JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY VOL. 72, NO. 25, 2018

PUBLISHED BY ELSEVIER

Association of Serum Cholesterol Efflux Capacity With Mortality in Patients With ST-Segment Elevation Myocardial Infarction

Maryse Guerin, PhD,[a,*] Johanne Silvain, MD, PhD,[a,b,*] Julie Gall, PhD,[a] Maryam Darabi, PhD,[a] Myriam Berthet, PhD,[a] Eric Frisdal, PhD,[a] Marie Hauguel-Moreau, MD,[a,b] Michel Zeitouni, MD,[a,b] Mathieu Kerneis, MD,[a,b] Benoit Lattuca, MD,[a,b] Delphine Brugier,[a,b] Jean-Philippe Collet, MD, PhD,[a,b] Philippe Lesnik, PhD,[a] Gilles Montalescot, MD, PhD[a,b]

ABSTRACT

BACKGROUND Serum cholesterol efflux capacity, a biomarker that integrates contributors and modulators of the initial step of the reverse cholesterol transport, has been associated with atherosclerosis independently of high-density lipoprotein (HDL) cholesterol level.

OBJECTIVES The authors evaluated the prognostic impact of serum cholesterol efflux capacity on mortality in a large cohort of patients hospitalized for an acute myocardial infarction (MI).

METHODS Serum cholesterol efflux capacity, cholesteryl ester transfer protein (CETP) activity, total cholesterol, low-density lipoprotein cholesterol, HDL cholesterol, and triglyceride levels were measured in 1,609 consecutive patients admitted with an acute MI. The primary endpoint was all-cause mortality evaluated at 6 years with a median follow-up of 1.9 years (interquartile range: 1.5 to 4.2 years). An analysis by quartile of serum cholesterol efflux capacity was also performed.

RESULTS In a fully adjusted model that included age, sex, traditional cardiovascular risk factors including lipid levels, and prognostic factors of MI, serum cholesterol efflux capacity was a strong predictor of survival (adjusted hazard ratio for mortality per 1-SD increase in serum cholesterol efflux capacity, 0.79; 95% confidence interval: 0.66 to 0.95; $p = 0.0132$). Patients displaying an elevated serum cholesterol efflux capacity had a marked lower rate of mortality at 6 years (adjusted hazard ratio: 0.54 [0.32 to 0.89]; $p = 0.0165$) as compared with patients with reduced serum cholesterol efflux capacity.

CONCLUSIONS Serum cholesterol efflux capacity, an integrative marker of reverse cholesterol transport pathway and efficacy, was inversely associated with all-cause mortality in MI patients independently of HDL cholesterol level and other risk factors. (J Am Coll Cardiol 2018;72:3259–69) © 2018 by the American College of Cardiology Foundation.

Listen to this manuscript's audio summary by Editor-in-Chief Dr. Valentin Fuster on JACC.org.

From the [a]INSERM UMRS1166, ICAN - Institute of CardioMetabolism and Nutrition, Hôpital Pitié-Salpêtrière, Sorbonne Université, Paris, France; and the [b]Sorbonne Université, ACTION Study Group, INSERM UMRS1166, ICAN - Institute of CardioMetabolism and Nutrition, Institut de Cardiologie, Hôpital Pitié-Salpêtrière, Paris, France. *Drs. Guerin and Silvain contributed equally to this work. This work was supported by Institut National de la Santé et de la Recherche Medicale (INSERM), the Institute of Cardiology and Metabolism (ICAN), the ACTION Coeur study group, and by the French National Agency through the national program Investissements d'Avenir Grant ANR-10-IAHU-05. Dr. Gall was a recipient of a research fellowship from the French Ministry of Research and Technology and from the New French Atherosclerosis Society (NSFA). Dr. Silvain has received research grants, honoraria, and/or travel support from Amgen, Algorythm, AstraZeneca, Bayer, Daiichi-Sankyo, Gilead Science, Sanofi, and WebMD. Dr. Zeitouni has received research grants from Servier and Fédération Française de Cardiologie. Dr. Kerneis has received research grants from Institut Servier, Fédération Française de Cardiologie, and Sanofi; and consulting fees from Bayer. Dr. Lattuca has received research grants from Biotronik, Daiichi-Sankyo, and Fédération Française de Cardiologie; consultant fees from Daiichi-Sankyo and Eli Lilly; and lecture fees from AstraZeneca, Daiichi-Sankyo, and Novartis. Dr. Collet has received research grants or honoraria from AstraZeneca, Bayer, Bristol-Myers Squibb, Daiichi-Sankyo, Eli Lilly, Fédération Française de Cardiologie, Lead-Up, Medtronic, Merck Sharp & Dohme, Sanofi, Servier, and WebMD. Prof. Montalescot has received research grants or honoraria from ADIR, Amgen, AstraZeneca, Bayer, Berlin Chimie AG, Boehringer Ingelheim, Bristol-Myers Squibb, Beth Israel Deaconess Medical,

ISSN 0735-1097/$36.00 https://doi.org/10.1016/j.jacc.2018.09.080

ABBREVIATIONS AND ACRONYMS

ABCA1 = ATP-binding cassette A1

apoB = apolipoprotein B

CETP = cholesteryl ester transfer protein

CI = confidence interval

HDL = high-density lipoprotein

HR = hazard ratio

LDL = low-density lipoprotein

MI = myocardial infarction

PCI = percutaneous coronary intervention

SR-BI = scavenger receptor class B member 1

STEMI = ST-segment elevation myocardial infarction

Low circulating levels of high-density lipoprotein (HDL) cholesterol represent a strong, independent risk factor for premature atherosclerosis and coronary heart disease (1). Although low-density lipoprotein (LDL) promotes atherosclerosis by delivering cholesterol to artery-wall macrophages, HDL's cardioprotective effects are mediated in part by the so called reverse cholesterol transport that represents its ability to remove cholesterol from macrophages in a centripetal movement from peripheral tissues, including the vessel wall, toward the liver for biliary secretion (2).

SEE PAGE 3270

The failure of HDL-raising drugs, such as niacin and more recently cholesteryl ester transfer protein (CETP) inhibitors (3), has shown that steady-state HDL cholesterol levels do not represent the most appropriate cardiovascular risk factor index (4). Efflux capacity, the capacity of HDL particles to mediate cholesterol efflux from macrophages, has been established as having a strong inverse association with both carotid intima-media thickness (5) and also with the incidence of cardiovascular events (6,7), independently of HDL cholesterol level. Moreover, recent studies suggested that the impaired efflux capacity of HDL particles may also be an independent risk factor for cardiovascular mortality in patients with chronic coronary artery disease (8,9). Cholesterol efflux capacity is also a new therapeutic target, and ongoing studies are evaluating innovative therapies trying to improve cholesterol efflux, thus reducing plaque burden and improving plaque stabilization rather than raising HDL alone (10).

The prevailing system measuring HDL efflux capacity that uses mouse macrophages and apolipoprotein B (apoB)-depleted serum is associated with its own limits, mostly inherent in the use of nonhuman macrophages (11-14) and nonconsideration of roles of both apoB lipoproteins (15) and CETP (16) in maintenance of cholesterol flow through the intravascular reverse cholesterol transport process (**Figure 1**). In this context, we developed a more comprehensive assay measuring the overall capacity of a given serum to mediate cholesterol efflux from macrophages and have demonstrated its relevance as a clinical tool for identification of patients at high risk of premature atherosclerosis (17). In the present study, we evaluated for the first time to our knowledge whether serum cholesterol efflux capacity has an independent prognostic value for all-cause mortality in patients hospitalized for an acute myocardial infarction (MI) and treated with primary percutaneous coronary intervention (PCI).

METHODS

STUDY POPULATION AND DATA COLLECTION. Between January 2003 and April 2014, 1,609 consecutive patients treated for an acute MI at the Pitié-Salpêtrière University Hospital, Paris, France, were enrolled in the ongoing ePARIS registry, a prospective registry with extensive clinical and biological data collection. Patients were included if they had an acute ST-segment elevation myocardial infarction (STEMI) treated by primary PCI, and biological sampling was obtained on arrival in the catheterization laboratory. Patients discharged without a final diagnosis of STEMI were excluded as well as the patients who did not consent to participate. Following revascularization, patients who survived and were discharged from hospital (95.2%) received a medical treatment including anti-ischemic, lipid-lowering, and antithrombotic drugs according to the current guidelines. All patients had follow-up during hospital stay, with completed baseline characteristics and pharmacological management. Clinical outcomes were obtained by telephone call by clinic research associates, during medical consultation or from pre-hospitalization medical reports. In the absence of direct contact with the patient, survival status was checked in the birth city hall registry.

STUDY ENDPOINT AND OBJECTIVES. The primary endpoint of the study was all-cause mortality evaluated for the entire study population at 6 years with a minimum of 1 year for the last patients included. Follow-up was continued until the last included patient reached 1 year of follow-up. The primary objective was to evaluate the independent impact of serum cholesterol efflux capacity from human

Brigham Women's Hospital, Cardiovascular Research Foundation, Celladon, CME Resources, Daiichi-Sankyo, Eli Lilly, Europa, Elsevier, Fédération Française de Cardiologie, Fondazione Anna Maria Sechi per il Cuore, Gilead, ICAN, Janssen, Lead-Up, Menarini, Medtronic, Merck Sharp & Dohme, Pfizer, Sanofi, Servier, The Medicines Company, TIMI Study Group, and WebMD. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

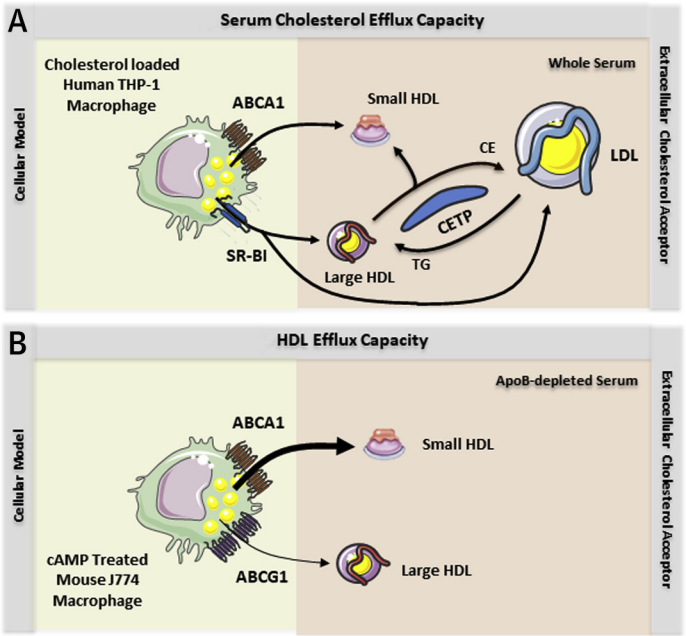
Manuscript received September 12, 2018; revised manuscript received September 28, 2018, accepted September 30, 2018.

macrophage cell line (17) on all-cause mortality. Secondary objectives included the evaluation of the impact of CETP activity on all-cause mortality and the prognostic value of various cholesterol efflux assays, including measurement of HDL cholesterol efflux (apoB-depleted serum) from human macrophage cell line, and the use of established cellular models each representative of 1 specific cholesterol efflux pathway known to significantly contribute to cholesterol efflux from human macrophage, scavenger receptor class B member 1 (SR-BI) or ATP-binding cassette A1 (ABCA1) (18) (**Figure 1**).

BLOOD SAMPLES AND BIOCHEMICAL MEASUREMENTS. Blood collected from all patients at admission to the catheterization laboratory by means of venipuncture was placed into gel-containing vacutainer tubes, immediately (within <1 h) centrifuged, then serum was stored at −80°C until used. Lipids were analyzed on an autoanalyser Konelab 20 (Thermo Electron Corporation/Thermo Fisher Scientific, Waltham, Massachusetts) and by using commercial kits from Roche Diagnostics (Risch-Rotkreuz, Switzerland) for total cholesterol and from Thermo Fisher Scientific for triglycerides and direct HDL cholesterol. LDL cholesterol was calculated using Friedewald formula when triglyceride levels were below 340 mg/dl or by using commercial kit from Thermo Fisher Scientific for direct LDL cholesterol when triglyceride levels were >340 mg/dl.

CHOLESTEROL EFFLUX MEASUREMENTS. Serum cholesterol efflux capacity was performed using cholesterol-loaded human THP-1 macrophages cell line as previously described (17,19,20). ^{3}H-cholesterol-labeled macrophages were incubated 4 h at 37°C in the presence of 40-fold diluted serum. Serum cholesterol efflux capacity was calculated as the amount of the label recovered in the medium divided by the total label in each well (radioactivity in the medium + radioactivity in the cells) obtained after lipid extraction from cells in a mixture of 3:2 hexane-isopropanol (3:2 v/v). The background cholesterol efflux obtained in the absence of any acceptor was subtracted from the efflux obtained with samples. A standard serum was tested in all experiments and was used calculate relative cholesterol efflux capacity of each sample. All efflux determinations were performed in triplicate for each sample with intra-assay and interassay coefficients of variation of 2% and 2.3%, respectively. To compare our serum cholesterol efflux assay to previous published data (5,6), we equally performed additional cholesterol efflux measurements using either apoB-depleted serum (HDL efflux capacity) as cellular cholesterol acceptor (20) or various specific cellular models representative of 1 specific efflux pathway to evaluate global efflux capacity via either ABCA1 or SR-BI (17-19) (see the Online Appendix for details).

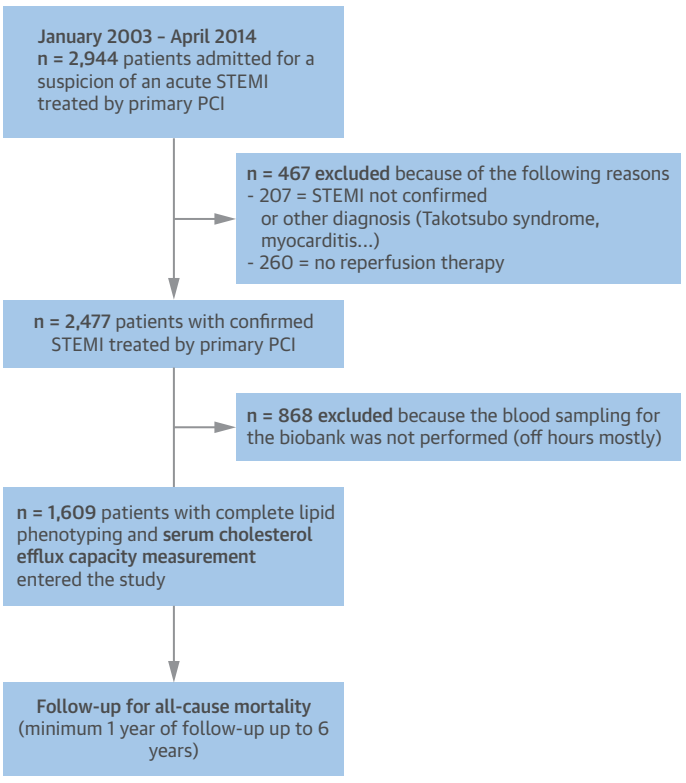
FIGURE 1 Simplified Schematic Representation of Serum Cholesterol Efflux Capacity



Serum cholesterol efflux capacity was measured in the presence of whole serum using cholesterol-loaded human macrophage **(A)**, and of standard system measuring HDL efflux capacity performed in the presence of apoB-depleted serum using cholesterol-loaded mouse macrophage in which ABCA1 is up-regulated by cAMP **(B)**. The **size of the arrow** indicates relative contribution of major cholesterol efflux pathways in both cellular models. Small HDL represents lipid-poor HDL; large HDL represents lipid-rich HDL. ABCA1 = ATP-binding cassette A1; ABCG1 = ATP-binding cassette G1; ApoB = apolipoprotein B; cAMP = cyclic adenosine monophosphate; CE = cholesteryl esters; CETP = cholesterol ester transfer protein; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SR-BI = scavenger receptor class B member 1; TG = triglycerides.

STUDY OVERSIGHT. The first 2 authors (M.G. and J.S.) designed the study, gathered and analyzed the data, and drafted the manuscript. The other authors contributed to data gathering, biological measurements, and critical revision of the manuscript. All the authors vouch for the data and analyses reported. The first 2 authors made the decision to submit the manuscript for publication. The study conforms to the principles outlined in the declaration of Helsinki. The ePARIS registry was declared to the French ministry of Health and Data Protection Authority (CNIL 1542887v0). Written informed consent was obtained from each patient participating in the registry.

FIGURE 2 Flow Chart of the Study



Between January 2003 and April 2014, 2,944 patients were screened and, finally, 1,609 patients with an acute ST-segment elevation myocardial infarction (STEMI) treated by primary percutaneous coronary intervention (PCI) were included in our analysis.

SAMPLE SIZE ESTIMATION. In our previous study (18), we observed a 20% standard deviation in serum cholesterol efflux in a cohort of primary prevention patients. On the basis of an alpha risk of 0.05 and a power of 80%, a minimum of 125 patients per group were then required to observe a significant difference of ≤5% in serum cholesterol efflux capacity between survivors and nonsurvivors. Considering an all-cause mortality rate of 10% after 2 years of follow-up in our population based on previous work on our registry (21), we estimated that a minimum of 1,250 subjects was needed in the final analysis study.

STATISTICAL ANALYSES. Normally distributed continuous variables are presented as mean ± SD, whereas continuous variables with skewed distribution (triglycerides) are given as median and interquartile range and were logarithmically transformed before analysis. Serum cholesterol efflux capacity was modelled either as a continuous variable or as quartiles. The qualitative variables presented as proportions were compared using the chi-square test. Comparisons between 2 groups of subjects were performed using an unpaired Student's *t*-test. Comparisons across quartiles of serum cholesterol efflux capacity were made with the use of the Jonckheere-Terpstra trend test. The survival curves for serum cholesterol efflux capacity were analyzed using the Kaplan-Meier method, and statistical assessment was performed using the log-rank test. The effects of different variables on all-cause mortality at 6 years were assessed by Cox regression analysis. Multivariable models included age and sex, cardiovascular risk factors (diabetes, hypertension, current smoking, obesity as defined by a body mass index >30 kg/m^2, LDL cholesterol levels, HDL cholesterol levels, log-transformed triglyceride levels, and status with regard to use of statins), and prognostic factors of MI (out-of-hospital cardiac arrest, Killip class ≥2, left ventricular ejection fraction <45%, symptom-to-balloon time >360 min, creatinine levels, previous cardiovascular events and status with regard to use of angiotensin-converting enzyme inhibitors/angiotensin II receptor blocker and beta blockers). Statistical analyses were performed using the R statistical software computer program version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). The results were considered to be statistically significant at $p < 0.05$.

RESULTS

STUDY POPULATION AND FOLLOW-UP. The flow chart of the study is presented in Figure 2. A total of 1,609 patients treated for an acute MI who underwent a complete lipid phenotyping were included in the analysis. The baseline characteristics and the lipid phenotyping of the population are presented in Table 1. The follow-up of the cohort was complete with a minimum of 1 year for the last patient enrolled and was stopped after 6 years with a median follow-up of 1.9 years (interquartile range: 1.5 to 4.2 years), during which 239 patients died (14.8%). Mean serum cholesterol efflux capacity was significantly lower in patients who died during follow-up as compared with those who survived (0.762 ± 0.140 and 0.818 ± 0.138; $p < 0.0001$), whereas HDL cholesterol levels and CETP activity were not (Table 1). When the population was divided into quartiles of serum cholesterol efflux capacity from the human macrophage cell line, we found a stepwise increase in lipid levels (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides) and CETP activity according to quartiles of serum

TABLE 1 Baseline Characteristics of the Study Population

	All Patients (N = 1,609)	Survivors at 6 Years (n = 1,370)	Death at 6 Years (n = 239)	p Value*
Age and sex				
Age, yrs	63.4 ± 14.1	61.8 ± 13.6	72.9 ± 13.3	<0.0001
Age >75 yrs	22.5	18.2	46.9	<0.0001
Men/women	1,218/391	1,053/317	165/74	0.0093
Traditional cardiovascular risk factor				
BMI, kg/m^2	26.0 ± 4.4	26.1 ± 4.4	25.3 ± 4.1	0.0137
Obesity (BMI >30 kg/m^2)	17.1	17.4	15.0	0.40
Dyslipidemia	42.4	43.1	38.5	0.19
Diabetes	18.5	18.7	17.2	0.57
Hypertension	47.6	46.2	55.6	0.0070
Smoke	40.3	43.2	23.8	<0.0001
Family history of coronary artery disease	20.5	22.4	9.6	<0.0001
Lipid phenotyping				
Triglycerides, g/l	0.82 (0.59–1.21)	0.82 (0.58–1.22)	0.80 (0.60–1.18)	0.36
Total cholesterol, g/l	1.68 ± 0.45	1.72 ± 0.43	1.50 ± 0.50	<0.0001
LDL cholesterol, g/l	1.13 ± 0.41	1.16 ± 0.39	0.97 ± 0.46	<0.0001
HDL cholesterol, g/l	0.35 ± 0.12	0.35 ± 0.12	0.34 ± 0.13	0.31
Cholesterol ester transfer protein activity	31.8 ± 10.9	32.0 ± 10.9	30.7 ± 10.6	0.08
Serum cholesterol efflux capacity	0.809 ± 0.139	0.818 ± 0.138	0.762 ± 0.140	<0.0001
Cardiac risk factor on arrival				
Out-of-hospital cardiac arrest	7.7	4.3	26.9	<0.0001
Previous cardiovascular events	19.4	18.2	25.9	0.0055
Creatinine clearance, ml/min	86.4 ± 42.4	91.1 ± 41.6	56.8 ± 34.8	<0.0001
Creatinine clearance <60 ml/min	27.7	22.5	60.7	<0.0001
Heart rate, beats/min	80.1 ± 17.5	79.5 ± 16.6	84.0 ± 21.5	0.0006
Systolic blood pressure, mm Hg	129.8 ± 26.4	130.6 ± 25.6	124.5 ± 30.5	0.0017
Left ventricular ejection fraction, %	49.6 ± 11.7	50.9 ± 10.6	41.0 ± 14.7	<0.0001
Left ventricular ejection fraction <45%	24.9	20.5	51.6	<0.0001
Killip class ≥2	15.3	11.6	37.9	<0.0001
STB time >360 min	38.3	38.8	35.3	0.32
Discharge therapy				
Statins	87.4	91.5	63.4	<0.0001
Beta-blockers	79.1	84.4	48.7	<0.0001
ACE inhibitor/ARB	81.2	85.5	56.6	<0.0001

Values are mean ± SD, %, n, or median (interquartile range). *p values indicate significant difference between patients who died and who survived at 6 years.

ACE = angiotensin-converting enzyme; ARB = angiotensin receptor II blocker; BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein; STB = symptom-to-balloon.

cholesterol efflux capacity from the human macrophage cell line (Table 2). By contrast, no relationship was observed when considering cardiovascular risk factors with the exception of systolic blood pressure and creatinine clearance.

ASSOCIATION OF CHOLESTEROL SERUM EFFLUX CAPACITY WITH ALL-CAUSE MORTALITY. We observed a stepwise inverse relationship between increasing quartiles of serum cholesterol efflux capacity and all-cause mortality at 6 years (hazard ratio [HR] for the highest versus lowest quartile of serum cholesterol efflux capacity: 0.40; 95% confidence interval [CI]: 0.27 to 0.58) (Central Illustration) that was sustained after adjustment for cardiovascular risk factors and established prognostic factors of MI (adjusted HR: 0.54; 95% CI: 0.32 to 0.89). Importantly, the results remained significant after adjustment for traditional risks factors without further correction for prognostic factors (Online Table 1). The results of the multivariate analysis with serum cholesterol efflux modelled as a continuous variable is presented in Table 3 and shows that elevated serum cholesterol efflux capacity was an independent predictor of survival after myocardial infarction. Additional analyses revealed that more potent serum efflux capacity was associated with in-hospital survival (adjusted HR: 0.63; 95% CI: 0.40 to 0.97; p = 0.038). Finally, in a

TABLE 2 Baseline Characteristics of the Study Population According to Quartiles of Serum Cholesterol Efflux Capacity

	Quartile 1 (n = 403)	Quartile 2 (n = 402)	Quartile 3 (n = 403)	Quartile 4 (n = 401)	p Value for Trend*
Serum cholesterol efflux capacity	0.395–0.723	0.724–0.804	0.805–0.890	0.891–1.841	
Age and sex					
Age, yrs	64.8 ± 13.9	63.3 ± 14.1	62.6 ± 14.2	63.0 ± 14.2	0.0151
Men/women	312/91	310/92	303/100	293/108	0.12
Traditional cardiovascular risk factor					
BMI, kg/m^2	25.7 ± 4.2	26.3 ± 4.2	26.1 ± 4.6	25.8 ± 4.4	0.63
Obesity, BMI>30 kg/m^2	12.9	21.0	17.7	16.8	0.27
Dyslipidemia	39.7	44.0	41.9	43.9	0.21
Diabetes	14.6	22.6	20.3	16.2	0.42
Hypertension	51.1	50.0	43.4	45.9	0.0417
Smoker	38.5	36.6	43.7	42.6	0.06
Family history of coronary artery disease	18.6	19.7	23.1	20.7	0.22
Lipid phenotyping					
Triglycerides, g/l	0.75 (0.55–1.04)	0.81 (0.58–1.16)	0.84 (0.59–1.29)	0.91 (0.65–1.37)	<0.0001
Total cholesterol, g/l	1.43 ± 0.46	1.66 ± 0.38	1.78 ± 0.40	1.87 ± 0.43	<0.0001
LDL cholesterol, g/l	0.94 ± 0.41	1.12 ± 0.35	1.20 ± 0.37	1.26 ± 0.42	<0.0001
HDL cholesterol, g/l	0.32 ± 0.11	0.34 ± 0.11	0.36 ± 0.12	0.37 ± 0.14	<0.0001
Cholesterol ester transfer protein activity	29.5 ± 10.6	31.4 ± 10.4	32.9 ± 10.6	33.3 ± 11.4	<0.0001
Cardiac risk factor on arrival					
Out-of-hospital cardiac arrest	10.8	8.5	6.7	4.8	0.06
Previous cardiovascular events	23.6	18.9	16.1	19.0	0.10
Creatinine clearance, ml/min	79.3 ± 41.0	89.1 ± 43.3	88.0 ± 41.8	89.3 ± 43.0	0.0016
Creatinine clearance <60 ml/min	32.8	25.8	26.6	25.6	0.07
Heart rate, beats/min	79.8 ± 17.8	79.6 ± 17.9	80.3 ± 17.3	80.9 ± 16.8	0.10
Systolic blood pressure, mm Hg	127.9 ± 26.6	127.7 ± 26.0	130.5 ± 26.5	133.0 ± 26.5	0.0019
Left ventricular ejection fraction, %	47.6 ± 12.5	50.2 ± 11.6	50.6 ± 11.8	50.0 ± 10.9	0.0120
Left ventricular ejection fraction <45%	30.0	21.9	21.6	26.3	0.20
Killip class ≥2	19.0	16.1	12.0	14.3	0.08
STB time >360 min	38.6	38.6	38.1	37.8	0.41
Discharge therapy					
Statins	81.4	88.3	90.8	89.0	0.0243
Beta-blockers	71.7	81.3	81.9	81.5	0.0100
ACE inhibitor/ARB	78.4	78.8	83.4	84.0	0.0493

Values are minimal and maximal values for each quartile, mean ± SD, n, %, or median (interquartile range). *p values indicate significant difference across quartiles of serum cholesterol efflux capacity.

Abbreviations as in Table 1.

landmark analysis, we demonstrate that serum cholesterol efflux is an independent marker of short-term prognosis at 30 days after the MI (adjusted HR: 0.32; 95% CI: 0.13 to 0.78; p = 0.012) (Online Figure 1), but also a marker of the long-term prognosis in MI survivors (excluding the patients who died within 48 h of hospitalization) were the association of a more potent serum efflux capacity with survival remained significant (adjusted HR: 0.51; 95% CI: 0.29 to 0.89; p = 0.018) (Online Figure 2).

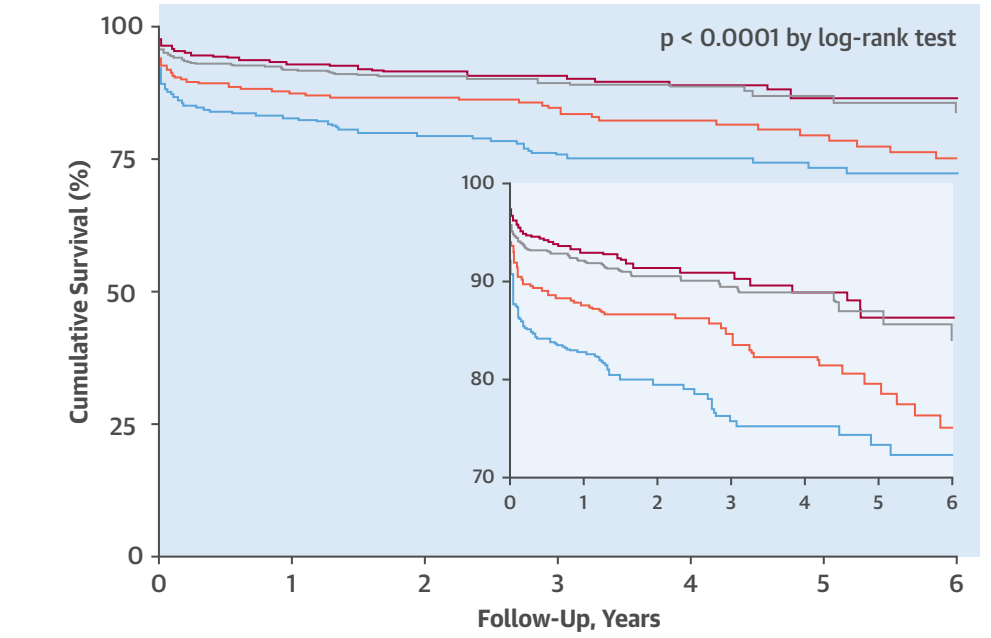
More importantly, among all the lipid parameters including HDL cholesterol, LDL cholesterol, triglycerides, and CETP activity, serum cholesterol efflux capacity was the only factor independently associated with survival when considering both unadjusted and adjusted models (**Figure 3**).

PROGNOSTIC VALUE OF OTHER CHOLESTEROL EFFLUX CAPACITY ASSAYS. Serum cholesterol efflux capacity from human macrophage cell line was the only biomarker to be independently associated with survival in adjusted analyses. Despite being significantly increased in patients who died versus those who survived, neither HDL efflux capacity (apoB-depleted serum used as cholesterol acceptor) (Online Figure 3) nor ABCA1-dependent or SR-BI-dependent serum efflux capacities were independent correlates of survival (**Figure 4**).

DISCUSSION

This study demonstrates for the first time to our knowledge that serum cholesterol efflux capacity,

CENTRAL ILLUSTRATION **Cholesterol Efflux and Mortality in Myocardial Infarction: Kaplan-Meier Cumulative Survival Curve**



No. at Risk

Q1	303		54
Q2	324		59
Q3	341		51
Q4	344		65

	Hazard Ratio	
	Unadjusted	Adjusted*
Q4	0.40 (0.27-0.58)	0.54 (0.32-0.89)
Q3	0.45 (0.31-0.65)	0.76 (0.48-1.21)
Q2	0.71 (0.52-0.97)	0.84 (0.55-1.27)
Q1	Reference	Reference

Guerin, M. et al. J Am Coll Cardiol. 2018;72(25):3259–69.

Mortality at 6 years according to quartiles of serum cholesterol efflux capacity. Patients with impaired serum cholesterol efflux capacity (quartile 1 [Q1]) had a worse prognosis than patients with effective level of cholesterol efflux capacity (Q3, Q4).

a biomarker that integrates circulating contributors and modulators of the initial step of reverse cholesterol transport, is inversely associated with all-cause mortality in a population-based cohort of patients admitted for an acute MI. Patients with the highest serum cholesterol efflux capacity had a marked decrease in all-cause mortality as compared to patients with the lowest serum cholesterol efflux capacity. This association persisted with several models of multivariable adjustment, including age, sex, traditional cardiovascular risk factors, but also circulating LDL, HDL cholesterol, triglycerides levels, and prognostic factors of MI.

Reduced HDL efflux capacity is independently associated with the progression of atherosclerosis measured by the carotid intima-media thickness and the likelihood of angiographic coronary artery disease (5), and was also found to be independent correlates of cardiovascular events in a large population-based cohort, the Dallas Heart Study (6). More recently, we developed a more comprehensive and humanized assay measuring the serum cholesterol efflux capacity and demonstrated its association with premature atherosclerosis (17). The present work goes further and adds to the current knowledge that reduced serum cholesterol efflux capacity

TABLE 3 Significant Predictors of All-Cause Mortality at 6 Years in Univariate and Multivariate Cox Regression Analyses

	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	p Value	HR (95% CI)	p Value
Male	0.69 (0.52–0.91)	0.0078	1.52 (1.04–2.24)	0.0322
Age	2.17 (1.89–2.49)	<0.0001	2.60 (2.06–3.27)	<0.0001
Creatinine	1.19 (1.13–1.26)	<0.0001	1.17 (1.06–1.31)	0.0033
Left ventricular ejection fraction <45%	3.84 (2.95–4.99)	<0.0001	1.94 (1.38–2.71)	0.0001
Killip class ≥2	4.18 (3.18–5.49)	<0.0001	1.64 (1.15–2.34)	0.0067
STB time >360 min	0.91 (0.69–1.19)	0.49	0.63 (0.44–0.90)	0.0116
Out-of-hospital cardiac arrest	6.55 (4.92–8.74)	<0.0001	6.83 (4.34–10.76)	<0.0001
Previous cardiovascular events, previous MACE	1.59 (1.19–2.12)	0.0018	1.25 (0.86–1.80)	0.24
Triglycerides	0.95 (0.84–1.08)	0.46	0.97 (0.79–1.19)	0.77
LDL cholesterol	0.60 (0.52–0.69)	<0.0001	0.91 (0.75–1.10)	0.35
HDL cholesterol	0.91 (0.80–1.04)	0.17	0.98 (0.79–1.20)	0.82
Diabetes	0.90 (0.64–1.26)	0.54	0.71 (0.41–1.25)	0.24
Hypertension	1.43 (1.11–1.84)	0.0062	1.09 (0.77–1.53)	0.63
Obesity	0.84 (0.57–1.23)	0.38	0.73 (0.40–1.33)	0.31
Smoking	0.44 (0.33–0.60)	<0.0001	1.34 (0.88–2.06)	0.17
Statins	0.18 (0.14–0.23)	<0.0001	0.44 (0.27–0.71)	0.0007
Beta-blockers	0.20 (0.15–0.26)	<0.0001	0.52 (0.34–0.78)	0.0015
ACE inhibitor/ARB	0.24 (0.19–0.31)	<0.0001	0.72 (0.46–1.15)	0.17
Cholesterol ester transfer protein activity	0.89 (0.78–1.01)	0.07	1.04 (0.86–1.26)	0.66
Serum cholesterol efflux capacity	0.66 (0.58–0.76)	<0.0001	0.79 (0.65–0.95)	0.0141

CI = confidence interval; HR = hazard ratio; MACE = major adverse cardiac event(s); other abbreviations as in Table 1.

represents a strong predictor of mortality after a MI independently of HDL and LDL cholesterol levels, and despite optimal secondary prevention treatments including statins.

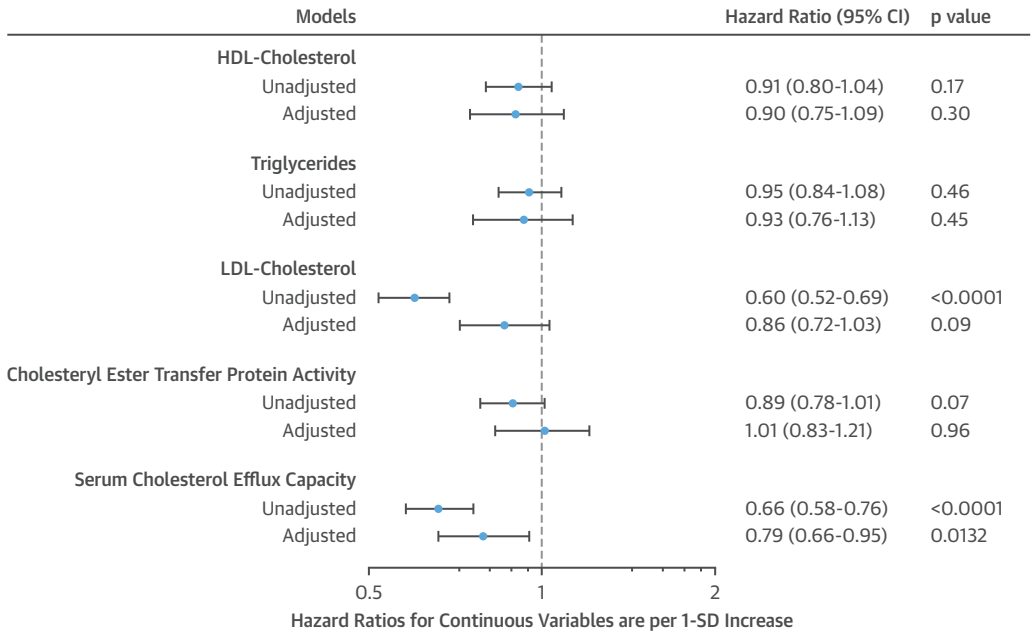
It is established that HDL particles in MI patients display an altered capacity to mediate cholesterol efflux from macrophages and have impaired anti-inflammatory functionality (22). These findings support the contention that dysfunctional HDL particles present within the intravascular compartment can contribute to the reduced serum cholesterol efflux capacity observed in the present study.

Numerous human studies support the concept that a reduction of CETP activity, a protein with a key role in reverse cholesterol transport and HDL remodeling (17), could prevent atherosclerosis (23). However, the inability of CETP inhibitors to decrease cardiovascular outcomes (24–26) may suggest that a residual CETP activity should be maintained in order to preserve its optimal physiological action (27). In our work, we found that reduced CETP activity was not independently related to outcomes despite being associated with a lower serum efflux capacity. Likewise, a large prospective observational trial of patients at high cardiovascular risk demonstrated that a low CETP level constitutes an independent risk factor for all-cause and cardiovascular mortality (26). Such results reinforce the importance of measuring serum cholesterol efflux capacity that integrates intravascular modulators of cholesterol efflux process and appears to be a more promising therapeutic target. Innovative therapies such as the infusion of reconstituted human apolipoprotein A-1, which showed promising results in term of improvement of the cholesterol efflux in post MI patients (10), is being tested on a large scale in the AEGIS-II phase 3 trial (Study to Investigate CSL112 in Subjects With Acute Coronary Syndrome; NCT03473223). The present study validates the hypothesis tested in the AEGIS-II trial, because impaired cholesterol efflux capacity seems to be associated with worse outcome. Whether the reversal of impaired cholesterol efflux will translate into an improvement of the prognosis of post MI patients remains to be demonstrated.

There is no gold standard method for ex vivo evaluation of cholesterol efflux capacity, and considerable differences do exist between research groups (11,28). The ideal cellular model would be primary human macrophages although their suitability for a large cohort is questionable due to considerable interindividual variability. In the present study, we used the human THP-1 macrophage, a model that is more integrative and relevant to human physiopathology measuring serum cholesterol efflux capacity including the contribution of ABCA1 and SR-BI (15,29), apoB-containing lipoproteins (16,30), and CETP activity (17) that are all important key players in the reverse cholesterol transport pathway. Nevertheless, we believe that the human THP-1 macrophage cell line cannot be considered as a superior model for efflux measurement because any cellular model used always represent artificial systems that are not directly reflecting the in vivo situation, but only the capacity of various components of serum to remove cholesterol from cells.

STUDY LIMITATIONS. First, our cohort is relatively modest as compared with previous studies evaluating the capacity of apoB-depleted serum to mediate cholesterol efflux and incidence of cardiovascular events (6) or mortality (8). However, we believe that this is compensated by the fact that our population is on average 20 years older than the population-based cohort previously studied (6,8), that the long follow-up and high event rate of our homogeneous post-MI population is quite high, providing sufficient power to conduct our analysis with multiple adjustments. Second, data on secondary cardiovascular events

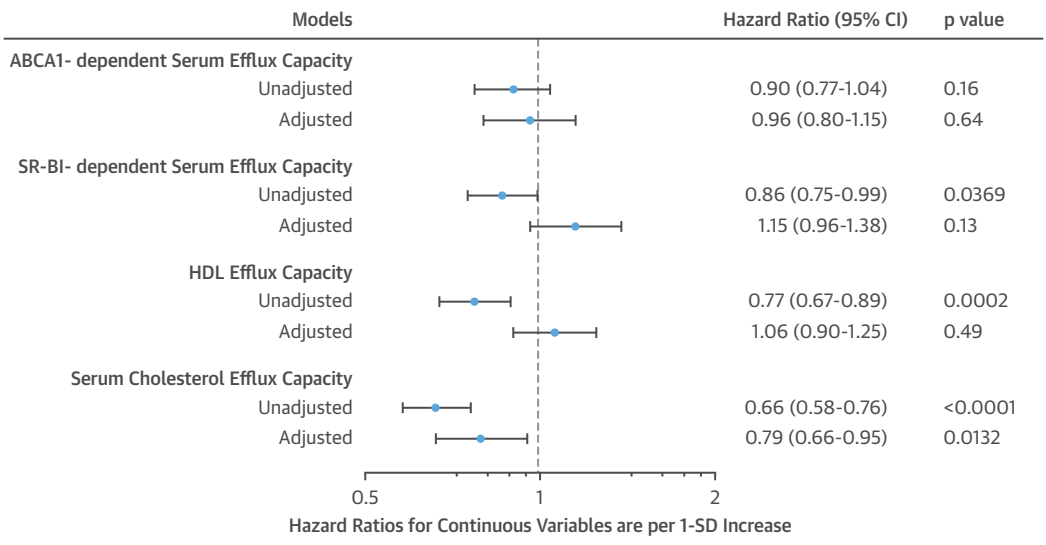
FIGURE 3 **HRs for All-Cause Mortality at 6 Years**



HRs are according to lipid level, CETP activity, and serum cholesterol efflux capacity. HRs for continuous variables are per 1-SD increase. CI = confidence interval; HR = hazard ratio; other abbreviations as in Figure 1.

were not available, and we cannot demonstrate an effect of serum cholesterol efflux capacity on a reduction of recurrent cardiovascular events; however, we believe that all-cause mortality is a stronger endpoint, and it includes cardiovascular mortality, which is largely predominant among the causes of death. Finally, measurements of apolipoprotein A-I were not available for correlation with cholesterol

FIGURE 4 **Impact of Serum Cholesterol Efflux Capacity Measured by Different Assays on All-Cause Mortality at 6 Years**



HRs for continuous variables are per 1-SD increase. Abbreviations as in Figure 3.

efflux capacity; instead, cholesterol ester transfer protein activity was measured as a more clinically relevant cofounding factor.

CONCLUSIONS

The present study demonstrates that serum cholesterol efflux capacity, which reflects flow of cholesterol through multiple intravascular components of the reverse cholesterol transport process, is independently associated with long-term survival in MI patients. These findings indicate that serum cholesterol efflux capacity is a useful biomarker to identify patients at higher risk of mortality after an acute coronary event. It also validates the hypothesis of an ongoing trial aiming to restore cholesterol efflux capacity with an innovative therapy in post-MI patients. Indeed, after the failure of niacin and CETP inhibitors, new treatments aiming to restore or improve serum cholesterol efflux capacity, and/or the overall efficacy of the reverse cholesterol transport pathway are needed to further improve the prognosis of patients who have had a major cardiovascular event.

ACKNOWLEDGMENTS The authors thank the nurses from the Institute of Cardiology for their critical assistance in this study. The authors are indebted to the patients for their cooperation.

ADDRESS FOR CORRESPONDENCE: Prof. Gilles Montalescot, ACTION Study Group, Bureau 1, 2ème étage, Institut de Cardiologie, Hôpital Pitié-Salpêtrière, 47-83 Boulevard de l'Hôpital, 75013 Paris, France. E-mail: gilles.montalescot@aphp.fr. Twitter: @ActionCoeur.

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Serum cholesterol efflux capacity, a biomarker that integrates modulators of reverse cholesterol transport, is associated with atherosclerosis independent of HDL cholesterol level, and impaired cholesterol efflux capacity is associated with increased mortality following acute coronary events.

TRANSLATIONAL OUTLOOK: Prospective trials are needed to assess the efficacy of restoring cholesterol efflux capacity on improving clinical outcomes among survivors of acute myocardial infarction.

REFERENCES

1. Voight BF, Peloso GM, Orho-Melander M, et al. Plasma HDL cholesterol and risk of myocardial infarction: a Mendelian randomisation study. Lancet 2012;380:572–80.

2. Shaw JA, Bobik A, Murphy A, et al. Infusion of reconstituted high-density lipoprotein leads to acute changes in human atherosclerotic plaque. Circ Res 2008;103:1084–91.

3. Tall AR, Rader DJ. Trials and tribulations of CETP inhibitors. Circ Res 2018;122:106–12.

4. Ronsein GE, Heinecke JW. Time to ditch HDL-C as a measure of HDL function? Curr Opin Lipidol 2017;28:414–8.

5. Khera AV, Cuchel M, de la Llera-Moya M, et al. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. N Engl J Med 2011;364:127–35.

6. Rohatgi A, Khera A, Berry JD, et al. HDL cholesterol efflux capacity and incident cardiovascular events. N Engl J Med 2014;371:2383–93.

7. Saleheen D, Scott R, Javad S, et al. Association of HDL cholesterol efflux capacity with incident coronary heart disease events: a prospective case-control study. Lancet Diabetes Endocrinol 2015;3:507–13.

8. Ritsch A, Scharnagl H, März W. HDL cholesterol efflux capacity and cardiovascular events. N Engl J Med 2015;372:1870–1.

9. Liu C, Zhang Y, Ding D, et al. Cholesterol efflux capacity is an independent predictor of all-cause and cardiovascular mortality in patients with coronary artery disease: a prospective cohort study. Atherosclerosis 2016;249:116–24.

10. Gibson CM, Korjian S, Tricoci P, et al. Safety and tolerability of CSL112, a reconstituted, infusible, plasma-derived apolipoprotein A-I, after acute myocardial infarction: the AEGIS-I trial (ApoA-I Event Reducing in Ischemic Syndromes I). Circulation 2016;134:1918–30.

11. Anastasius M, Kockx M, Jessup W, et al. Cholesterol efflux capacity: an introduction for clinicians. Am Heart J 2016;180:54–63.

12. Jessup W, Gelissen IC, Gaus K, Kritharides L. Roles of ATP binding cassette transporters A1 and G1, scavenger receptor BI and membrane lipid domains in cholesterol export from macrophages. Curr Opin Lipidol 2006;17:247–57.

13. Adorni MP, Zimetti F, Billheimer JT, et al. The roles of different pathways in the release of cholesterol from macrophages. J Lipid Res 2007;48:2453–62.

14. Larrede S, Quinn CM, Jessup W, et al. Stimulation of cholesterol efflux by LXR agonists in cholesterol-loaded human macrophages is ABCA1-dependent but ABCG1-independent. Arterioscler Thromb Vasc Biol 2009;29:1930–6.

15. Vergeer M, Korporaal SJ, Franssen R, et al. Genetic variant of the scavenger receptor BI in humans. N Engl J Med 2011;364:136–45.

16. Hoang A, Drew BG, Low H, et al. Mechanism of cholesterol efflux in humans after infusion of reconstituted high-density lipoprotein. Eur Heart J 2012;33:657–65.

17. Villard EF, El Khoury P, Duchene E, et al. Elevated CETP activity improves plasma cholesterol efflux capacity from human macrophages in women. Arterioscler Thromb Vasc Biol 2012;32:2341–9.

18. Gall J, Frisdal E, Bittar R, et al. Association of cholesterol efflux capacity with clinical features of metabolic syndrome: relevance to atherosclerosis. J Am Heart Assoc 2016;5:e004808.

19. Villard EF, El Khoury P, Frisdal E, et al. Genetic determination of plasma cholesterol efflux capacity is gender-specific and independent of HDL-cholesterol levels. Arterioscler Thromb Vasc Biol 2013;33:822–8.

20. El Khoury P, Waldmann E, Huby T, et al. Extended-release niacin/laropiprant improves overall efficacy of postprandial reverse cholesterol transport. Arterioscler Thromb Vasc Biol 2016;36:285–94.

21. Silvain J, Nguyen LS, Spagnoli V, et al. Contrast-induced acute kidney injury and mortality in ST elevation myocardial infarction treated with primary percutaneous coronary intervention. Heart 2018;104:767–72.

22. Annema W, Willemsen HM, de Boer JF, et al. HDL function is impaired in acute myocardial infarction independent of plasma HDL cholesterol levels. J Clin Lipidol 2016;10:1318–28.

23. Le Goff W, Guerin M, Chapman MJ. Pharmacological modulation of cholesteryl ester transfer protein, a new therapeutic target in atherogenic dyslipidemia. Pharmacol Ther 2004;101:17–38.

24. Barter PJ, Caulfield M, Eriksson M, et al. Effects of torcetrapib in patients at high risk for coronary events. N Engl J Med 2007;357:2109–22.

25. Schwartz GG, Olsson AG, Abt M, et al. Effects of dalcetrapib in patients with a recent acute coronary syndrome. N Engl J Med 2012;367:2089–99.

26. Lincoff AM, Nicholls SJ, Riesmeyer JS, et al. Evacetrapib and cardiovascular outcomes in high-risk vascular disease. N Engl J Med 2017;376:1933–42.

27. Schwartz CC, VandenBroek JM, Cooper PS. Lipoprotein cholesteryl ester production, transfer, and output in vivo in humans. J Lipid Res 2004;45:1594–607.

28. Rohatgi A. High-density lipoprotein function measurement in human studies: focus on cholesterol efflux capacity. Prog Cardiovasc Dis 2015;58:32–40.

29. Williams DL, Connelly MA, Temel RE, et al. Scavenger receptor BI and cholesterol trafficking. Curr Opin Lipidol 1999;10:329–39.

30. Tréguier M, Moreau M, Sposito A, Chapman MJ, Huby T. LDL particle subspecies are distinct in their capacity to mediate free cholesterol efflux via the SR-BI/Cla-1 receptor. Biochim Biophys Acta 2007;1771:129–38.

KEY WORDS CETP, cholesterol efflux, HDL cholesterol, mortality, myocardial infarction

APPENDIX For an expanded Methods section as well as a supplemental table and figures, please see the online version of this paper.