 – 1414) $\text{nmol}/\text{min}/\text{mL}$, $p=0.015$. In contrast to AryIE activity, patients who had HF events, did not have lower paraoxonase activity on baseline [median (IQR) of 754 (291–1023) vs 883 (368–1270), $p=0.123$] or follow up [474 (286 – 1044) vs 915 (405–1424) $\text{nmol}/\text{min}/\text{mL}$, $p=0.301$]. No significant correlation was found between either baseline or follow up paraoxonase activity and HF events or HF related hospitalization.

Results validation and association with Apolipoprotein A1

We validated our results using a longer follow up time (5 years) of our previously published cohort of 830 patients with HF undergoing elective coronary angiography enrolled from the Cleveland Clinic Genbank database (Online supplement). The mean age was 67 ± 11 years, 60% were males and 76% had coronary artery disease (Online table 2). Mean AryIE activity was 94.2 ± 26.3 , and mean apolipoprotein A1 was 113.6 ± 25.2 mg/dL . There was a positive correlation between apolipoprotein A1 and AryIE activity ($\beta=0.42$, $R^2=0.16$, $p<0.001$). In this cohort, both AryIE activity and apolipoprotein A1 were associated with 5-year all-cause mortality. However, when both included in one model, only AryIE activity was significantly associated with all-cause mortality (Online table 3).

DISCUSSION

We demonstrate that in an outpatient population of chronic HF, impaired anti-oxidant function of HDL as measured by serum AryIE activity was associated with adverse HF events and HF related hospitalizations. We also observed a significant decrease in AryIE activity overtime, mainly in patients who developed adverse HF events. We didn't see any correlation between HF events or hospitalizations and paraoxonase activity. Furthermore, using our previously published cohort we found AryIE activity to be independent of

apolipoprotein A1 levels in predicting adverse outcomes. These findings are supportive of our initial observations, and confer HDL as an important mediator that may serve to prevent disease progression in HF.

Patients with HF have increased oxidative stress in comparison to healthy population.(11, 12) Furthermore, HF patients with high oxidative stress, have higher disease severity, (11–13) hospitalization and adverse outcomes.(11) Myocardial ischemia, toxins, and infection, are main factors suggested to increase oxidative stress in HF patients.(1) Increased oxidative stress in cardiomyocytes can cause exaggerated autophagy, apoptosis or necrosis, which subsequently cause progressive loss of cardiomyocytes and left ventricular remodeling.(1, 2, 14–16) On the other hand, progression of HF with increased volume status and myocardial stretching, increases myocardial oxidative stress and mitochondrial dysfunction.(13, 17) Thus, anti-oxidant activity may play a major role in modifying oxidative stress and subsequently, may prevent progression of HF.(17)

Circulating PON-1 is the major “detoxification” protein that is bound to apolipoprotein A1 in the HDL particle.(3) PON-1 has been shown to be involved in inhibiting LDL oxidation, cholesterol efflux, monocyte binding and transmigration, plaque inflammation, as well as destroying oxidized phospholipids and thus decrease atherosclerosis and plaque formation. (18, 19) Furthermore, by hydrolyzing homocysteine thiolactones, PON-1 may prevent endothelial dysfunction and vascular damage.(7, 20, 21) The clinical significance of PON-1 activity is still controversial. Concordant with the athero-protective role of ArylE activity, several studies have shown low activity to be associated with increased CAD risk, CAD severity, endothelial dysfunction,(22) worse collateral flow and adverse cardiovascular outcomes.(7, 19, 20, 23–25) However, in contrary to this, few other studies showed high activity to be associated with increased CAD severity, coronary calcification and increased risk of myocardial infarction,(19, 26, 27) while other studies have not shown any such associations.(28, 29)

Only few studies assessed serum ArylE activity in the setting of HF. In our previous report, we found patients with HF to have diminished ArylE activity in comparison to healthy control group.(8) We also showed that diminished ArylE activity is associated with all-cause mortality, myocardial infarction, and stroke in patients with ischemic cardiomyopathy.(3) We have largely related this to the anti-atherogenic role of PON-1 since the study population included mainly patients with ischemic HF. However, in this group of patients with mixed ischemic and non-ischemic HF, we also showed a significant association with adverse HF events (death, cardiac transplantation, LVAD). Furthermore, using long term data from our previously cohort, (3) we found ArylE activity to carry more prognostic information above and beyond the levels of apolipoprotein A1. Taken together, the association of diminished serum ArylE activity and adverse outcomes is likely independent of the underlying etiology of HF, and more reflective of anti-oxidative properties of HDL than the levels of apolipoprotein A1. It’s possible that PON-1, in addition to its anti-atherosclerotic effect, plays a direct protective role on cardiac myocyte by decreasing oxidative injury and thus alters the progression of HF.

It is still unclear what the factors that influence diminished serum AryIE activity are. In our population, only ACEi/ARBs use and baseline HDL-cholesterol levels were independent predictors of high baseline AryIE activity. However, no factor was associated with increased AryIE activity on follow up. Several other studies have shown an increase in AryIE activity after statin treatment, (30–32) however, only one was a randomized control study.(30) Since diminished PON-1 activity is linked to adverse outcomes in patients with HF, targeting those with diminished PON-1 activity may provide a protective role in HF. Although previous trials that used anti-oxidants or statins, failed to show mortality benefit, (33–38) these trials were testing the effect of the intervention regardless of the oxidative status of the patient. Thus, the beneficial effect could be masked by those with low oxidative status, who may not benefit from the intervention. Whether identifying the heightened risk patients, like those with diminished PON-1 activity, could affect HF outcomes need to be further investigated.

Interestingly, we observed a discordance between the two PON-1 activities in predicting adverse HF outcomes. These findings are consistent with our previous observations of no association between paraoxonase activity and subclinical myocardial necrosis and adverse cardiovascular outcomes in patients with CAD undergoing coronary angiogram.(3) The unique relationship between the 2 PON-1 activity measurements is largely the result of a very strong association between serum paraoxonase activity and its underlying genetic determinants, which may also explain why serum AryIE activity is normally distributed whereas serum paraoxonase activity was not.

In clinical practice, clinicians tend to treat HF patients based on their ejection fraction, without considering the underlying mechanism. Our findings are important in the context that, among different HF patients, there is a variable involvement of different mechanisms, like inflammation,(39–41) oxidative stress, (11, 12) microvascular rarefaction,(42) pressure overload,(43) endothelial dysfunction,(1)...etc. Thus, profiling HF patients by a more mechanistic driven way, may help identifying heightened risk patients and provide a more individualized HF therapy. Whether treating patients with low PON-1 activity with statins, or other medications have an impact on outcome need to be further investigated.(44)

Study strengths and limitations

Our study is the first study to assess serial PON-1 activity in outpatients with HF and correlate that with their prospective outcomes. However, the small sample size and low number of patients with follow up samples are the major limitations of our study. Also, it's worth noting that, serum paraoxonase and AryIE activities used substrates that are not the endogenous substrates for PON-1, but are used because they are not readily influenced by other esterases/lactonases in serum, and are presumed to reflect the underlying catalytic activity of PON-1.(8) We did not assess genotyping or circulating levels of PON-1 that may also impact the prognostic value of AryIE activity in this population.

CONCLUSION

Diminished serum PON-1 activity, measured by AryIE activity, is associated with adverse outcomes in stable outpatients with HF.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

AryIE	Arylesterase
PON-1	paraoxonase-1
HF	heart failure
EF	ejection fraction
CAD	Coronary artery disease

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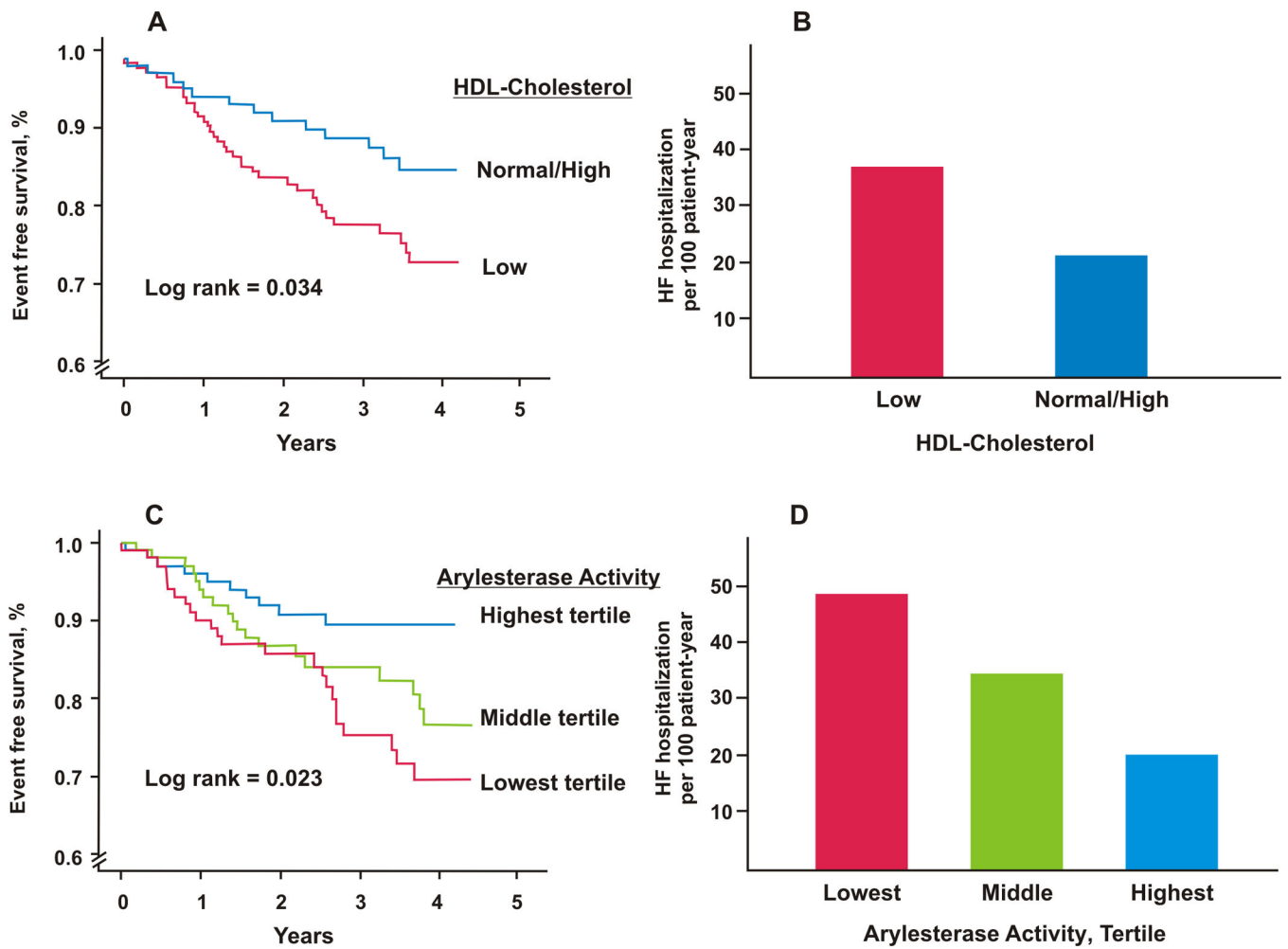


Figure 1. Baseline HDL-Cholesterol levels association with: A) HF events (Death, transplant, or LVAD), and B) HF related hospitalizations. Low HDL-Cholesterol was defined as HDL-Cholesterol <40 mg/dL for men or <50 mg/dL for women. Baseline Arylesterase activity association with: C) HF events, and D) HF related hospitalizations.

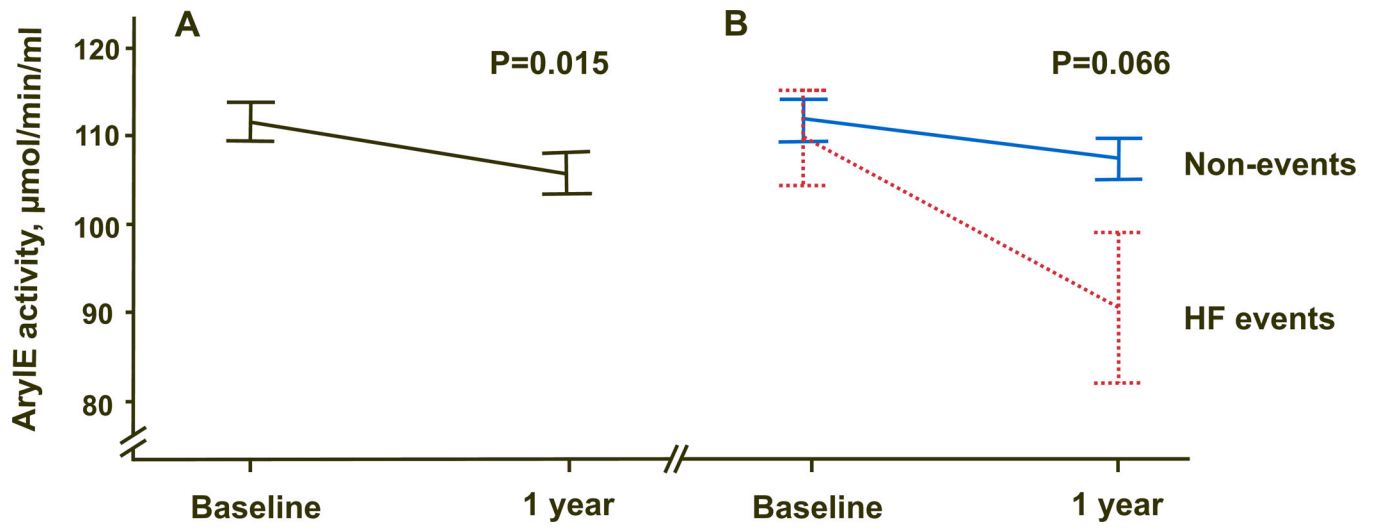


Figure 2. AryIE activity decreased over time in patients with available follow up blood samples (n=145) in A) overall population, B) stratified by event status.

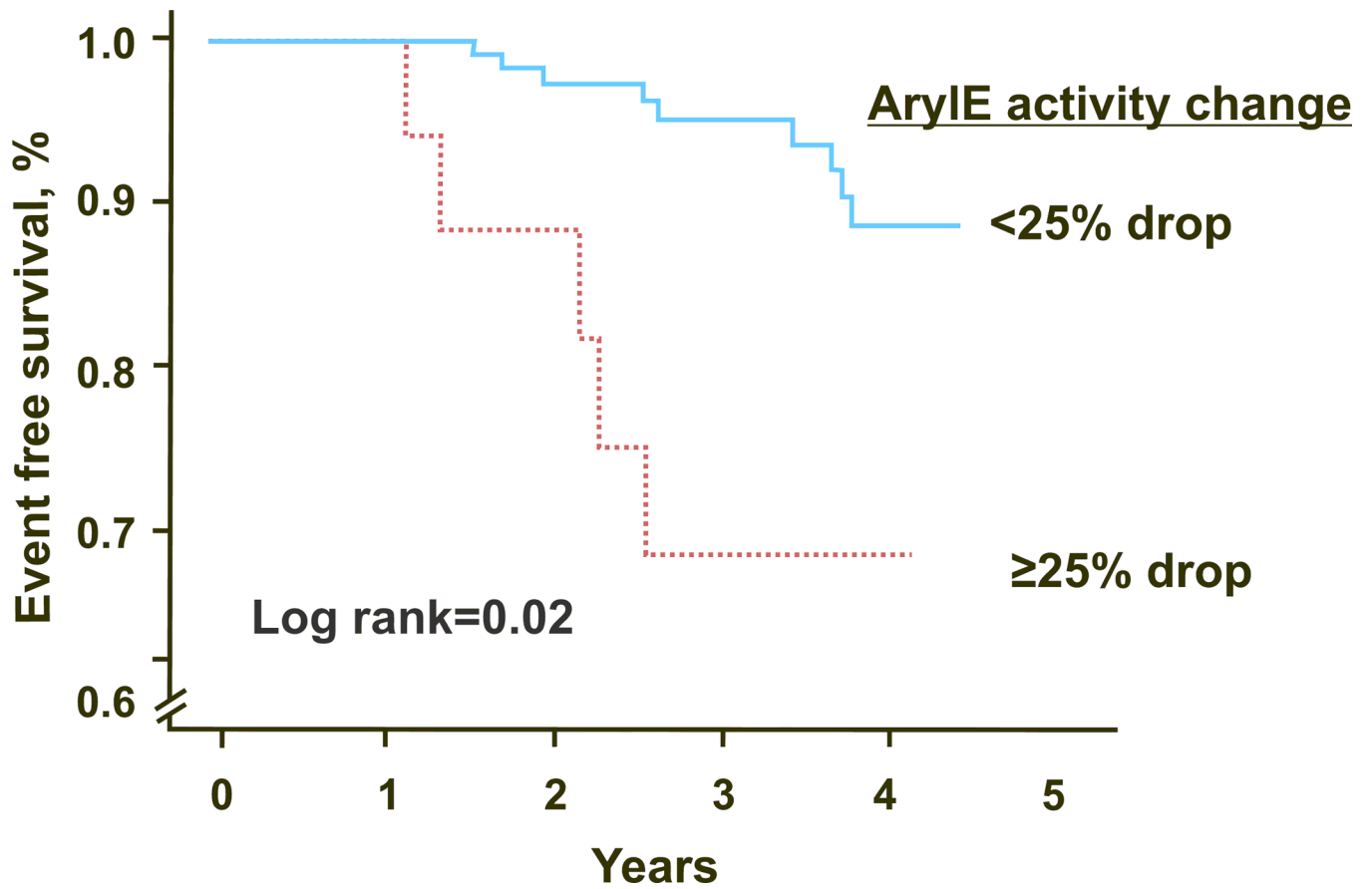


Figure 3. Correlation between AryIE activity change between baseline and one year follow up samples, and long term HF events (Death, transplant or LVAD).

Table 1

Baseline Characteristics

ArYE activity, $\mu\text{mol/L}/\text{min}/\text{mL}$	Total	Tertile 1 <97	Tertile 2 97-120	Tertile 3 120	p value
Number	299	100	99	100	
Age, years	57 \pm 12	56 \pm 13	57 \pm 13	57 \pm 12	0.93
Male, %	64	71	64	57	0.15
Black, %	44	48	34	50	0.04
Body mass index, kg/m ²	31 \pm 7	31 \pm 7	32 \pm 7	31 \pm 7	0.69
Systolic blood pressure, mmHg	113 \pm 19	112 \pm 18	112 \pm 18	115 \pm 22	0.42
Diabetes, %	33	35	31	32	0.81
Smoking, %	31	33	34	25	0.36
Coronary artery disease, %	40	40	46	35	0.29
Creatinine clearance, mL/min	93 \pm 43	92 \pm 46	94 \pm 42	93 \pm 40	0.92
Ejection Fraction, %	30 \pm 15	29 \pm 15	31 \pm 16	31 \pm 14	0.51
Ejection Fraction 40%	29	27	29	29	0.95
B-type Natriuretic Peptide, pg/mL	163 (57 – 564)	233 (75 – 684)	147 (57 – 543)	110 (39 – 301)	0.003
ACE inhibitors or ARBs, %	78	73	75	87	0.03
Beta blockers, %	91	90	93	90	0.72
Hydralazine, %	19	16	18	24	0.34
Nitrate, %	23	16	29	25	0.1
Statins, %	54	50	62	51	0.16
High density lipoprotein cholesterol, mg/dL	41 \pm 13	38 \pm 11	40 \pm 13	46 \pm 15	<0.001

AryIE activity, μmol/L/min/mL	Total	Tertile 1 <97	Tertile 2 97-120	Tertile 3 120	p value
Low density lipoprotein cholesterol, mg/dL	97 ± 36	93 ± 38	96 ± 30	101 ± 38	0.33

Abbreviations: AryIE: Arylesterase. ACE: Angiotensin converting enzyme. ARB: Angiotensin II receptor blockers.

Table 2

AryIE activity and adverse HF outcomes.

AryIE activity	Continuous variable, per 10 $\mu\text{mol/L/min/mL}$	Tertile 1 <97	Tertile 2 97–120	Tertile 3 120
Baseline levels (n=299)				
Events, %		23.5	18.8	9.9
Unadjusted model, HR(95%CI)	$\beta = -0.12^*$	2.6 (1.3 – 5.5) [*]	2 (0.9 – 4.2)	Reference
Adjusted model 1, HR(95%CI)	$\beta = -0.11^*$	2.2 (1.04 – 4.7) [*]	1.7 (0.8 – 3.8)	Reference
Adjusted model 2, HR(95%CI)	$\beta = -0.11^*$	2.4 (1.03 – 5.4) [*]	1.8 (0.7–4.2)	Reference
HF admission				
Per 100-patient-year		49	31.4	20.2
Unadjusted model, Incidence rate ratio (95CI%)	$\beta = -0.1^*$	2.2 (1.2– 4.1) [*]	1.5 (0.8–1.5)	Reference
Adjusted model 1, Incidence rate (95CI%)	$\beta = -0.1^*$	1.9 (1.004– 3.6) [*]	1.4 (0.7 – 2.8)	Reference
Adjusted model 2, Incidence rate (95CI%)	$\beta = -0.1^*$	3.4 (1.7– 6.7) [*]	1.9 (0.9–3.9)	Reference
Follow up levels (n=145)				
Events, %		14.9	10.2	4.1
Unadjusted model, HR(95%CI)	$\beta = -0.22^*$	4.2 (0.9– 20.3)	2.9 (0.6– 15.1)	Reference
Adjusted model for baseline levels, HR(95%CI)	$\beta = -0.29^*$	6 (1.04– 34.7) [*]	3.6 (0.7– 20.1)	Reference
HF admission				
Per 100-patient-years		30.1	33.8	23.2
Unadjusted model, Incidence rate (95CI%)	$\beta = -0.02$	1.2 (0.4–3.3)	1.3 (0.48– 3.7)	Reference
Adjusted for baseline levels, Incidence rate (95CI%)	$\beta = -0.01$	1.1 (0.35– 3.2)	1.2 (0.43– 3.6)	Reference

Model 1: Age, male, systolic blood pressure, diabetes, creatinine clearance and coronary artery disease.

Model 2: Model 1 + HDL-cholesterol levels (only 253 patients had available HDL-cholesterol).

*
p<0.05**Abbreviations:** AryIE: Arylesterase. HR: hazard ratio. CI: confidence interval.