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The PCSK9-LDL Receptor Axis and Outcomes in Heart Failure

BIOSTAT-CHF Subanalysis

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ABSTRACT

BACKGROUND Proprotein convertase subtilisin/kexin type 9 (PCSK9) binds low-density lipoprotein receptor (LDLR), preventing its recycling. PCSK9 is a risk predictor and a biotarget in atherosclerosis progression.

OBJECTIVES The aim of this study was to determine whether the PCSK9-LDLR axis could predict risk in patients with heart failure (HF).

METHODS The BIOSTAT-CHF (Biology Study to Tailored Treatment in Chronic Heart Failure) is a multicenter, multinational, prospective, observational study that included patients with worsening HF signs and/or symptoms. The primary endpoints were all-cause mortality and the composite of mortality or unscheduled hospitalizations for HF. We implemented Cox proportional hazard regression to determine the simultaneously adjusted effect of PCSK9 and LDLR on both outcomes when added to the previously validated BIOSTAT-CHF risk scores.

RESULTS This study included 2,174 patients (mean age: 68 ± 12 years; 53.2% had a history of ischemic heart disease). Median (interquartile range) PCSK9 and LDLR levels were 1.81 U/ml (1.45 to 2.18) and 2.98 U/ml (2.45 to 3.53), respectively. During follow-up, 569 deaths (26.2%) and 896 (41.2%) composite endpoints were ascertained. A multivariable analysis, which included BIOSTAT-CHF risk scores, LDLR, and statin treatment as covariates, revealed a positive linear association between PCSK9 levels and the risk of mortality (hazard ratio [HR]: 1.24; 95% confidence interval [CI]: 1.04 to 1.49; $p = 0.020$) and the composite endpoint (HR: 1.21; 95% CI: 1.05 to 1.40; $p = 0.010$). A similar analysis for LDLR revealed a negative association with mortality (HR: 0.86; 95% CI: 0.76 to 0.98; $p = 0.025$) and the composite endpoint (HR: 0.92; 95% CI: 0.83 to 1.01; $p = 0.087$). Including PCSK9 and LDLR improved risk score performance.

CONCLUSIONS The PCSK9-LDLR axis was associated with outcomes in patients with HF. Future studies must assess whether PCSK9 inhibition will result in better outcomes in HF. (J Am Coll Cardiol 2017;70:2128–36)

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Major advances in heart failure (HF) management have been achieved over the past 3 decades by targeting 2 main pathways activated in HF, namely the renin-angiotensin-aldosterone system and the sympathetic nervous system (1–5). Nevertheless, HF remains a syndrome with high morbidity and mortality, poor quality of life, and high health care costs (6).

Targeting alternative pathways that participate in the HF syndrome seem the next logical approach. One such pathway is atherosclerosis progression; however, the administration of statins in HF has led to debatable results. Indeed, the 2 major randomized trials that studied the effect of statin treatment in patients with chronic HF did not demonstrate sufficient evidence of benefit (7,8). However, at least in Controlled Rosuvastatin in Multinational Trial in Heart Failure the secondary endpoint of HF hospitalization was significantly reduced by rosuvastatin (7). Also, some reports on real-life data have shown a positive association between statins and outcomes (9).

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A new biotarget for treating atherosclerosis progression is proprotein convertase subtilisin/kexin type 9 (PCSK9) (10,11). Secreted into the plasma by the liver, PCSK9 binds the low-density lipoprotein (LDL) receptor (LDLR) at the surface of hepatocytes. This binding prevents LDLR recycling and enhances its degradation in endosomes and lysosomes, which results in reduced LDL-cholesterol clearance (12). The recent Glavov (13) and Fourier (14) studies have demonstrated that PCSK9 inhibition with monoclonal antibodies could reduce the atherosclerosis disease burden and cardiovascular events.

The potential of PCSK9 as a biotarget in HF is unknown. Here, we hypothesized that, similar to what was shown in patients with coronary artery disease, elevated levels of PCSK9 in HF are associated with outcomes. Accordingly, we aimed to decipher the value of the PCSK9-LDLR axis for predicting risk in patients with HF in the multicenter BIOSTAT-CHF (Biology Study to Tailored Treatment in Chronic Heart Failure) cohort (15).

METHODS

BIOSTAT-CHF COHORT. BIOSTAT-CHF was a multicenter, multinational, prospective, observational study that included 2,516 patients with worsening signs and/or symptoms of HF from 69 centers in 11 European countries. The recruitment period was 24 months (December 2010 to December 2012) (15). The median follow-up was 21 months (interquartile range [IQR]: 15 to 27 months). The ethics committees of participating institutions approved this study, and all patients provided written consent to participate.

Eligible patients, exclusion criteria, and characteristics of the BIOSTAT-CHF cohort have been described elsewhere (15). In brief, the majority of patients were hospitalized for acute HF; the remainder presented with worsening signs and/or symptoms of HF at outpatient clinics. Approximately one-half of the patients were classified as New York Heart Association functional class III. Blood was drawn within days of the worsening HF event (either in- or outpatient).

All deaths and hospitalizations were recorded. The primary outcomes of interest were the time to all-cause mortality and the time to a composite of death or unscheduled hospitalization for HF.

PCSK9 AND LDL RECEPTOR ASSAYS. PCSK9 and LDLR were measured using the Proseek Multiplex

ABBREVIATIONS AND ACRONYMS

CI = confidence interval
HF = heart failure
HR = hazard ratio
IDI = integrated discrimination improvement
IQR = interquartile range
LDLR = low-density lipoprotein receptor
LVEF = left ventricular ejection fraction
NRI = net reclassification improvement
NT-proBNP = N-terminal pro-B-type natriuretic peptide
PCSK-9 = proprotein convertase subtilisin/kexin type 9

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CVDIII panel (Olink Proteomics AB, Uppsala, Sweden) according to the manufacturer's instructions. The Proximity Extension Assay technology used for the Proseek Multiplex protocol has been well described (16). In brief, pairs of oligonucleotide-labeled antibody probes bind to their targeted protein; if the 2 probes are brought in close proximity, the oligonucleotides will hybridize in a pair-wise manner. The addition of a DNA polymerase leads to a proximity-dependent DNA polymerization event, generating a unique polymerase chain reaction target sequence. The resulting DNA sequence is subsequently detected and quantified using a microfluidic real-time polymerase chain reaction instrument (Biomark HD, Fluidigm). Data are then quality controlled and normalized using an internal extension control and an inter-plate control to adjust for intrarun and interrun variation. The final assay readout is presented in normalized protein expression (NPX) values, which is an arbitrary unit on a log2 scale in which a high value corresponds to a higher protein expression. All assay validation data (e.g., detection limits, intra-assay and interassay precision data) are available on manufacturer's website.

STATISTICAL ANALYSIS. Continuous variables are expressed as mean ± SD or median (IQR) per variable distribution. Discrete variables are presented as percentages. Baseline characteristics among PSCK9 and LDLR quartiles were compared with analysis of variance, Kruskal-Wallis, or chi-square tests, as appropriate.

The bivariate correlation of the 2 exposures was assessed with Spearman rank correlation coefficient. A multivariable linear regression analysis was performed to determine the association of LDLR with PCSK9 while adjusting for age, sex, ischemic heart disease, LDL-cholesterol, HDL-cholesterol, and estimated glomerular filtration rate.

The Cox proportional hazard regression was used to determine the simultaneously adjusted effect of PCSK9 and LDLR on all-cause mortality and on the composite of mortality and HF hospitalization. Each of these models included the use of statins and tertiles of the previously derived BIOSTAT-CHF risk score as covariates (17). Briefly, the BIOSTAT-CHF risk score for each endpoint was calculated as the probability of achieving the endpoint at a 2-year follow-up. The BIOSTAT-CHF risk score for mortality included age, blood urea nitrogen, N-terminal pro-B-type natriuretic peptide (NT-proBNP), serum hemoglobin, and the use of a beta-blocker. The BIOSTAT-CHF risk score for the composite endpoint included age, previous HF-related hospitalization, presence of edema, systolic blood pressure, and the estimated glomerular filtration rate (17). Results are expressed as hazard ratios (HRs) with their respective 95% confidence intervals (CIs). The proportionality assumption, tested by means of the Schoenfeld residuals, was met for all models. In a sensitivity analysis, LDL-cholesterol was added as an additional covariate to the previous prognostic models.

Measures of performance were assessed by means of Δ C-statistics, integrated discrimination improvement (IDI), and net reclassification improvement (NRI) (%). To match the scope of the BIOSTAT-CHF risk score, these indices were calculated at a horizon of 2 years. Two variations of the analysis are presented: 1) PCSK9, LDLR, and the use of statins are compared over the BIOSTAT-CHF risk score alone; and 2) PCSK9 is contrasted against LDLR, use of statins, and the BIOSTAT-CHF risk score (18).

We set a 2-sided p value of <0.05 as the threshold for statistical significance. Stata 14.2 (Stata Statistical Software, Release 14, 2015, StataCorp LP, College Station, Texas), was used for the main analysis. Risk reclassification analyses were implemented in R (version 3.40, R Foundation for Statistical Computing, Vienna, Austria) with the survIDINRI and SurvC1 modules.

RESULTS

A total of 2,174 patients were included in this analysis (Online Figure 1). The mean age of the sample was 68 ± 12 years; 581 (49.7%) were female; 1,156 (53.2%) had history of ischemic heart disease; and 1,727 (88.8%) exhibited left ventricular ejection fraction (LVEF) ≤40% (4.5% had LVEF in the mid-range and 6.7% had preserved LVEF). The median (IQR) values of PCSK9, LDLR, and NT-proBNP were 1.81 U/ml (1.45 to 2.18), 2.98 U/ml (2.45 to 3.53), and 4,148 pg/ml (2,330 to 8,136), respectively. The baseline values of PCSK9 and LDLR across quartiles are shown in **Tables 1 and 2**. Overall, patients in the higher PCSK9 quartiles displayed a higher prevalence of ischemic heart disease and prior coronary revascularization and higher values of creatinine (**Table 1**). In contrast, the disease severity surrogates were inversely related to the LDLR quartiles. Indeed, those in the lower LDLR quartiles were older, more frequently males, and exhibited higher proportions of prior renal failure and atrial fibrillation. Similarly, the lower LDLR quartile displayed lower hemoglobin and higher values of NT-proBNP (**Table 2**). Regarding medications, higher proportions of patients used statins in the upper quartiles of the 2 studied exposures

TABLE 1 Demographic, Clinical, Laboratory and Treatment Characteristics Relative to PCSK9 Quartiles

	PCSK9				
	First Quartile (0.59–1.45 U/ml) (n = 544)	Second Quartile (1.45–1.81 U/ml) (n = 543)	Third Quartile (1.81–2.18 U/ml) (n = 544)	Fourth Quartile (2.18–5.43 U/ml) (n = 543)	p Value
Age, yrs	69 ± 12	69 ± 13	67 ± 12	68 ± 12	0.08
Male	413 (75.9)	401 (73.8)	391 (71.9)	388 (71.5)	0.32
Ischemic heart disease	273 (50.7)	273 (51.3)	277 (52.7)	333 (61.8)	0.001
Dilated cardiomyopathy	167 (30.7)	186 (34.3)	157 (28.9)	156 (28.7)	0.17
Hypertension	347 (63.8)	322 (59.3)	323 (59.4)	355 (65.4)	0.09
Diabetes mellitus	173 (31.8)	172 (31.7)	171 (31.4)	185 (34.1)	0.77
Renal failure	162 (29.8)	153 (28.2)	131 (24.1)	169 (31.1)	0.06
Atrial fibrillation	243 (44.7)	266 (49.0)	254 (46.7)	228 (42.0)	0.12
Heart rate, beats/min	81 ± 19	80 ± 20	79 ± 20	80 ± 19	0.67
LVEF, %*	32 ± 11	30 ± 10	31 ± 10	31 ± 11	0.28
Laboratory					
Hemoglobin, g/dl	13.0 ± 1.9	13.1 ± 1.8	13.2 ± 1.9	13.4 ± 2.0	0.07
Serum creatinine, mg/dl	1.28 ± 0.52	1.27 ± 0.53	1.27 ± 0.67	1.38 ± 0.74	0.008
NT-proBNP, ng/l	3975 (2,288–7,751)	4654 (2,364–8,475)	3784 (2,242–8,105)	3,949 (2,360–8,000)	0.61
LDLc, mmol/l†	2.54 ± 0.99	2.50 ± 0.98	2.65 ± 1.11	2.67 ± 1.14	0.21
Treatment					
Diuretics	543 (99.8)	543 (100.0)	544 (100.0)	542 (99.8)	0.57
MRA	270 (49.6)	290 (53.4)	300 (55.1)	282 (51.9)	0.31
Beta-blocker	454 (83.5)	451 (83.1)	443 (81.4)	459 (84.5)	0.59
ACEI/ARB	390 (71.7)	387 (71.3)	383 (70.4)	399 (73.5)	0.72
Statins	257 (47.3)	251 (46.1)	301 (55.3)	340 (62.2)	<0.001

Values are mean ± SD, n (%), or median (interquartile range). U/ml, is a normalized protein expression arbitrary unit (see Methods for details). *Data available in 1,946 patients. †Data available in 1,000 patients.

ACEI = angiotensin converting-enzyme inhibitor; ARB = angiotensin receptor 2 inhibitor; IQR = interquartile range; LDLc = low-density lipoprotein cholesterol; LVEF = left ventricular ejection fraction; MRA = mineralocorticoid receptor antagonist; PCSK9 = proprotein convertase subtilisin/kexin type 9; NTpro-BNP = N-terminus pro B-type natriuretic peptide; SD = standard deviation.

(Tables 1 and 2). LVEF categories, as defined in the European Society of Cardiology Guidelines (1), were similarly distributed across PCSK9 and LDLR quartiles (p = 0.365 and p = 0.193, respectively).

CIRCULATING PCSK9 PREDICTORS. Online Figure 2 shows the identified predictors of circulating PCSK9 levels. The model's adjusted R^2 value was 0.422. A closer look at the positive correlation between PCSK9 and LDLR is depicted in Online Figure 3 (r = 0.59; p < 0.001). Exploratory analyses across NT-proBNP quartiles with PCSK9 and LDLR did not show prognostic differences on all-cause mortality or the composite endpoint.

MORTALITY ENDPOINT. During a median follow-up of 1.78 years (IQR: 1.29 to 2.25 years), 569 deaths (26.2%) were registered. Multivariable analysis that included as covariates the BIOSTAT-CHF risk score for mortality, LDLR, and statin treatment, revealed a positive linear association between PCSK9 and the risk of mortality (p = 0.020) (Figure 1A). A similar analysis revealed a negative linear association between LDLR and mortality (p = 0.025) (Figure 1B). The estimated HRs from regression modeling are shown in Table 3. Because PCSK9 and LDLR levels were highly correlated, we excluded 1 exposure at a time to assess, on the other one, if multicollinearity was artificially changing in the direction of the effect. Under this premise, a positive association for all-cause mortality was confirmed for PCSK9 and a negative one for LDLR (results not shown). Indeed, both variables' linear trajectories showed opposite directions, with increasing risk at higher PCSK9 levels and lower risk at higher LDLR levels (Figure 1).

In exploratory fashion, several interactions were tested, all with negative results: PCSK9 quartiles versus LDLR (p = 0.388); LDLR quartiles versus PCSK9 (p = 0.143); statins versus PCSK9 (p = 0.432); statins versus LDLR (p = 0.860); ischemic heart disease versus PCSK9 (p = 0.271); and ischemic heart disease versus LDLR (p = 0.079).

The added value in performance for PCSK9, LDLR, and statin treatment over the BIOSTAT risk score was confirmed by Δ C-statistic (0.0120 [0.002 to 0.022]; p = 0.019), IDI (0.3 [0.0 to 1.1]), and NRI (6.0 [0.0 to

TABLE 2 Demographic, Clinical, Laboratory, and Treatment Characteristics Relative to LDLR Quartiles

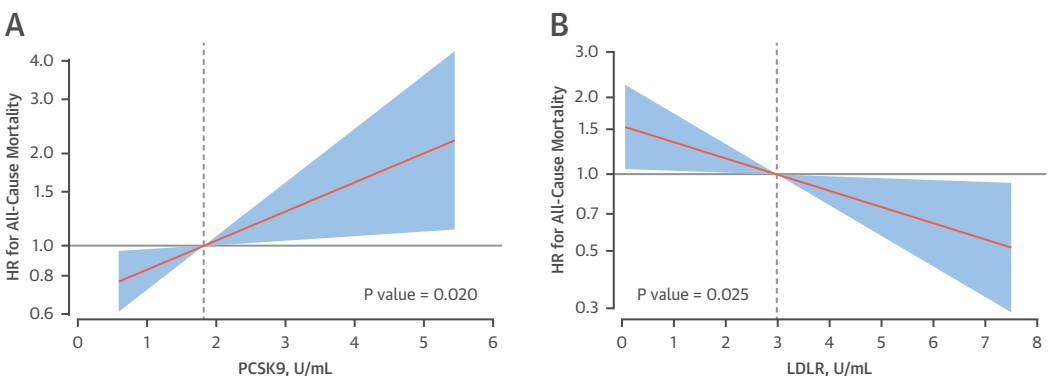
	LDLR				
	First Quartile (0.07–2.44 U/ml) (n = 544)	Second Quartile (2.45–2.98 U/ml) (n = 543)	Third Quartile (2.98–3.53 U/ml) (n = 544)	Fourth Quartile (3.53–7.48 U/ml) (n = 543)	p Value
Age, yrs	70 ± 12	69 ± 12	68 ± 12	66 ± 12	<0.001
Male	425 (78.1)	409 (75.3)	397 (73.0)	362 (66.7)	<0.001
Ischemic heart disease	280 (52.3)	295 (55.5)	288 (54.0)	293 (54.8)	0.76
Dilated cardiomyopathy	154 (28.3)	161 (29.7)	163 (30.0)	188 (34.6)	0.12
Hypertension	349 (64.2)	314 (57.8)	327 (60.1)	357 (65.7)	0.03
Diabetes mellitus	167 (30.7)	162 (29.8)	177 (32.5)	195 (35.9)	0.15
Renal failure	180 (33.1)	153 (28.2)	136 (25.0)	146 (26.9)	0.02
Atrial fibrillation	285 (52.4)	259 (47.7)	250 (46.0)	197 (36.3)	<0.001
Heart rate, beats/min	80 ± 19	80 ± 20	81 ± 21	80 ± 19	0.94
LVEF, %*	31 ± 11	30 ± 11	31 ± 11	31 ± 10	0.50
Laboratory					
Hemoglobin, g/dl	12.8 ± 1.9	13.2 ± 1.9	13.2 ± 1.9	13.5 ± 1.9	<0.001
Serum creatinine, mg/dl	1.32 ± 0.59	1.28 ± 0.71	1.31 ± 0.60	1.29 ± 0.58	0.66
NT-proBNP, ng/l	4,339 (2,420–8,068)	5,302 (2,767–9,449)	3,632 (2,358–7,340)	3,499 (1,775–7,337)	<0.001
Treatment					
Diuretics	543 (99.8)	543 (100.0)	544 (100.0)	542 (99.8)	0.57
MRA	297 (54.6)	299 (55.1)	277 (50.9)	269 (49.5)	0.18
Beta-blocker	440 (80.9)	462 (85.1)	448 (82.4)	457 (84.2)	0.25
ACEI/ARB	396 (72.8)	383 (70.5)	382 (70.2)	398 (73.3)	0.58
Statins	268 (49.9)	279 (51.3)	278 (51.1)	324 (59.7)	0.003

Values are mean ± SD, n (%), or median (interquartile range). U/ml, is a normalized protein expression arbitrary unit (see Methods for details). *Data available in 1,946 patients. LDLR = low-density lipoprotein receptor; other abbreviations as in Table 1.

11.9]). Similarly, PCSK9 produced a better risk reclassification over LDLR, statin treatment, and the BIOSTAT-CHF risk score as evidenced by IDI (0.6 [0.1 to 1.8]) and NRI (11.0 [1.0 to 18.3]).

COMPOSITE ENDPOINT. At a median follow-up of 1.53 years (IQR: 0.67 to 2.15 years), we ascertained 896 (41.2%) composite endpoints (composite of death or HF-related hospitalization). In a multivariable

FIGURE 1 Multivariable Analyses for All-Cause Mortality



(A) HR gradient (**shaded area** = 95% CI), with the median PCSK9 value (1.81 U/ml) as reference. Analysis adjusted for LDLR, statins, and the BIOSTAT-CHF risk score for mortality. **(B)** HR gradient (**shaded area** = 95% CI), with the median LDLR value (2.98 U/ml) as reference. Analysis adjusted for PCSK9, statins, and the BIOSTAT-CHF risk score for mortality. U/ml is a normalized protein expression arbitrary unit (see Methods). BIOSTAT-CHF = Biology Study to Tailored Treatment in Chronic Heart Failure; CI = confidence interval; HR = hazard ratio; LDLR = low-density lipoprotein receptor. PCSK9 = proprotein convertase subtilisin/kexin type 9.

TABLE 3 Regression Modeling for All-Cause Mortality and the Composite Endpoint of Mortality and/or HF-Related Hospitalization

Endpoint	HR	95% CI	p Value
All-cause mortality			
PCSK9	1.24	1.04–1.49	0.020
LDLR	0.86	0.76–0.98	0.025
Statins	1.03	0.87–1.22	0.725
Risk score*			<0.001†
Tertile 1	1.00		
Tertile 2	2.35	1.76–3.13	<0.001
Tertile 3	6.41	4.92–8.36	<0.001
Mortality/HF-related hospitalization			
PCSK9	1.21	1.05–1.40	0.011
LDLR	0.92	0.83–1.01	0.087
Statins	1.25	1.09–1.42	0.001
Risk score‡			<0.001†
Tertile 1	1.00		
Tertile 2	2.87	2.32–3.56	<0.001
Tertile 3	6.13	5.00–7.52	<0.001

*BIOSTAT-CHF score for mortality includes: age, blood urea nitrogen, NT-pro-BNP, serum hemoglobin, and use of beta-blocker. †Omnibus p value. ‡BIOSTAT-CHF score for mortality/HF-related rehospitalization includes: age, previous HF-related hospitalization, presence of edema, systolic blood pressure, and estimated glomerular filtration rate.

CI = confidence interval; HF = heart failure; HR = hazard ratio; other abbreviations as in Tables 1 and 2.

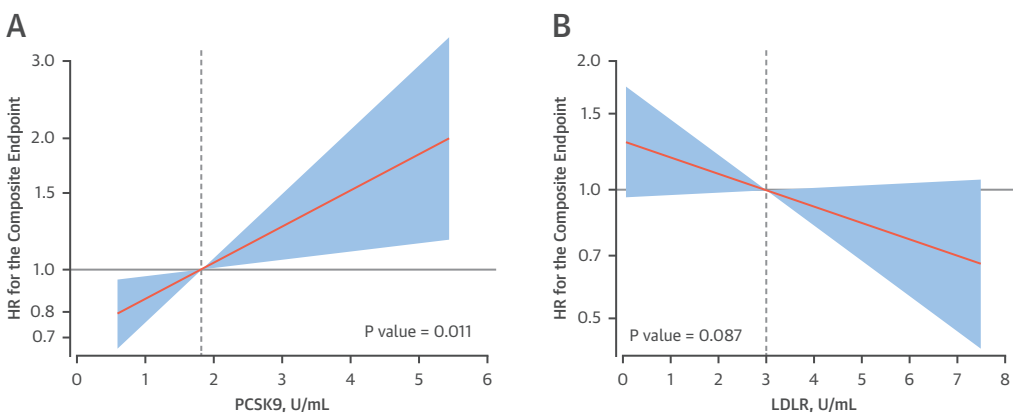
context, PCSK9 showed a linear and positive association with the risk of the combined endpoint (p = 0.011) (Figure 2A), independent of the effect of BIOSTAT-CHF risk score for the composite endpoint, LDLR, and statin treatment; LDLR, however, showed a negative but borderline association (p = 0.087) (Figure 2B). The estimated HRs from regression modeling are shown in Table 3. The direction of the effect for both PCSK9 and LDLR were confirmed by excluding one of them at a time to rule out changes from multicollinearity.

In exploratory fashion, several interactions were tested, all with negative results: PCSK9 quartiles versus LDLR (p = 0.200); LDLR quartiles versus PCSK9 (p = 0.929); statins versus PCSK9 (p = 0.485); statins versus LDLR (p = 0.403); ischemic heart disease vs PCSK9 (p = 0.170); and ischemic heart disease vs LDLR (p = 0.211).

The added value in performance for PCSK9, LDLR, and statin treatment over the BIOSTAT-CHF risk score was confirmed by Δ C-statistic (0.014 [0.006 to 0.022]; p < 0.001), IDI (0.8 [0.2 to 1.8]), and NRI (10.8 [2.9 to 15.0]). Similarly, PCSK9 produced a better risk reclassification over LDLR, statin treatment, and the BIOSTAT-CHF risk score both by IDI (0.6 [0.0 to 1.5]) and NRI (9.8 [0.4 to 17.7]).

SENSITIVITY ANALYSIS. In a sensitivity analysis in which plasma LDL-cholesterol was added as an additional covariate, the results were consistent with the main findings, despite a drop in sample numbers (n = 1,000) from missing LDL-cholesterol values. Circulating PCSK9 and LDLR levels were independently associated with mortality risk and the composite endpoint (Online Table 1).

FIGURE 2 Multivariable Analyses for the Composite Endpoint (Mortality or HF-Related Hospitalization)



(A) HR gradient (**shaded area** = 95% CI), with the median PCSK9 value (1.81 U/ml) as reference. Analysis adjusted for LDLR, statins, and the BIOSTAT-CHF risk score for the composite endpoint. **(B)** HR gradient (**shaded area** = 95% CI), with the median LDLR value (2.98 U/ml) as reference. Analysis adjusted for PCSK9, statins, and the BIOSTAT-CHF risk score for the composite endpoint. U/ml is a normalized protein expression arbitrary unit (see Methods). HF = heart failure; other abbreviations as in Figure 1.

CENTRAL ILLUSTRATION **PCSK9 Across the Cardiovascular Continuum**

CARDIOVASCULAR CONTINUUM				
	Dyslipidemia	Asymptomatic Atheroma	Symptomatic Ischemic Heart Disease	Heart Failure
Circulating PCSK9	✓	✓	✓	✓
PCSK9 and Outcomes	–	–	✓	✓
Value of PCSK9 Inhibition	✓	✓	✓	?

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In heart failure, we proved evidence of elevated proprotein convertase subtilisin/kexin type 9 (PCSK9) levels, which were associated with poor outcomes. There is no evidence of PCSK9 inhibition in heart failure.

DISCUSSION

The present report is the first to show that the PCSK9-LDLR axis was associated with poor outcomes in patients with HF. Our subanalysis of the BIOSTAT-CHF cohort indicated that soluble PCSK9 was positively associated with both all-cause mortality and the composite endpoint of mortality or HF-related rehospitalizations in patients with worsening HF. In contrast, as pathobiologically expected, circulating LDLR was inversely associated with the same 2 endpoints. Interestingly, the predictive value of PCSK9 improved after adjusting for traditional prognosticators and soluble LDLR.

The predictive value of circulating PCSK9 concentrations was previously described in other clinical settings of cardiovascular pathology (19,20). In the context of HF, a comprehensive multivariable analysis with validated BIOSTAT-CHF risk scores showed that higher PCSK9 was associated with an increased incidence of the composite endpoint; this relationship was independent of serum LDL-cholesterol, statin use, or ischemic HF etiology. Thus, PCSK9 may be considered a risk predictor across the cardiovascular disease continuum, from asymptomatic dyslipidemia, through subclinical and clinical atherosclerosis, and in HF, based on our findings (**Central Illustration**).

Secreted PCSK9 follows 2 possible tracks: the first is to bind immediately to LDLRs in the liver, and the second is to enter the systemic circulation (12). Once bound, the PCSK9/LDLR complex is endocytosed, taken into the lysosomes, and undergoes degradation (21). The presence of PCSK9 enhances LDLR degradation; therefore, this track reduces the LDLR abundance on the cell surface (22). In the plasma, circulating PCSK9 can bind to LDLRs on the membranes of various organ systems, such as the liver, intestines, kidneys, lungs, pancreas, and adipose tissues (23–27). In the present report, although the ability for risk prediction of PCSK9 and LDLR was opposite when included in comprehensive multivariable models, their circulating concentrations showed a significantly positive correlation. This finding may reflect the already recognized complexity of the PCSK9-LDLR axis (28). Indeed, Tavori et al. (28) report that, in addition to the straightforward mechanism of action (PCSK9 terminating the lifecycle of LDLR), there are more complex interactions among PCSK9, LDLR, and plasma lipoprotein levels, including: 1) the presence of both parallel and reciprocal regulation of surface LDLR and plasma PCSK9; 2) a correlation between PCSK9 and LDL-cholesterol levels dependent, not only on the fact that PCSK9 removes hepatic LDLR, but also because that up to 40% of plasma PCSK9 is physically associated with LDL; and 3) an association between plasma PCSK9 production and the assembly and secretion of triglyceride-rich lipoproteins.

Despite numerous advances in HF treatments, which block both the sympathetic nervous system and the renin-angiotensin-aldosterone system, the

morbidity and mortality of patients with HF remain unacceptably high (29). Our finding that PCSK9 could predict risk in HF may serve as a basis for designing prospective studies that aim to inhibit PCSK9, either with specific PCSK9 neutralizing monoclonal antibodies or with the administration of small, interfering RNAs that specifically bind and inhibit translation of PCSK9 messenger RNAs (13,14,30).

In patients with acute coronary syndromes, the potential benefit of treatment with PCSK9 antibodies may be 2-fold, because it could both reduce LDL-cholesterol and stabilize plaques (10). Indeed, PCSK9 adversely affects coronary plaques through several pathways, including proinflammatory LDL oxidation and direct modification of plaque composition. Moreover, PCSK9 is associated with the inflammatory response, which is largely based on nuclear factor-κB-mediated expression of proinflammatory genes, including cytokines, chemokines, and adhesion molecules (31,32). The PCSK9-induced nuclear factor-κB pathway can upregulate tissue factor expression, which enhances the thrombotic substrate in atherosclerotic plaques (33). In the context of HF, it is reasonable to speculate that plaque stabilization, combined with reductions in the prothrombotic and proinflammatory states, accomplished by inhibiting PCSK9, might represent a new avenue of treatment for preventing disease progression, when the current optimal medical treatment is insufficient. It is unclear whether this strategy will be useful across all HF etiologies, or only for those of ischemic origin; this issue should be investigated in future, well-designed, prospective clinical trials.

Two randomized clinical trials that explored reducing cholesterol levels with rosuvastatin in patients with HF did not demonstrate a clear benefit (7,8). Rosuvastatin is a hydrophilic statin, which relies on active transport into hepatocytes to exert its effect and has poor penetration into extrahepatic tissues; thus, it has less risk of adverse effects but also very low uptake by cardiac muscle. No randomized studies have been performed with lipophilic statins (simvastatin, atorvastatin), which tend to achieve higher levels of exposure in nonhepatic tissues, have very high cardiac muscle uptake, and have shown some benefit in real-life scenarios (9,34). Current guidelines do not recommend the use of statins in patients with HF (1). Whether interfering with cholesterol-related mechanistic pathway may be beneficial in selected patients with HF is not a totally settled issue (35).

STUDY LIMITATIONS. First, the data presented here are valid for patients with worsening HF, mainly from reduced LVEF. It remains to be determined whether soluble PCSK9 is also a good predictor for patients with stable chronic HF. Available data in mice in the context of coronary artery disease showed that plasma PCSK9 concentration was mostly elevated in the early hours after an acute coronary syndrome (36). The second limitation was that the assay used to measure PCSK9 and LDLR was designed for research only, and its use cannot be recommended in clinical practice. Nevertheless, both exposures were measured with state-of-the-art proteomics technology, currently available and well validated (16). The last limitation was that genetic mutations that might determine PCSK9 levels were not measured in patients enrolled in the BIOSTAT-CHF trial. PCSK9 gain-of-function mutations have been associated with increased severity in coronary atherosclerosis (37).

CONCLUSIONS

The PCSK9-LDLR axis has been investigated expeditiously, from discovery to targeted therapy in dyslipidemia and coronary atherosclerosis. Here, we provided the first evidence of PCSK9 participation in HF. Indeed, HF risk was positively associated with circulating PCSK9 and negatively associated with LDLR in patients with worsening HF. Future studies are needed to better understand the PCSK9-LDLR axis in HF and to assess whether PCSK9 inhibition or silencing might lead to better outcomes in HF.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: PCSK9, which binds the LDLR and prevents its recycling, is a predictor of risk and therapeutic target in patients with atherosclerosis. The risk of heart failure progression is also associated with circulating PCSK9 levels and inversely associated with LDLR levels.

TRANSLATIONAL OUTLOOK: Future research should investigate whether outcomes in patients with heart failure can be improved by inhibiting PCSK9, either with specific PCSK9-neutralizing monoclonal antibodies or with interfering RNAs.

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KEY WORDS heart failure, LDLR, low-density lipoprotein receptor, PCSK9, proprotein convertase subtilisin/kexin type 9

APPENDIX For supplemental figures and a table, please see the online version of this article.