ORIGINAL RESEARCH

Impaired High-Density Lipoprotein Anti-Oxidative Function Is Associated With Outcome in Patients With Chronic Heart Failure

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***Background*—**Oxidative stress is mechanistically linked to the pathogenesis of chronic heart failure (CHF). Antioxidative functions of high-density lipoprotein (HDL) particles have been found impaired in patients with ischemic cardiomyopathy; however, the impact of antioxidative HDL capacities on clinical outcome in CHF patients is unknown. We therefore investigated the predictive value of antioxidative HDL function on mortality in a representative cohort of patients with CHF.

***Methods and Results*—**We prospectively enrolled 320 consecutive patients admitted to our outpatient department for heart failure and determined antioxidative HDL function using the HDL oxidative index (HOI). During a median follow-up time of 2.8 (IQR: 1.8-4.9) years, 88 (27.5%) patients reached the combined cardiovascular endpoint defined as the combination of death due to cardiovascular events and heart transplantation. An HOI ≥1 was significantly associated with survival free of cardiovascular events in Cox regression analysis with a hazard ratio (HR) of 2.28 (95% CI 1.48-3.51, $P<0.001$). This association remained significant after comprehensive multivariable adjustment for potential confounders with an adjusted HR of 1.83 (95% CI 1.1-2.92, $P=0.012$). Determination of HOI significantly enhanced risk prediction beyond that achievable with N-terminal pro-B-type natriuretic peptide indicated by improvements in net reclassification index (32.4%, $P=0.009$) and integrated discrimination improvement (1.4%, $P=0.04$).

***Conclusions*—**Impaired antioxidative HDL function represents a strong and independent predictor of mortality in patients with CHF. Implementation of HOI leads to a substantial improvement of risk prediction in patients with CHF. **(*J Am Heart Assoc.* 2016;5:e004169 doi: 10.1161/JAHA.116.004169)**

Key Words: heart failure • high-density lipoprotein cholesterol • oxidative stress • prognosis

Although modern treatment approaches lead to marked improvements in outcome of patients with chronic heart failure (CHF) during the last decades, this group of patients is still affected by high mortality rates.[1] Consequently, strategies to identify patients at very high risk for cardiovascular events may help to intensify treatment strategies tailored to the need of the individual patient. In addition, exploration of novel markers predictive of mortality and cardiovascular events helps to improve the knowledge of underlying pathophysiologic mechanisms. Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) production and antioxidative defenses, is known to be a key mediator in the pathogenesis of CHF.[2,3] Potential sources of increased ROS generation in the failing heart are the mitochondrial electron transport chain (ETC), NADPH oxidases, nitric oxide synthases, and xanthine oxidase.[4-6] Excessive release of ROS leads to left ventricular remodeling and dysfunction by cellular damage through a number of pathways including direct damage to proteins, membranes, DNA, and RNA or indirect damage through the activation of proinflammatory and proapoptotic pathways.[7]

Besides promoting cellular cholesterol efflux and reverse cholesterol transport, high-density lipoprotein (HDL) exerts versatile protective functions on the cardiovascular system including antioxidative activity by binding and removing oxidative molecules.[8] Thus, HDL is believed to play an important role in diminishing elevated oxidative stress levels. However, it has become evident that protective properties of HDL become functionally impaired under certain conditions such as inflammation and tissue injury, and oxidatively modified HDL may even promote pro-oxidative processes.[9-15] A previously conducted case-control study published by Patel et al showed an inverse correlation between the prevalence of CHF and the

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Accompanying Tables S1 through S4 and Figure S1 are available at http://jaha.ahajournals.org/content/5/12/e004169/DC1/embed/inline-supplementary-material-1.pdf

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ability of HDL to promote reverse cholesterol transport or to prevent lipid oxidation.[16] In addition, paraoxonase-1 (PON-1) activity, which represents an HDL-bound key mediator to prevent oxidative modifications, has previously been linked to outcome in CHF.[17] In the light of these findings, and considering the crucial role of oxidative processes implicated in the pathophysiology of CHF, it is tempting to speculate that HDL functionality not only is significantly impaired but may also be associated with outcome in this highly vulnerable population. Based on the aforementioned background, we aimed to determine the prognostic value of antioxidant capacity of HDL in patients with CHF.

Methods

Study Sample

Patients enrolled in this prospective observational study were recruited at the outpatient department for heart failure at the Medical University of Vienna between January 2008 and July 2013. Inclusion criteria were defined as New York Heart Association functional classification (NYHA) ≥2 and the presence of either an N-terminal pro-B-type natriuretic peptide level ≥500 pg/mL or an echocardiographic left ventricular ejection fraction (LVEF) <40% at time of enrollment. LVEF was assessed using biplane Simpsons method. Exclusion criteria were defined as age <18 years, presence of a severe life-threatening condition other than CHF (eg, malignancies), chronic inflammatory diseases, and refusal of informed consent. Treatment and diagnosis were in accordance with current heart failure guidelines.[18] Baseline demographics and clinical history were assessed by trained researchers using a standardized questionnaire. All patients gave written informed consent. The study complies with the Declaration of Helsinki and was approved by the ethics committee of the Medical University of Vienna.

Follow-Up and Study Endpoints

A combination of death due to cardiovascular events and heart transplantation (HTX) was chosen as primary study endpoint. Mortality was assessed browsing local community registries (Statistik Austria), and death certificates were reviewed and categorized into cardiovascular and noncardiovascular causes of death. HTX was assessed during follow-up visits at the outpatient department.

Measurement of Laboratory Parameters and High-Density Lipoprotein Oxidative Index

Fasting venous samples were drawn on the day of study enrollment. Routine laboratory measurements were assessed according to local laboratory procedures on fresh samples. Samples were stored at −80°C and used for further analyses immediately after thawing. HDL antioxidative capacity, the ability of HDL to inactivate previously oxidized LDL, was measured using a well-validated cell-free assay comparable to the cell-based assays as previously described.[13,14,16,19-22] The assay is based on the conversion of 2′,7′-dichlorodihydrofluorescein diacetate (DCF) into its fluorescent form in the presence of oxidized LDL. In accordance with prior study we used apolipoprotein (apo)-B-depleted patient serum, which includes HDL, apo-A1, apo-A2, and HDL-associated particles and determined the ability to attenuate or aggravate previously oxidized low-density lipoprotein cholesterol (LDL).[20] Briefly, purified LDL (Merck, Millipore, Darmstadt, Germany) was diluted in PBS to a final cholesterol concentration of 100 μg/mL prior to a 6-hour oxidation in 100 μmol/L $CuSO_4$ (Merck) at 37°C. Oxidized LDL- (final concentration 1.4 μg/mL) and HDL-containing supernatant (15 μL) and DCF-DA (final concentration 2.9 μg/mL) were added to separate wells of 96-well black microplates with clear bottoms (Corning, Amsterdam, The Netherlands) and incubated at 37°C with PBS to a final volume of 175 μL for 1 hour. The fluorescence signal was measured at an excitation of 485 nm and emission wavelength of 530 nm using a Synergy H1 Hybrid Microplate Reader (Biotek, Winooski, VT). All patient samples were run in duplicate, and mean fluorescence was recorded. The high-density oxidative index (HOI) was determined by calculating the ratio of fluorescence in the presence of apo-B-depleted patient samples divided by the fluorescence of oxidized LDL alone and log-transformed before analysis. A HOI <1.0 stands for antioxidative HDL function, whereas a HOI ≥1.0 indicates pro-oxidative HDL.[12,13,21,22] This definition is based on the functional properties of HDL and describes whether the respective apo-B-depleted patient sample attenuates or aggravates experimental oxidative stress. To correct for interassay variability across different plates, fluorescence value was normalized by the fluorescence of a pooled serum control from 3 healthy volunteers added on each plate. Interassay coefficient of variation (CV) was 7.8%, whereas intra-assay CV was 5.9%.

PON-1 activity was determined using a highly sensitive fluorometric assay (EnzChek® Paraoxonase Assay Kit, Invitrogen, Carlsbad, CA). NT-proBNP was analyzed on an Elecsys 2010 NT-proBNP ELISA (Roche Diagnostics, Mannheim, Germany). Plasma concentrations of interleukin-6 (IL-6) were measured using a human Quantikine high-sensitivity ELISA (R&D Systems, Minneapolis, MN).

Statistical Analysis

The Kolmogorov-Smirnov test was applied to test for normal distribution of data. Categorical data are presented as counts

and percentages, continuous data as median and interquartile range (IQR). Comparison between groups was performed using Mann-Whitney U test and chi-squared test. The Spearman rho correlation coefficient was used to assess associations between baseline variables. Receiver operating characteristic (ROC) curves and the Fisher exact test were applied to estimate the optimal risk cutoff of the HOI. Cox proportional hazard regression models were used to estimate the effect of variables on outcome. Noncardiovascular events were specified as censored data when analyzing cardiovascular death and HTX. Continuous variables were log-transformed before being entered into the model. Results were reported as the hazard ratio (HR) for categorical variables and as HR per 1 increase of a log-transformed standard deviation (HR per 1 SD) for continuous variables. In the multivariable model HOI was adjusted for established CHF risk predictors and potential confounders associated with the HOI, including age, sex, NYHA class, NT-proBNP, LVEF (continuous), estimated glomerular filtration rate, body mass index, HDL-cholesterol, CRP, IL-6, PON-1 activity, diabetes mellitus, and atrial fibrillation. To account for competing risk of endpoints, data were analyzed for cause-specific and subdistribution hazard models as recommended by Austin et al.[23] In addition, cumulative incidence functions for the cardiovascular endpoint and noncardiac death are shown in Figure S1. Kaplan-Meier curves were constructed to estimate event rates according to an HOI above or below 1. The Harrell C-statistic was applied to evaluate the discriminatory power to predict cardiovascular events, and models were compared with the likelihood-ratio test. An improvement in individual risk stratification was analyzed using net reclassification improvement (NRI) and integrated discrimination increment (IDI).[24] Risk categories for the categorical NRI were set as 0% to 30% (low), 30% to 60% (middle), and >60% (high).[25] A *P*-value of <0.05 (2-tailed) was considered statistically significant. All statistical analyses were performed using SPSS 20.0 (IBM SPSS, Armonk, NY), STATA 12 (StataCorp, College Station, TX), and R (R Development Core Team, Vienna, Austria) including the PredictABLE package.[26]

Results

Baseline Characteristics

The final study cohort comprised 320 patients with a diagnosis of CHF consisting of 147 (46%) patients with ischemic etiology and 173 (54%) patients with nonischemic etiology of CHF. Detailed baseline characteristics according to patients with an HOI above or below 1 are shown in Table 1. In brief, patients had a median age of 65 years (IQR 57-71 years), 260 (81%) were male, 145 (45.3%) were in NYHA class III/IV, and patients had a median NT-proBNP level of 1214 (IQR 477-2654) pg/mL.

With respect to antioxidative capacity of HDL, we found that 223 (69.7%) patients had a preserved antioxidative function with an HOI <1, and 97 (30.3%) patients presented pro-oxidative HDL serum measurements with an HOI ≥1. The median PON-1 activity was 0.032 U/μL (IQR 0.027-0.308 U/μL). Patients with an HOI ≥1 had significantly higher levels of C-reactive protein (CRP; 0.47 mg/L [IQR 0.22-0.73 mg/L] vs 0.27 mg/L [IQR 0.13-0.62 mg/L], *P*=0.003), HbA1c (6.2% [IQR 5.7% to 7.1%] vs 6.0% [5.6% to 6.7%], *P*=0.035), NT-proBNP (1393 pg/mL [IQR 663-3392 pg/mL] vs 1095 pg/mL [IQR 403-2229 pg/mL], *P*=0.025) and were more often in NYHA class III/IV (55% vs 41%, *P*=0.025). Furthermore, patients with an HOI ≥1 showed a significantly lower PON-1 activity (0.029 U/μL [0.025-0.036 U/μL] vs 0.033 U/μL [0.028-0.04 U/μL], *P*<0.001). With respect to inflammatory markers, HOI showed weak significant correlations with IL-6 (r=0.16, *P*=0.01) and CRP (r=0.248, *P*<0.001). No differences in HOI (*P*=0.73) or PON-1 activity (*P*=0.409) were found between patients with ischemic and nonischemic etiology of CHF. There was no difference in serum HDL-cholesterol levels between patients with an HOI ≥1 or an HOI <1. However, PON-1 activity showed a weak but significant correlation with HDL-cholesterol levels (r=0.177, *P*=0.001).

Survival Analysis

The median follow-up period was 2.8 years (95% CI 1.8-4.9 years), corresponding to a total of 1096 patient years. During this observation time, 88 (27.5%) patients reached the combined cardiovascular endpoint, and 116 (36.3%) patients died from all causes. The optimal risk cutoff of the HOI using ROC analysis and Fisher exact test was 0.995 with a sensitivity of 42.5% and a specificity of 73.9%. An HOI ≥1 had a significant association with survival free of cardiovascular mortality and HTX of patients in unadjusted Cox regression analysis with a HR of 2.28 (95% CI 1.48-3.51, *P*<0.001; Table 2). Corresponding Kaplan-Meier curves stratified by an HOI ≥1 or <1 are shown in Figure 1. The association of HOI ≥1 with cardiovascular events remained significant after multivariable adjustment for age, sex, NYHA class, NT-proBNP, LVEF, estimated glomerular filtration rate, body mass index, HDL-cholesterol, CRP, IL-6, PON-1 activity, diabetes mellitus, and atrial fibrillation with an adjusted HR of 1.83 (95% CI 1.14-2.92, *P*=0.012; Table 2). Analysis for subdistribution and cause-specific hazard models showed a significant association of an HOI ≥1 with the cardiovascular endpoint but not with noncardiac death in multivariable analysis (Table S1). No difference in the predictive value of an HOI ≥1 was found between ischemic and nonischemic etiology of CHF (*P* for interaction 0.60). However, we

Table 1. Baseline Characteristics

	All Patients	HOI <1	HOI ≥1	
N (%)	320 (100)	223 (69.7)	97 (30.3)	*P* Value
Clinical characteristics				
Age, median years (IQR)	65 (57-71)	65 (55-70)	65 (59-73)	0.09
Male sex, n (%)	260 (81)	176 (79)	84 (87)	0.11
Body mass index, kg/m^2 (IQR)	28 (25-31)	28 (25-31)	28 (26-32)	0.29
Ischemic CHF, n (%)	147 (46)	101 (45)	46 (47)	0.73
NYHA III/IV, n (%)	145 (45)	92 (41)	53 (55)	0.027*
LVEF				
Mild, n (%)	67 (21)	50 (22)	17 (18)	0.34
Moderate, n (%)	124 (39)	80 (36)	44 (45)	0.10
Severe, n (%)	91 (28)	64 (29)	27 (28)	0.90
SBP, mm Hg (IQR)	125 (113-142)	125 (114-144)	123 (110-138)	0.25
Heart rate, bpm (IQR)	70 (62-79)	70 (63-79)	69 (62-80)	0.97
Active smoker, n (%)	69 (22)	52 (23)	17 (18)	0.51
Comorbidities				
Previous MI, n (%)	133 (42)	95 (43)	38 (39)	0.57
Atrial fibrillation, n (%)	137 (43)	87 (39)	50 (52)	0.037*
Hypertension, n (%)	247 (77)	173 (78)	74 (76)	0.80
Diabetes mellitus, n (%)	119 (37)	77 (35)	42 (43)	0.14
COPD, n (%)	80 (25)	51 (23)	29 (30)	0.18
Laboratory parameters				
NT-proBNP, pg/mL (IQR)	1214 (477-2654)	1095 (403-2229)	1393 (663-3392)	0.025*
eGFR (MDRD), mL/min (IQR)	61.4 (46.7-78)	65.9 (47.5-80)	58.30 (42.9-74.6)	0.33
Cholesterol, mg/dL (IQR)	178 (149-210)	181 (152-210)	172 (143-207)	0.16
LDL-cholesterol, mg/dL (IQR)	99.5 (78.7-126.6)	100.6 (81.8-125.5)	94.40 (76.9-127)	0.28
HDL-cholesterol, mg/dL (IQR)	43 (36-52)	43 (37-52)	41 (35-52)	0.08
Triglycerides, mg/dL (IQR)	154 (99-213)	159 (103-218)	134 (94-197)	0.08
HbA1c, % (IQR)	6.0 (5.6-6.8)	6.0 (5.6-6.7)	6.2 (5.7-7.1)	0.035*
Erythrocytes, 10^{12}/L (IQR)	4.6 (4.2-5.0)	4.6 (4.2-4.9)	4.7 (4.3-5.1)	0.05
Leukocytes, 10^9/L (IQR)	7.17 (6.07-8.57)	7.07 (6.02-8.45)	7.25 (6.21-8.65)	0.47
CRP, mg/L (IQR)	0.34 (0.14-0.67)	0.27 (0.13-0.62)	0.47 (0.22-0.73)	0.003*
Interleukin-6, pg/mL (IQR)	1.21 (0.61-2.69)	1.12 (0.59-2.51)	1.45 (0.64-3.70)	0.041*
PON-1 activity, U/μL (IQR)	0.032 (0.027-0.038)	0.033 (0.028-0.04)	0.029 (0.025-0.036)	<0.001*
Cardiac medication				
β-Blockers, n (%)	311 (97)	217 (97)	94 (97)	0.84
ACE-inhibitors/ARBs, n (%)	100 (100)	100 (100)	100 (100)	0.89
Aldosterone antagonist, n (%)	209 (65)	144 (65)	65 (67)	0.67
Diuretics, n (%)	165 (52)	110 (49)	55 (57)	0.23
Statins, n (%)	191 (60)	130 (58)	61 (63)	0.44

Baseline characteristics according to patients stratified by a high-density lipoprotein oxidative index below or above 1. Comparisons between groups were performed using chi-squared test and Mann-Whitney U test as appropriate. Continuous data are presented as median and interquartile range, categorical data as counts and percentages. ACE indicates angiotensin-converting enzyme; ADP, adenosine diphosphate receptor; ARB, angiotensin receptor blockers; bpm, beats per minute; CHF, chronic heart failure; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HOI, high-density lipoprotein oxidative index; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; MI, myocardial infarction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association functional classification; SBP, systolic blood pressure.

*P<0.05.

Table 2. Survival Analysis

	Unadjusted		Multivariable*	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Cardiovascular mortality and HTX				
HDL oxidative index ≥1	2.28 (1.48-3.51)	<0.001†	1.83 (1.14-2.92)	0.012†
Paraoxonase activity‡	0.72 (0.57-0.9)	0.004†	0.95 (0.37-1.21)	0.68
All-cause mortality				
HDL oxidative index ≥1	1.88 (1.30-2.72)	0.001†	1.454 (0.96-2.17)	0.08
Paraoxonase activity‡	0.72 (0.60-0.88)	0.001†	0.88 (0.33-1.1)	0.27

Unadjusted and multivariable Cox proportional hazard models were used to estimate the effect of high-density lipoprotein oxidative index on survival of patients. CI indicates confidence interval; HDL, high-density lipoprotein; HR, hazard ratio; HTX, heart transplantation.

*Adjusted for age, sex, New York Heart Association functional classification, N-terminal pro-B-type natriuretic peptide, left ventricular ejection fraction, estimated glomerular filtration rate, body mass index, high-density lipoprotein, C-reactive protein (CRP), interleukin-6 (IL-6), paraoxonase activity, type 2 diabetes mellitus, and atrial fibrillation.

†$P<0.05$.

‡Results for paraoxonase activity are represented as hazard ratio per increase of 1 standard deviation.

observed a significant interaction between IL-6 and an HOI ≥1 versus <1 (P=0.044). Stratification into tertiles of IL-6 revealed that the HOI ≥1 versus <1 had increasing HRs with higher levels of IL-6 and a significant association with the cardiovascular endpoint in the highest IL-6 tertile (≥2.09 pg/mL) with a crude HR of 2.91 (95% CI 1.62-5.24, P<0.001; Table S2). We further analyzed the prognostic value of PON-1 activity in our cohort. PON-1 activity showed a significant inverse association with the cardiovascular endpoint with a HR per 1 SD of 0.72 (95% CI 0.57-0.9, P=0.004; Table 2) in an unadjusted Cox regression, but this association turned nonsignificant after multivariable adjustment with an adjusted HR per 1 SD of 0.95 (95% CI 0.37-1.21, P=0.68; Table 2).

HOI and Risk Prediction

In a next step we aimed to determine whether measurement of the HOI has advantages over conventional risk-prediction strategies. First, we stratified patients into 4 groups according to combined strata of HOI above and below 1 and NT-proBNP above and below 2065 pg/mL, which was the optimal cutoff to predict cardiovascular events calculated with CART analysis. Patients with an HOI ≥1 and NT-proBNP >2065 pg/mL had a HR of 6.8 (95% CI: 2.9-16.1, P<0.001) for the combined cardiovascular endpoint compared to patients with an HOI <1 and NT-proBNP <2065 pg/mL. The predictive value of HOI ≥1 was independent of the NT-proBNP category (P for interaction 0.282, Figure 2). Second, the additional prognostic value beyond that assessable with NT-proBNP was confirmed by significant improvements in C-statistic (AUC 0.730 [95% CI 675-0.786] vs 0.742 [95% CI 0.686-0.798], P=0.001), category-free NRI (32.4% [95% CI 19.9% to 44.9%]), categorical NRI (12.8% [95% CI 3.3% to 22.4%], P=0.009) and IDI (1.4% [95% CI 0.7% to 2.1%], P=0.04; Table 3 and Table S3). Third, an HOI ≥1 provided additional prognostic information even over a comprehensive multivariable model comprising all parameters used in multivariable Cox regression analysis (see above) indicated by significant improvements in C-statistic (AUC 0.763 [95% CI 0.713-0.813] vs 0.768 [95% CI 0.717-0.818], P=0.01), and category-free NRI of 30.4% (95% CI 17.8% to 43.1%; P=0.01; Table 3 and Table S4).

Discussion

The current study demonstrates a strong association between impaired antioxidative capacity of serum HDL and clinical outcome in heart failure patients. Survival free of the combined cardiovascular endpoint of cardiovascular mortality and HTX was significantly lower in patients with pro-oxidative HDL serum measurements reflected by a HOI ≥1 and remained significant after adjustment for potential confounders. Furthermore, determination of the HOI led to considerable improvements of conventional risk prediction in CHF patients.

Inverse correlations between HDL-cholesterol levels and the risk for development of cardiovascular diseases are well investigated.[27] However, HDL-cholesterol-raising therapies failed to improve outcome in patients with coronary artery disease: a large percentage of individuals develop cardiovascular diseases despite normal or even high HDL-cholesterol levels, and the prognostic validity of HDL in patients with already existing cardiovascular diseases seems to be diminished.[28-31] This supposed failure of the HDL hypothesis encouraged more in-depth investigation of underlying mechanisms, and recent studies suggest that not merely quantity but rather functionality of HDL may be more relevant for the protection against cardiovascular disease.[10,12,32] Accordingly,

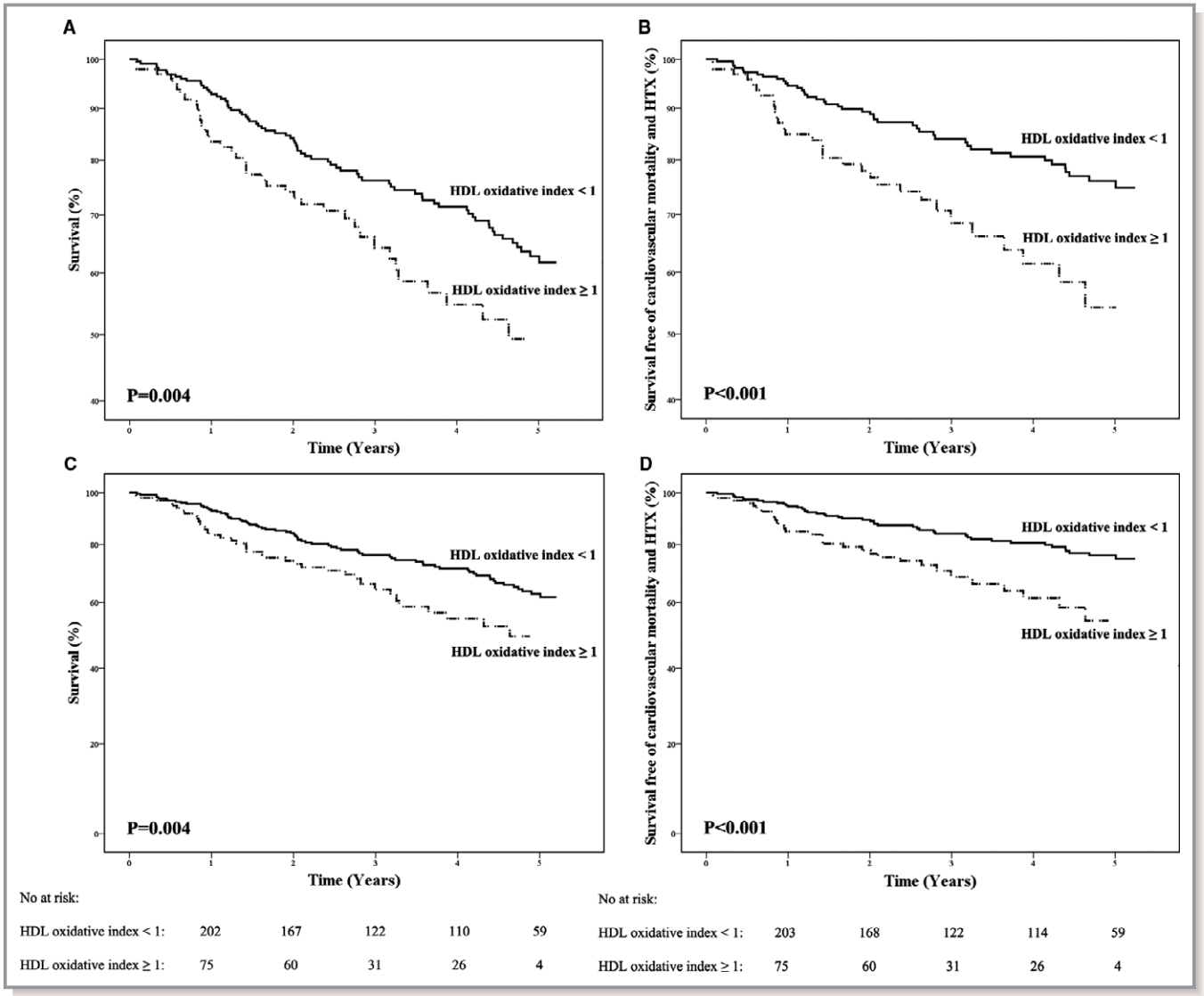

Figure 1. Kaplan-Meier plots showing the crude cumulative survival free from all-cause mortality (A and B) and cardiovascular mortality (C and D) according to the high-density lipoprotein (HDL) oxidative index above or below 1.

we observed that an impairment of antioxidative function of HDL determined by an HOI ≥1 was independent of HDL-cholesterol serum levels in our cohort and was a significant predictor of cardiovascular events even though HDL itself was not associated with outcome in these patients.

So far, antioxidative properties of HDL have been found impaired in common CHF-related comorbidities such as chronic kidney disease and type 2 diabetes mellitus, and determination of the HOI turned out to be a useful predictor of cardiovascular events and mortality in patients with acute coronary syndrome.[13,15,33,34] With respect to CHF, a previously conducted case-control study has reported reduced HDL antioxidative capacity in patients with ischemic CHF.[16] The present study extends this initial observation, showing that an impaired HDL antioxidative capacity is associated with higher mortality in CHF patients independent of traditional cardiovascular risk factors and irrespective of the underlying etiology.

Besides promoting reverse cholesterol transport, HDL particles have been shown to possess antioxidative, anti-inflammatory, antithrombotic, and antiapoptotic properties.[35] In light of the pivotal role of oxidative stress and inflammation in the development and progression of CHF, the assumption that an impaired antioxidative capacity of HDL may affect clinical outcome in patients with CHF seems likely.[2,3] The pathophysiological mechanisms for the loss of antioxidative function of HDL have not been fully elucidated yet, but there is increasing evidence that alterations in HDL protein composition and protein quality are crucially involved.[36,37] Conditions of infection, inflammation, or tissue injury are

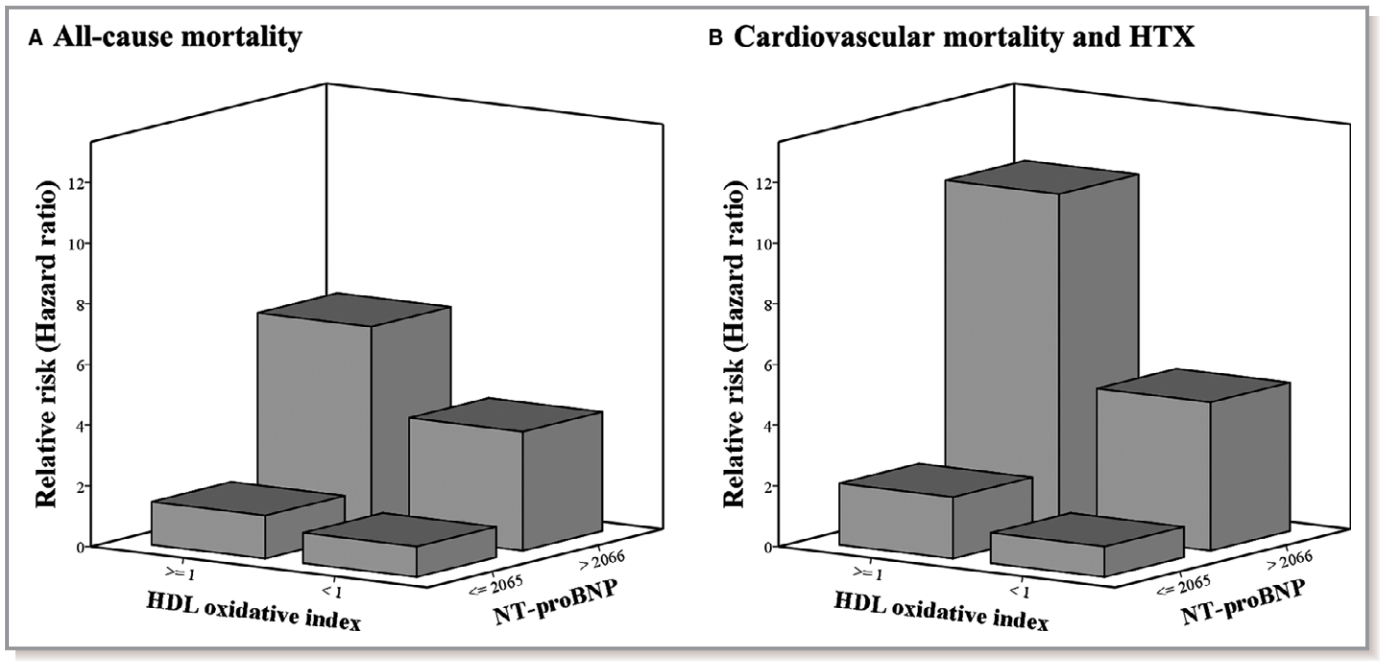

Figure 2. Relative risk of mortality (A) and the combined cardiovascular endpoint (B) according to combined strata of the high-density lipoprotein oxidative index (HOI) and N-terminal pro-B-type (NT-proBNP) natriuretic peptide. HTX indicates heart transplantation.

thought to trigger structural alterations of HDL particles.[38] Accordingly, the association of an HOI ≥1 with cardiovascular events was more pronounced in patients with high levels of the proinflammatory cytokine IL-6. These results further support the assumption of inflammatory processes being crucially involved in impairment of the antioxidative capacity of HDL. Interestingly, HOI ≥1 was still associated with cardiovascular outcome after multivariable adjustment for the inflammatory biomarkers CRP and IL-6. As a result, the effects represented by the HOI as a global measure of oxidative capacity seem to be beyond the inflammatory pathways indicated by the measured biomarkers. Oxidative stress itself is considered to render HDL dysfunctional by provoking posttranslational modifications of HDL-associated proteins.[37,38] Myeloperoxidase (MPO), which is mainly secreted by activated neutrophils, has been demonstrated to mediate these structural and functional alterations of HDL.[39] Furthermore, the presence of serum amyloid A, a protein family of major acute-phase reactants on HDL, has been reported to impair HDL's antioxidative functionality.[40] In contrast, HDL loading with the protein sphingosine-1-phosphate (S1P), has recently been demonstrated to correct for HDL dysfunction.[41] As an important finding of our study, no differences in the predictive value were observed between patients with ischemic and nonischemic etiology of CHF. This suggests the assumption that the link between impaired HDL antioxidative capacity and poor outcome in CHF cannot be solely attributed to atherosclerosis and coronary vascular events, as shown by previous studies, although these may be important confounders.[9,12] In this respect, several lines of evidence indicate a direct involvement of oxidative stress and excess ROS production in left ventricular dysfunction and cardiac remodeling by inducing rather unspecific damage on cellular membranes, proteins, and DNA.[2] These alterations result in multifaceted maladaptive changes including extracellular matrix remodeling, hypertrophy of cardiomyocytes, and impaired excitation-contraction coupling.[7] It is therefore tempting to speculate that a preserved antioxidative function of HDL can prevent the various maladaptive responses to excessive ROS production involved in left ventricular remodeling.

HDL particles are composed of lipids and more than 100 different proteins and exert their protective functions by the collaboration of essential apolipoproteins and associated enzymes.[38] The HDL-binding glycoprotein PON-1 is shown to be critical for the antioxidative capacity of HDL.[42] A previously published association of low PON-1 activity and poor long-term prognosis in patients with CHF was visible in our cohort in unadjusted Cox regression analysis but turned nonsignificant after adjustment for potential confounders including implementation of HOI in our multivariable model.[17] Although we saw a significant association between PON-1 activity and the measured antioxidative capacity in the unadjusted model, this relationship was no longer observed after multivariable adjustment. PON-1 is thought to be mainly responsible for preventing oxidative modifications, but HOI appeared to be a more robust risk predictor, sustaining adjustment for NT-proBNP and other potential confounders. The stronger

Table 3. Metrics of Discrimination and Reclassification

Harrel C-Statistic	AUC	Δ-AUC	*P* Value
HOI	0.596 (0.542-0.649)		
NT-proBNP	0.730 (0.675-0.786)		
HOI in addition to NT-proBNP	0.742 (0.686-0.798)	0.012	For comparison: 0.001*
Multivariable model†	0.763 (0.713-0.813)		
HOI in addition to multivariable model†	0.768 (0.717-0.818)	0.005	For comparison: 0.02*
Net reclassification index	Improvement (95% CI)		
HOI in addition to NT-proBNP	32.4% (19.9-44.9%)		0.009*
HOI in addition to multivariable model†	30.4% (17.8-43.1%)		0.016*
Categorical NRI‡	Improvement (95% CI)		
HOI in addition to NT-proBNP	12.8% (3.3-22.4%)		0.009*
HOI in addition to multivariable model†	0.3% (−7.9% to 8.5%)		0.95
Integrated discrimination increment	Improvement (95% CI)		
HOI in addition to NT-proBNP	1.4% (0.7-2.1%)		0.04*
HOI in addition to multivariable model†	0.7% (0.2-1.2%)		0.16

An improvement in individual risk stratification beyond that assessable with N-terminal pro-B-type natriuretic peptide and the multivariable model was calculated using Harrel C-statistic, category-free and categorical net reclassification index, and integrated discrimination improvement. AUC indicates area under the curve; CI, confidence interval; HOI, high-density lipoprotein oxidative index; NRI, net reclassification index; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

*$P<0.05$.

†The multivariable model includes age, sex, New York Heart Association functional classification, N-terminal pro-B-type natriuretic peptide, left ventricular ejection fraction, estimated glomerular filtration rate, body mass index, high-density lipoprotein, C-reactive protein, paraoxonase activity, diabetes mellitus, and atrial fibrillation.

‡Full reclassification tables according to risk categories 0% to 30% (low), 30% to 60% (middle), and >60% (high) are shown in Tables S3 and S4.

performance of HOI to prevent or enhance the formation and inhibit or aggravate the biologic activity of oxidized LDL could be due to additional components attached to HDL. These enzymes include apolipoprotein A-I, apolipoprotein J, platelet-activating factor acetylhydrolase (PAF-AH), LCAT, and glutathione (GSH) selenoperoxidase.[43,44] Thus, HDL particles contain at least 2 apolipoproteins and 4 enzymes that protect LDL from oxidation, demonstrating the complexity of the antioxidative capacity. Determination of the global antioxidative function using HOI may therefore represent a reliable surrogate of the multifaceted alterations in HDL structure and function in response to oxidative stress.

Strategies to improve the antioxidative function of HDL may be beneficial for patients with CHF. In this respect, exercise training has been shown to significantly improve HDL functionality.[45] Notably, exercise training represents a proven intervention strategy in patients with CHF, leading to enhanced survival rates and quality of life.[46] The exercise-mediated effect on HDL function is still not elucidated, but it is likely that exercise training positively affects the antioxidative capacity because an exercise-induced reduction of lipid peroxidation has been observed, which is a general measure of oxidative load.[45] One may therefore speculate that strategies to improve the antioxidative capacity of HDL represent a potential treatment target for future therapies in patients with CHF.

Limitations

A potential limitation of this study is that antioxidative capacity of HDL was determined in apolipoprotein B–depleted serum samples instead of isolated HDL. Although polyethylene glycol precipitation is generally accepted as a reproducible and rapid method to extract HDL from patient serum, it remains unclear if antioxidant effects are influenced by other non-HDL-associated proteins, which might interact with the applied assay.[12,16] However, prior studies showed strong correlations of antioxidant results from HDL samples isolated by different techniques.[47,48] Therefore, the use of apolipoprotein B–depleted serum samples is applicable as long as this method is consistently applied for all samples.[48] Naturally, measuring the antioxidative capacity of HDL using an in vitro cell-free assay may be limited by the necessity of an artificial environment. However, the assay harbors the advantage of being applicable to large patient cohorts, with potential clinical importance given by the robust association with cardiovascular outcome.

Accurate statistical evaluation of novel biomarkers comprises estimation of additional predictive performance in comparison to established risk-prediction strategies.[24] However, the generally recommended indices of reclassification are subjected to criticisms regarding their statistical properties, as their values are difficult to interpret. For instance, the

category-free NRI summarizes broad changes in risk models, which may incorporate irrelevant information and overestimate the added usefulness of a marker, whereas the categorical NRI strongly depends on clinically meaningful risk categories.[49] However, with a full disclosure of the reclassification risk (Tables S3 and S4), a careful selection of risk categories as recommended by Leening et al,[25] and consistent improvements of the HOI in C-statistic, NRI and IDI may overcome statistical concerns.

Endothelial dysfunction plays an important role in the pathophysiology and progression of CHF. Because HDL is capable of ameliorating endothelial dysfunction by enhancing endothelial nitric oxide synthesis, this may be a possible link between impaired HDL function and adverse outcome in our patient population.[50] However, we did not perform examinations such as flow-mediated dilation of the brachial artery to investigate a potential association between the HOI and endothelial dysfunction.

Conclusion

The identification of impaired antioxidative HDL function as a risk prediction marker for poor clinical outcome beyond the known risk factors complements the knowledge on risk stratification in CHF. This suggests that antioxidant properties of HDL rather than total HDL serum levels may be considered for an improved risk assessment. Considering the robust effect of impaired antioxidant HDL function on clinical outcome in CHF, this high-risk patient population might benefit from a specific treatment to restore and maintain antioxidant HDL capacity.

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Disclosures

None.

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Supplemental Material

Table S1. Analysis for subdistribution and cause-specific hazard models for the cardiovascular endpoint and noncardiac death.

	Subdistribution Hazard Model				**Cause-specific Hazard Model**			
Variable	Cardiovascular mortality + HTX		Noncardiac Death		Cardiovascular mortality + HTX		Noncardiac Death	
	Adj. HR (95% CI)	P-Value	Adj. HR (95% CI)	P-Value	Adj. HR (95% CI)	P-Value	Adj. HR (95% CI)	P-Value
HOI ≥1	1.79 (1.14 - 2.82)	**0.012**	0.54 (0.21 - 1.39)	0.20	1.73 (1.07 - 2.78)	**0.024**	0.78 (0.35 - 1.78)	0.56

Table S2. Subgroup analysis of the predictive value of a high-density lipoprotein oxidative index ≥1 stratified by tertiles of Interleukin-6 in Cox regression hazard analysis.

Cardiovascular mortality and HTX	**HOI ≥1**	
Interleukin-6 tertiles	HR (95% CI)	P-Value
1st tertile (≤0.78 pg/ml)	1.45 (0.53 - 3.95)	0.474
2nd tertile (0.79 - 2.08 pg/ml)	2.08 (0.84 - 5.12)	0.111
3rd tertile (≥2.09 pg/ml)	2.91 (1.62 - 5.24)	**<0.001**

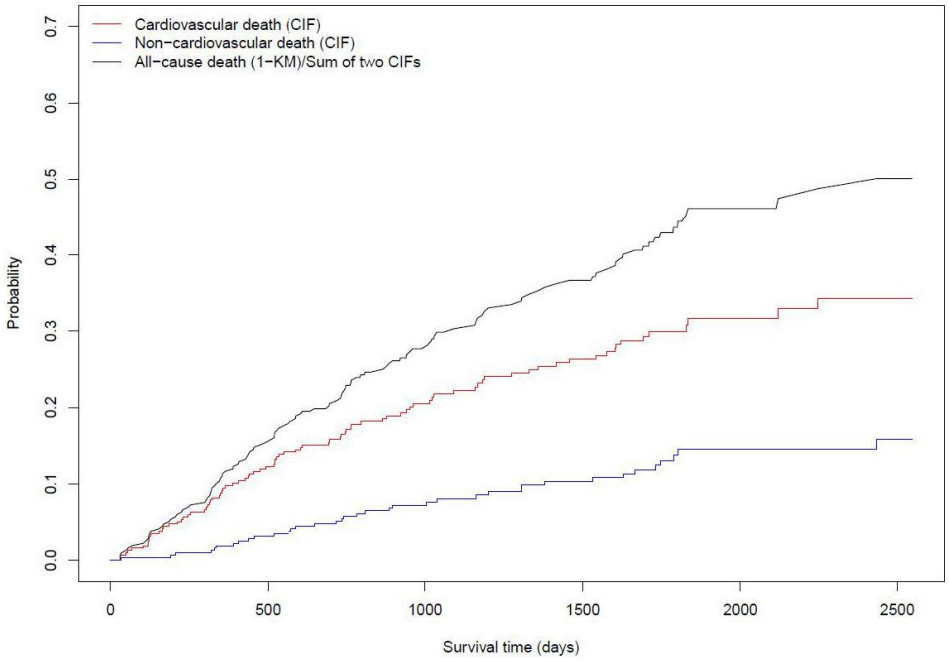
Table S3. Categorical Net reclassification improvement (HOI in addition to NT-proBNP)

Categorical NRI				Increased risk	Decreased risk	Net reclassified
Events (N=88)	Updated model					
Initial model	0-30%	30-60%	>60%			
0-30%	28	6	0	6	0	
30-60%	1	32	6	6	1	
>60%	0	2	13	0	2	
Total				12 (13.6%)	3 (3.4%)	9 (10.2%)
No events (N=232)	Updated model					
Initial model	0-30%	30-60%	>60%			
0-30%	156	11	0	11	0	
30-0%	17	46	1	1	17	
>60%	0	1	4	0	1	
Total				12 (5.2%)	18 (7.8%)	6 (2.6%)
Net reclassification improvement: 12.8% (95% CI: 3.3% - 22.4%), P=0.009						

Table S4. Categorical Net reclassification improvement (HOI in addition to the multivariable model)

Categorical NRI				Increased risk	Decreased risk	Net reclassified
Events (N=88)	Updated model					
Initial model	0-30%	30-60%	>60%			
0-30%	27	2	0	2	0	
30-60%	1	27	4	4	1	
>60%	0	4	23	0	4	
Total				6 (6.8%)	5 (5.7%)	1 (1.1%)
No events (N=232)	Updated model					
Initial model	0-30%	30-60%	>60%			
0-30%	163	7	0	7	0	
30-0%	4	48	2	2	4	
>60%	0	3	3	0	3	
Total				9 (3.8%)	7 (3%)	-2 (-0.8%)
Net reclassification improvement: 0.3% (96% CI: -7.9% - 8.5%), P=0.949						

Figure S1. Cumulative Incidence functions.



Cumulative Incidence functions for the cardiovascular endpoint, non-cardiac death and all-cause death.