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Low-density lipoprotein electronegativity and risk of death after acute coronary syndromes: A case-cohort analysis

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ABSTRACT

Background and aims: Low-density lipoprotein (LDL)-cholesterol (LDL-C) promotes atherosclerotic cardiovascular disease (ASCVD), with changes in LDL electronegativity modulating its pro-atherogenic/pro-thrombotic effects. Whether such alterations associate with adverse outcomes in patients with acute coronary syndromes (ACS), a patient population at particularly high cardiovascular risk, remains unknown.

Methods: This is a case-cohort study using data from a subset of 2619 ACS patients prospectively recruited at four university hospitals in Switzerland. Isolated LDL was chromatographically separated into LDL particles with increasing electronegativity (L1-L5), with the L1-L5 ratio serving as a proxy of overall LDL electronegativity. Untargeted lipidomics revealed lipid species enriched in L1 (least) vs. L5 (most electronegative subfraction). Patients were followed at 30 days and 1 year. The mortality endpoint was reviewed by an independent clinical endpoint adjudication committee. Multivariable-adjusted hazard ratios (aHR) were calculated using weighted Cox regression models.

Results: Changes in LDL electronegativity were associated with all-cause mortality at 30 days (aHR, 2.13, 95% CI, 1.07–4.23 per 1 SD increment in L1/L5; p=.03) and 1 year (1.84, 1.03–3.29; p=.04), with a notable association with cardiovascular mortality (2.29; 1.21–4.35; p=.01; and 1.88; 1.08–3.28; p=.03). LDL electronegativity superseded several risk factors for the prediction of 1-year death, including LDL-C, and conferred improved discrimination when added to the updated GRACE score (area under the receiver operating characteristic curve

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0.74 vs. 0.79, p=.03). Top 10 lipid species enriched in L1 vs. L5 were: cholesterol ester (CE) (18:2), CE (20:4), free fatty acid (FA) (20:4), phosphatidyl-choline (PC) (36:3), PC (34:2), PC (38:5), PC (36:4), PC (34:1), triacylglycerol (TG) (54:3), and PC (38:6) (all $p < .001$), with CE (18:2), CE (20:4), PC (36:3), PC (34:2), PC (38:5), PC (36:4), TG (54:3), and PC (38:6) independently associating with fatal events during 1-year of follow-up (all $p < .05$).

Conclusions: Reductions in LDL electronegativity are linked to alterations of the LDL lipidome, associate with all-cause and cardiovascular mortality beyond established risk factors, and represent a novel risk factor for adverse outcomes in patients with ACS. These associations warrant further validation in independent cohorts.

1. Introduction

The identification of novel cardiovascular risk factors is limited by tradeoffs between statistical power, resource availability (e.g., complexity of sample preparation and analysis), and costs. Case-cohort studies have emerged as an attractive epidemiological approach to study the association between exposures and disease outcomes [1,2], particularly when a full-cohort design is not feasible due to resource constraints [3]. The case-cohort design, first proposed by Prentice [4], delineates the framework of an observational study in which a random subset of the full cohort is selected, while all newly occurring cases within the original cohort are concurrently included. This study design provides high efficiency and flexibility enabling the cost-effective investigation of multiple exposures while minimizing the risk of selection bias owing to outcome-dependent sampling, as it may occur in nested case-control studies, providing precise estimates of exposure-outcome associations of the full-cohort [3,5].

Low-density lipoprotein (LDL) indubitably promotes the initiation and progression of atherosclerotic cardiovascular disease (ASCVD), with alterations in LDL quality representing an important but understudied determinant of ASCVD risk [6–8]. In patients with a recent acute coronary syndrome (ACS) or established ASCVD, interventions that lower levels of LDL-cholesterol (LDL-C) improve cardiovascular outcomes, but the residual risk remains high [9,10]. Observational studies examining the relationship between baseline LDL-C levels and mortality in patients with ACS have yielded discordant outcomes: one study has reported a counterintuitive inverse relationship [11], while others did not establish any association [12,13], potentially due to other factors influencing short-to-mid-term outcomes post-ACS. Whilst traditional cardiovascular risk factors, such as those informing the Framingham risk score [14], are undeniably linked to long-term risk of cardiovascular events, biomarkers other than cholesterol levels have been shown to determine 1-year outcomes after the index ACS [12,15,16].

LDL particles ship their water-insoluble lipid cargo in a polar shell of apolipoproteins which serve as a molecular fingerprint to direct them to specific cell types. A single apolipoprotein (apo) B_{100} molecule encircles the LDL particle and stabilizes the outer unilamelar layer consisting of amphiphilic phospholipids, sphingolipids and unesterified cholesterols, with its hydrophobic core containing a conglomerate of cholesteryl esters and triacylglycerols [17]. Beyond the presence of additional proteins (e.g., apoC-III) and the degree of sialylation, lipid composition represents a major determinant of LDL charge [18], with electronegative properties of LDL particles impinging on their pro-atherogenic and pro-thrombotic effects [19,20], the latter being particularly relevant in patients with a recent ACS. However, the association of altered LDL electronegativity and mortality in these patients is uncertain, and data on corresponding changes in the LDL particles' lipidome remain limited.

To address this knowledge gap, we aimed (1) to determine the associations of LDL electronegativity with all-cause and cardiovascular mortality, (2) to test its predictive utility beyond and above established risk scores, and (3) to study the lipidome of least (L1) and most electronegative (L5) LDL particles in patients with ACS who were prospectively recruited at four university hospitals in Switzerland.

2. Patients and methods

2.1. Study design and participants

This is a case-cohort study nested within the prospective, multicentre SPUM-ACS study. The design of case-cohort studies [1–5], and the study population have been described previously [12,16,21]. Briefly, from October 2012 until December 2017 a total of 2619 patients with ACS aged ≥18 years presenting within 5 days after pain onset were recruited at four university hospitals in Switzerland (SPUM-ACS study; Cohort II; ClinicalTrials.gov Identifier: NCT01000701). EDTA-plasma samples were obtained prior to coronary angiography. Data on baseline demographics, risk factors, and medication were entered by trained personnel using a centralized data entry system. All patients were followed at 30 days and 1 year. The mortality endpoint was reviewed by an independent event adjudication committee comprising 3 certified cardiologists using predefined adjudication forms and blinded to patients' baseline characteristics. Of all 1272 patients with follow-up data and ≥2.0 ml EDTA-plasma for fast protein liquid chromatography (FPLC) available, a subcohort was drawn using simple random sampling. The oversampling of cases inherent to the case-cohort design was accounted for by fitting weighted Cox proportional hazard regression models [22,23]. All participants provided informed consent. The study was conducted according to the Declaration of Helsinki, and was approved by the institutional review board. We followed the principles outlined by the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) initiative for the reporting of cohort studies. The STROBE checklist and details on case-cohort sampling methods are provided in the Supplemental Material.

2.2. Assessment of LDL-cholesterol, electronegativity and lipidome

Informed by standard lipid panels, levels of low-density lipoprotein cholesterol (LDL-C) were calculated using the Sampson equation [24]. Isolation of LDL was achieved by sequential KBr-based ultracentrifugation (1.019–1.063 g/mL), and dissolved into its 5 subfractions with increasing electronegativity (L1 to L5) using anion-exchange columns on a fast protein liquid chromatography (FPLC) system [19,20,25]. The effluent, yielded by a multistep sodium chloride gradient, was monitored at 280 nm, and concentrations of L1 and L5 (least and most electronegative subfraction, respectively) were calculated, as previously described [19,20,25], with the L1-L5 ratio serving as a proxy of overall LDL electronegativity. Least and most electronegative LDL particles (L1 and L5, respectively) of a subcohort of patients were subjected to untargeted lipidomics, as previously reported (Supplemental Material) [26]. Only lipid species with known annotation (according to LIPID MAPS® Structure Database; accessed on March 22, 2022; Supplemental Table 1) were considered for statistical analyses [27]. LDL electronegativity and lipidomics data were generated by blinded study personnel.

2.3. Statistical analysis

Continuous data are presented as mean and standard deviations (SD) or median and interquartile ranges (IQRs), and categorical variables as counts and percentages (%). Given the oversampling of cases, covariate

balance was assessed by calculating standardized mean differences (SMD). We estimated crude (HR) and adjusted hazard ratios (aHR) with 95% confidence intervals (CIs) for 30-day and 1-year mortality per SD increment of each biomarker in the full cohort by fitting weighted Cox proportional hazard regression models [22,23]. Model 1 represents the crude regression model, model 2 includes sex (categorical) and age (continuous), and model 3 includes sex (categorical), age (continuous), high-sensitivity cardiac troponin T (hs-cTnT; continuous), diabetes (categorical), estimated glomerular filtration rate (eGFR; continuous), statin use (categorical), and LDL-C levels (continuous), as specified in the figure legend. Model 4 additionally accounts for GRACE 2.0 risk for 1-year death (<3% low, 3–8% intermediate and >8% high risk) [16,28]. In sensitivity analyses, all models were additionally adjusted for the time elapsed between symptom onset and blood sampling (continuous) (Supplemental Material). Where data on covariates in the respective regression models were missing (Supplemental Table 2), we applied multiple imputation by chained equations (MICE; Supplemental Tables 3–5). Independent variables included in the multivariable-adjusted regression models were ranked based on their Wald χ^2 value, as reported [1]. Potential effects of MICE on main results were explored in additional sensitivity analyses using complete cases (Supplemental Tables 5 and 7). To assess whether the L1-L5 ratio

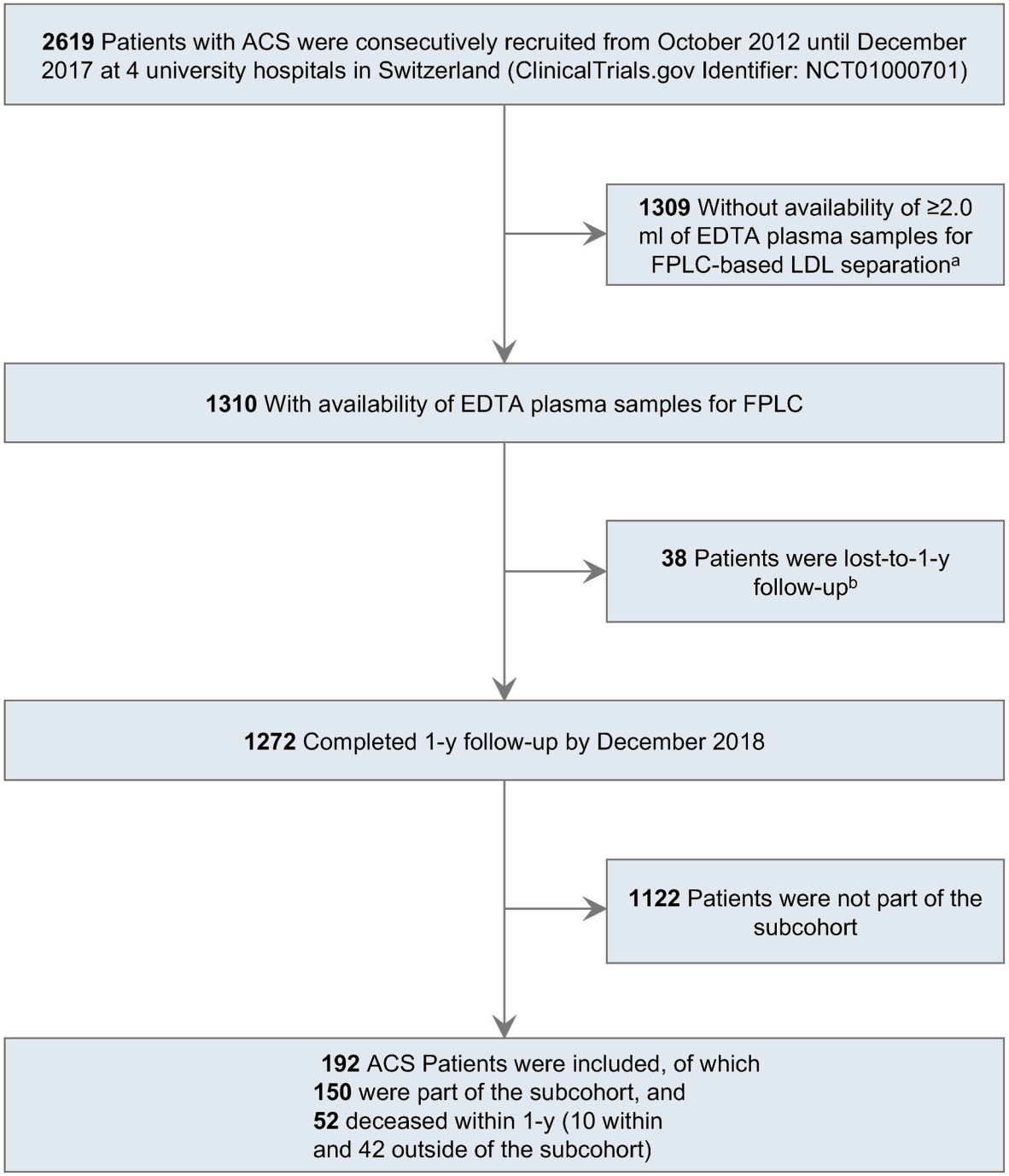

Fig. 1. Flow diagram of the SPUM-ACS case-cohort study.
[a]Defined as grossly haemolytic or plasma volume <2.0 ml. [b]Patients who failed to complete the study such as those who withdrew consent or were lost to follow-up were censored at the date of last contact or the date of the assessment of the survival status, whichever occurred later.

improves the performance of the GRACE 2.0 or Framingham risk score to predict 1-year death while accounting for different sampling weights, time-dependent area under the receiver operating characteristic curve (AUC) and goodness of fit (likelihood function) of both risk prediction models (i.e., GRACE 2.0 or the Framingham and the biomarker-enhanced GRACE 2.0 or Framingham model) were calculated following stratified superpopulation sampling [14,15,29]. The difference in the AUC at 1 year was calculated using a resampling approach with 5000 replicates [30]. Lipidomics data were analyzed using the R package MetaboDiff (version 0.9.5) [31], in which correction for multiple comparisons was achieved by the Benjamini Hochberg method [32]. Additional analyses were focused on the associations of top 10 lipid species enriched in least (L1) electronegative LDL particles with outcomes of interest by fitting conditional logistic regression models, further adjusting for sex (categorical) and age (continuous) (Supplemental Tables 7–8). Statistical significance for all analyses was established at $p < .05$. All analyses were performed in R version 4.2.1. A detailed description is provided in the Supplemental Material.

3. Results

3.1. Study population

The present case-cohort study is based on data from a subset of 2619 patients with ACS recruited between October 17, 2012 and December 31, 2017. Among the final study population comprising 1272 patients a total of 52 deaths (4.09%) occurred within 1 year of follow-up (Fig. 1). At baseline, cases differed in several clinical features associated with increased cardiovascular risk as compared to patients sampled in the subcohort (Table 1). Patients who had a fatal event during follow-up presented at a higher age (median [IQR], 76.45 (67.67–82.22) and 73.95 (65.58–80.97) years; SMD, 0.22), with lower estimated glomerular filtration rate (eGFR; 62.16 (44.66–78.86) and 78.15 (59.26–86.96) ml/min/1.73 m^2, 0.55) and were more often diabetics (n (%), 22 (42) and 45 (30), 0.26). Moreover, they showed higher LDL-C levels (102.03 (72.52–123.19) and 87.66 (67.05–119.46) mg/dl, 0.19), were more frequently assigned to the GRACE high-risk group (37 (82) and 70 (54), 0.75) and had a notable shift in the electronegative properties of isolated LDL particles, implying the presence of less negatively charged LDL (L1-L5 ratio, 4.79 (2.80–8.55) and 3.83 (2.03–7.73), 0.236).

Table 1
Baseline characteristics of SPUM-ACS case cohort participants.

Characteristics	No. (%)				
	All				
	Subcohort	All-cause death[a]	SMD	Cardiovascular death[a]	SMD
	(n = 150)	(n = 52)		(n = 48)	
Age, y	73.95 (65.58–80.97)	76.45 (67.67–82.22)	0.218	75.05 (67.05–82.22)	0.159
Female	43 (28.7)	16 (30.8)	0.046	14 (29.2)	0.011
ST-segment deviation	75 (55.1)	26 (55.3)	0.003	25 (56.8)	0.034
Current smoker	41 (27.7)	11 (23.9)	0.087	11 (26.2)	0.034
Estimated glomerular filtration rate, ml/min/1.73 m^2	78.15 (59.26–86.96)	62.16 (44.66–78.86)	0.550	64.39 (48.88–78.86)	0.505
Type 2 diabetes	45 (30.0)	22 (42.3)	0.258	20 (41.7)	0.245
Dyslipidemia	98 (65.3)	32 (61.5)	0.079	30 (62.5)	0.059
Hypertension	105 (70.0)	39 (75.0)	0.112	35 (72.9)	0.065
Previous percutaneous intervention	32 (21.3)	12 (23.1)	0.042	11 (22.9)	0.038
Previous coronary artery bypass grafting	12 (8.0)	5 (9.6)	0.057	4 (8.3)	0.012
Family history of coronary artery disease	15 (10.1)	5 (9.8)	0.009	4 (8.5)	0.054
Peripheral arterial disease	17 (11.3)	6 (11.5)	0.006	5 (10.4)	0.029
Cerebrovascular disease	9 (6.0)	3 (5.8)	0.010	3 (6.2)	0.010
Heart failure	3 (2.0)	1 (1.9)	0.006	1 (2.1)	0.006
History of malignancy	12 (8.0)	7 (13.5)	0.177	4 (8.3)	0.012
High-sensitivity cardiac troponin, ng/l	256.00 (86.75–715.50)	449.00 (185.50–1270.00)	0.186	453.00 (216.00–1270.00)	0.192
Lipid panel					
Total cholesterol, mg/dl	150.81 (128.29–192.09)	154.29 (132.25–181.75)	0.126	160.48 (132.25–181.75)	0.106
LDL-C, mg/dl	87.66 (67.05–119.46)	102.03 (72.52–123.19)	0.191	103.21 (73.75–123.19)	0.179
LDL-subfractions[b]					
L1 LDL, %	64 (47–78)	66 (53–85)	0.235	66 (54–81)	0.267
L2 LDL, %	4 (2–10)	3 (2–9)	0.112	3 (2–9)	0.134
L3 LDL, %	4 (1–12)	4 (1–11)	0.014	4 (1–10)	0.017
L4 LDL, %	2 (0–11)	1 (0–10)	0.225	1 (0–10)	0.232
L5 LDL, %	16 (10–25)	14 (9–23)	0.227	14 (9–23)	0.240
Overall LDL electronegativity					
L1-L5 ratio	3.83 (2.03–7.73)	4.79 (2.80–8.55)	0.236	4.79 (2.80–8.55)	0.232
Medication at presentation					
Aspirin	59 (48.8)	23 (56.1)	0.147	20 (54.1)	0.106
ACEi or ARB	75 (62.0)	28 (68.3)	0.133	25 (67.6)	0.117
Betablocker	45 (37.2)	20 (48.8)	0.236	17 (45.9)	0.178
Statin	64 (52.9)	18 (43.9)	0.181	17 (45.9)	0.139
GRACE 2.0 risk category for 1-y death[c]			0.657		0.745
Low-risk	15 (11.5)	1 (2.2)		1 (2.4)	
Intermediate-risk	45 (34.6)	7 (15.6)		5 (11.9)	
High-risk	70 (53.8)	37 (82.2)		36 (85.7)	

Continuous data are mean (SD) or median (IQR) if skewed and categorical data are n (%). ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; GRACE 2.0, Global Registry of Acute Coronary Events; HDL-C, high-density lipoprotein cholesterol; hs-cTnT, high-sensitivity cardiac troponin; LDL-C, low-density lipoprotein cholesterol, SMD standardized mean difference. SI conversion factors: To convert cholesterol levels to millimoles per liter, multiply by 0.0259. The number of events occurring within/outside the subcohort are specified in Fig. 1.

[a] Includes cases within and outside the subcohort and study participants may have had multiple incident events.

[b] Percentages may not total 100 owing to rounding.

[c] Defined as <3% (low-risk), 3–8% (intermediate-risk), or >8% (high-risk).

3.2. Independent association of altered LDL electronegativity and fatal outcomes

In unadjusted analyses, reductions in overall LDL electronegativity, as assessed by the L1-L5 ratio, were strongly linked to mortality from any and cardiovascular causes at both 30 days (hazard ratio (HR), 2.18, 95% CI, 1.28–3.69, p=.0041; and 2.31, 1.37–3.90 per 1 SD increment, p=.0020) and 1 year (aHR, 1.91, 1.20–3.04, p=.0065; and 1.89, 1.19–3.02 per 1 SD increment, p=.0077), which remained consistent in multivariable-adjusted analyses accounting for established clinical risk factors (adjusted [a]HR, 2.13, 1.07–4.23, p=.032; and 2.29, 1.21–4.35, p=.011; 1.84, 1.03–3.29, p=.038; and 1.88, 1.08–3.28, p=.027; Fig. 2A and B; Supplemental Table 3). These associations were robust in sensitivity analyses considering an alternative set of potential confounders, including the time elapsed between symptom onset and blood sampling (aHR, 2.18, 1.09–4.39, p=.029; 1.86, 1.05–3.31, p=.033; 2.34, 1.19–4.60, p=.015; 1.89, 1.07–3.33, p=.028; Supplemental Table 4). Complete-case analyses yielded similar results (Supplemental Table 5). Conversely, no associations between baseline LDL-C levels and all-cause or cardiovascular mortality were observed at 30 days or 1 year (Supplemental Fig. 1; Supplemental Tables 6–7).

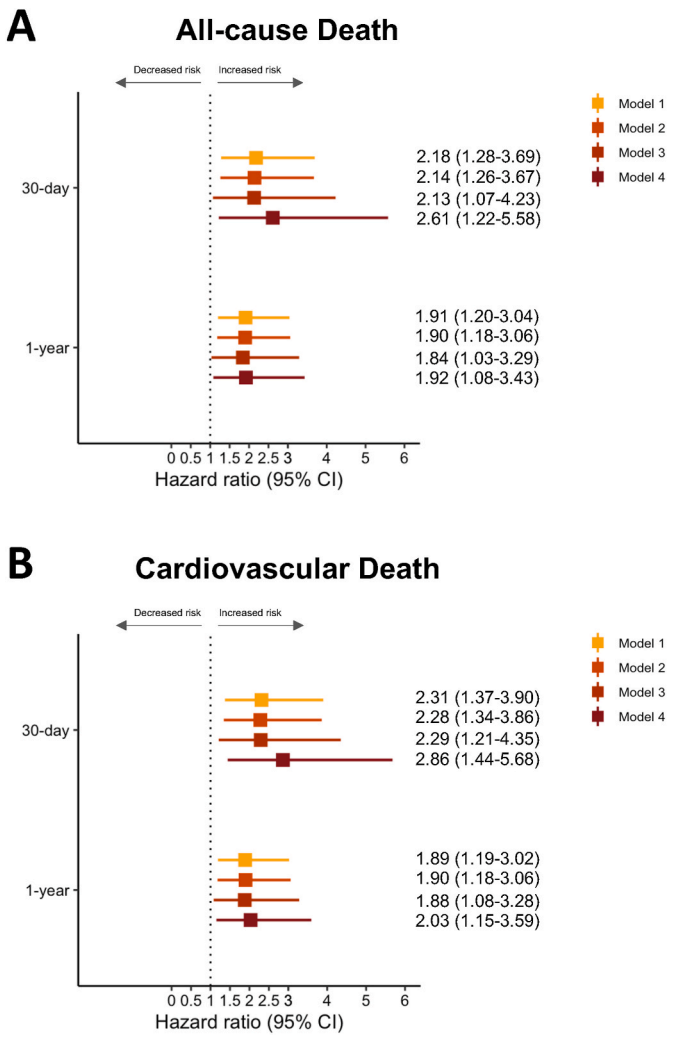

Fig. 2. Associations of reduced LDL electronegativity with mortality.
Data are HRs and 95% confidence intervals of death from any (A) or cardiovascular causes (B) at 30 days and 1 year per SD increment in the L1-L5 ratio. Model 1 represents the crude regression model; model 2 includes sex (categorical) and age (continuous); model 3 includes sex (categorical), age (continuous), high-sensitivity cardiac troponin T (continuous), diabetes (categorical), estimated glomerular filtration rate (continuous), statin use (categorical), and levels of LDL-C (continuous); model 4 includes sex (categorical), age (continuous), high-sensitivity cardiac troponin T (continuous), diabetes (categorical), estimated glomerular filtration rate (continuous), statin use (categorical), levels of LDL-C (continuous), and GRACE 2.0 1-year mortality risk estimates (categorical).

3.3. LDL electronegativity and 1-year mortality risk beyond and above the updated GRACE or Framingham risk score

Among all variables included in the multivariable-adjusted regression model to predict 1-year outcomes, including established clinical risk factors, such as LDL-C, the L1-L5 ratio was the second highest-ranked predictor of death from any cause (Fig. 3A), and superseded several clinical risk factors for the prediction of cardiovascular death (Fig. 3B). Notably, changes in LDL electronegativity associated with 1-year mortality risk beyond the one estimated by GRACE 2.0 (aHR, 1.92, 1.08–3.43, p=.027), suggesting a potential predictive utility of this biomarker over and above the updated GRACE risk score. Indeed, adding the L1-L5 ratio to the GRACE model increased its goodness of fit (p < .001) and resulted in improved discriminatory performance as compared to the original GRACE 2.0 risk score (AUC, 0.78 vs. 0.74, p=.03; Fig. 4A). Conversely, adding the L1-L5 ratio to the Framingham risk score did not improve its discriminatory performance (AUC, 0.59 vs. 0.62, p=.83; Fig. 4B).

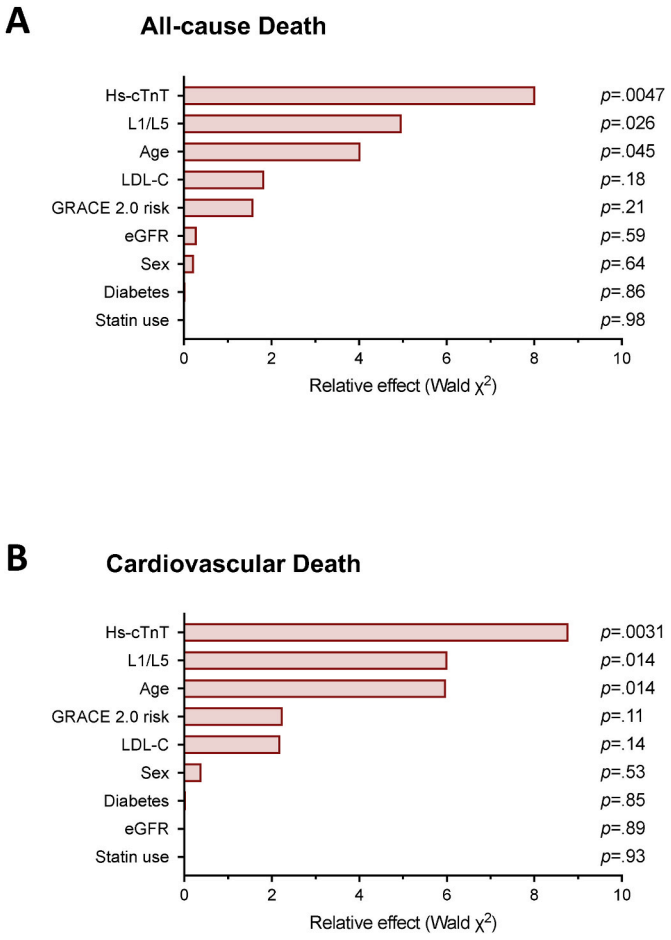

Fig. 3. Relative effect of each independent variable on model output.
Ranking of clinical features associated with increased cardiovascular risk by their effect on the prediction of 1-year death from any (A) and cardiovascular causes (B).

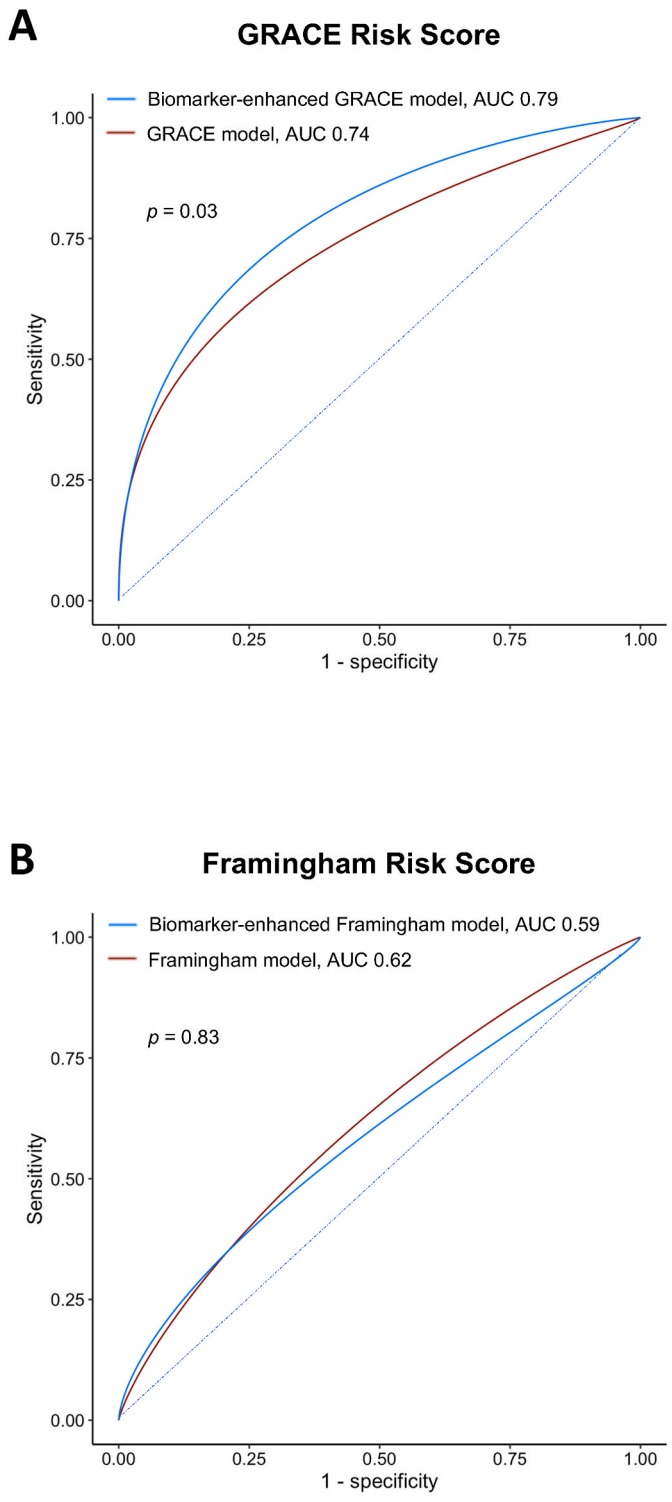

Fig. 4. Smoothed receiver operating characteristic curve of the GRACE 2.0 and biomarker (L1-L5 ratio)-enhanced GRACE 2.0 risk score (A) or Framingham and biomarker-enhanced Framingham risk score (B) for the prediction of 1-year death. Paired ROC curves were compared by bootstrapping using 5000 replicates. GRACE denotes global registry of acute coronary events.

3.4. Lipidome of LDL particles differs according to their electronegative properties and determines mortality risk

Lipidomic analyses of least (L1) and most (L5) electronegative LDL particles unveiled abundancy of fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, and sterol lipids (Fig. 5; Supplemental Table 1). The top 10 differentially expressed lipid species, ranked by the mean difference between least (L1) and most (L5) electronegative LDL particles, were: cholesterol ester (CE) (18:2), CE (20:4), free fatty acid (FA) (20:4), phosphatidylcholine (PC) (36:3), PC (34:2), PC (38:5), PC (36:4), PC (34:1), triacylglycerol (TG) (54:3), and PC (38:6) (all $p < .001$; Supplemental Table 8). In exploratory analyses, 8 out of these 10 lipid species enriched in L1 LDL showed an independent association with mortality from any and cardiovascular causes at 1 year (CE (18:2), adjusted odds ratio (aOR), 4.23 and 3.99; CE (20:4), 6.21 and 5.44; PC (36:3), 5.32 and 4.86; PC (34:2), 4.76 and 4.36; PC (38:5), 9.05 and 7.63; PC (36:4), 5.34 and 4.75; TG (54:3), 4.66 and 4.25; PC (38:6), 5.48 and 5.03 per 1 SD increment in each lipid species; $p < .05$; Supplemental Table 9).

4. Discussion

In a prospective cohort of contemporary patients with ACS, we demonstrate for the first time that attenuated LDL electronegativity, as assessed by the L1-L5 ratio, unfavorably associates with all-cause and cardiovascular mortality after the index ACS, over and above the updated GRACE risk score. Moreover, our data suggest that the lipidome of LDL particles markedly differs depending on their charge, with the majority of highest-ranked lipid species independently associating with adverse events. Importantly, LDL electronegativity represented the second highest-ranked predictor of all-cause death (Fig. 3A), and superseded several clinical risk factors for the prediction of cardiovascular death at 1 year (Fig. 3B). While the exact mechanism underpinning this phenomenon warrants further study, it is interesting to note that electronegative properties of LDL particles determine their LDL-receptor affinity, with changes in LDL charge impinging on their prothrombotic and pro-atherogenic effects [19,20].

Despite contemporary advances to further decrease LDL-C, the residual risk of death in patients following a recent ACS remains substantial [9,10]. In fact, while PCSK9 inhibition on top of statin-therapy associates with a marked risk reduction in myocardial infarction, ischemic stroke, and coronary revascularization, the short-to-mid-term mortality benefit of aggressive LDL-C lowering appears marginal [33]. In the present study, baseline LDL-C levels showed no association with all-cause or cardiovascular mortality at 1 year after the index ACS, yet hazard ratios nominally increased across follow-up periods within each model (Supplemental Tables 6–7; Supplemental Fig. 1), suggesting a potential association at long-term follow-up. Whilst interleukin-6 (IL6)-driven LDL-receptor upregulation may attenuate a potential association in the acute setting [34], results remained intriguingely consistent after controlling for high-sensitivity cardiac troponin T (hs-cTnT), the latter showing release kinetics similar to those of IL6 following acute myocardial ischaemia [35].

Importantly, the L1-L5 ratio provided additive predictive utility beyond the updated GRACE (Fig. 4A) but not Framingham risk score, emphasizing the importance of this biomarker for the prediction of adverse events in patients with ACS. Indeed, while the Framingham risk score shows good discriminatory performance for the prediction of 10-year cardiovascular disease (CVD) events (defined as a composite of clinical events related to coronary heart disease, cerebrovascular disease, peripheral artery disease, and heart failure) in individuals free of CVD [14], it performs less well in patients with established ASCVD, with a c statistic of 0.59 to predict 10-year mortality in patients with ACS [36], as similarly observed in magnitude in the present analysis focusing on 1-year outcomes (Fig. 4B). This might be due to several factors, including the different study population (healthy individuals vs. patients with ACS), distinct endpoints (10-year CVD events vs. 1-year mortality), and different study settings (primary vs. secondary prevention). As such, the GRACE risk score, derived and validated in patients with ACS [15], represents the primary tool to assess mortality risk in our study population.

Our study reinforces the concept of LDL quality as an important

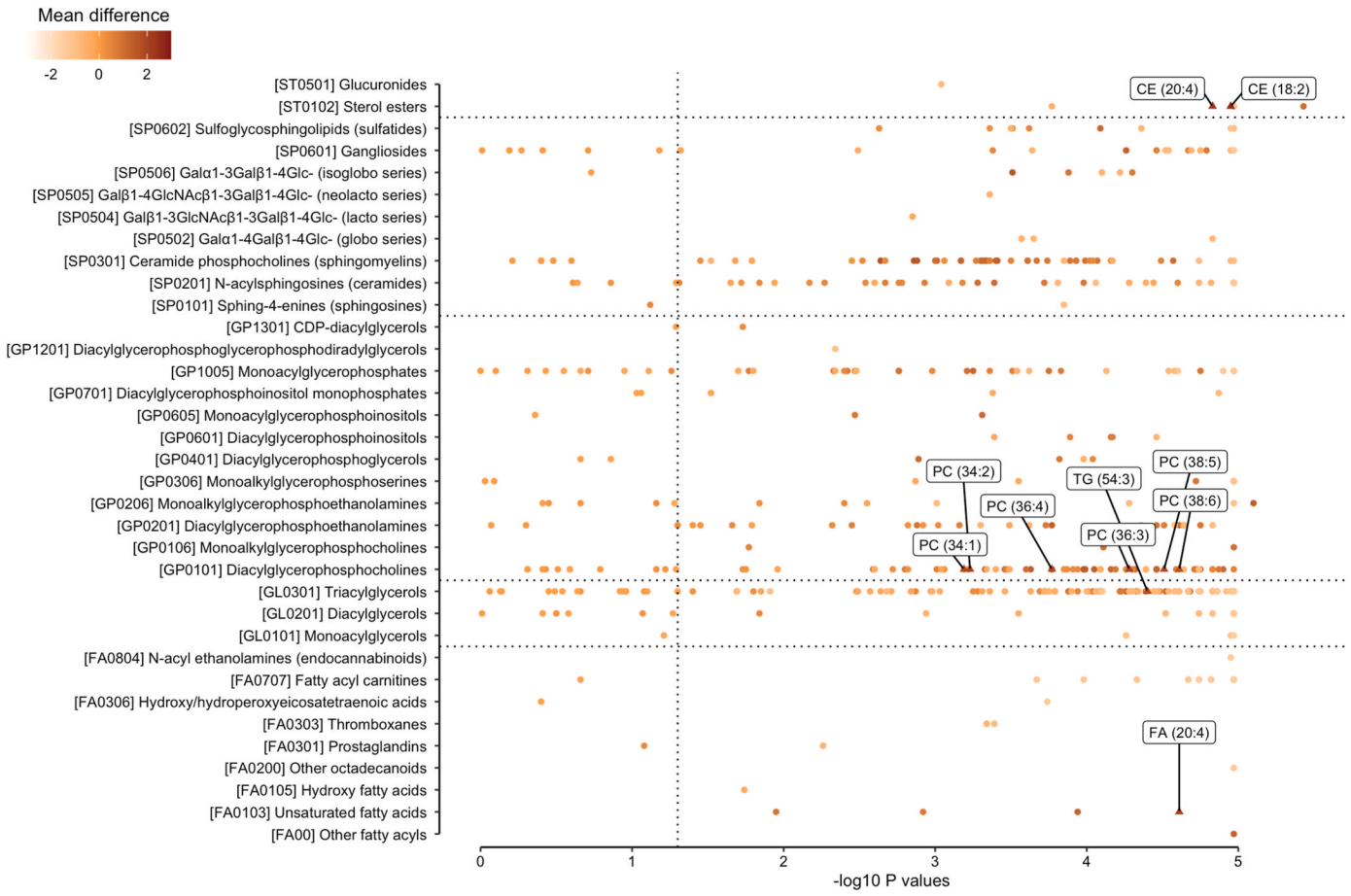

Fig. 5. Customized Manhattan plot for the comparative analysis of 482 annotated lipid species of least (L1) *vs.* most electronegative (L5) LDL particles. Individual lipid species arranged by 25 lipid classes (y-axis) and -log10 *p* values (x-axis) for their association with L1 *vs.* L5 LDL particles. The threshold of the false discovery rate of less than 0.05 is signified by the vertical dashed line. Color indicates the magnitude of mean difference in normalized expression with triangularly shaped dots representing the 10 highest-ranked lipid species. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

determinant of outcomes in patients with established ASCVD [6], a patient population at particularly high cardiovascular risk. In fact, lipidomic analyses revealed an enrichment in atherogenic lipid species in least (L1) electronegative lipid particles (Supplemental Table 8), with the majority thereof independently associating with risk of mortality from any and cardiovascular causes (Supplemental Table 9). For instance, L1 was found to be highly enriched in cholesterol ester (CE) (18:2) and CE (20:4), lipid species known to be abundantly expressed in atherosclerotic plaques [37,38]. Of note, LDL particles rich in CE (18:2) are susceptible to aggregate and thus become entrapped subendothelially [6], with oxidatively modified derivates driving chronic inflammatory processes within the vessel wall [39], thereby providing a potential soil for progressive ASCVD.

To the best of our knowledge, this is the first cohort study examining the relationship of LDL electronegativity with adverse outcomes in patients with established ASCVD. The independent association of the L1-L5 ratio with multiple endpoints using different regression models that account for a variety of potential confounders, and its additive predictive value beyond an established risk prediction model provide high internal validity of our findings. Nonetheless, external validation studies are warranted to confirm the independent association of reduced LDL electronegativity with mortality in patients with ACS.

4.1. Conclusions

In contemporary patients with ACS, a reduction in overall LDL electronegativity associates with all-cause and cardiovascular mortality beyond established risk factors, including LDL-C, and provides additional prognostic utility beyond the updated GRACE score (Fig. 6). Electronegative properties of LDL particles are tightly linked to alterations in their lipidome and represent a novel determinant of mortality risk in patients with ACS. These findings should stimulate further research into LDL quality as a potential risk factor of ASCVD initiation and accelerated disease progression. Ultimately, these efforts may open novel therapeutic avenues that go beyond LDL-C lowering for secondary prevention of ASCVD.

4.2. Strengths and limitations

To the best of our knowledge, this is the first cohort study on LDL electronegativity in patients with ACS. Our study has several strengths, including that it is based on a large, multicentre, prospective cohort of contemporary patients with ACS, with robust follow-up data on prespecified end points, and independent clinical event adjudication [16,21]. By applying a case-cohort design, we could optimize study resources while preserving the benefits of cohort studies to make patient population-based inferences [3]. The present study has several

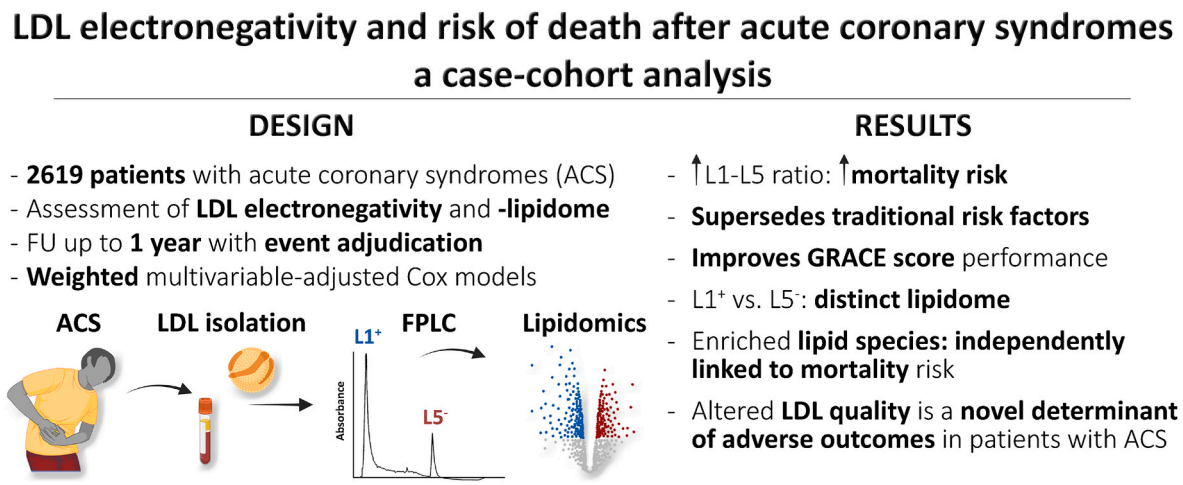

Fig. 6. In prospectively recruited patients with acute coronary syndromes (ACS), a reduction in overall low-density lipoprotein (LDL) electronegativity emerged as a strong predictor of all-cause and cardiovascular mortality independent of established risk factors, including LDL-C, and provided additional prognostic utility beyond the updated GRACE risk score.
Changes in the electronegative properties of LDL particles are tightly linked to alterations in their lipidome and represent a novel determinant of mortality risk in patients with established atherosclerotic cardiovascular disease (ASCVD). These observations should trigger further research into LDL quality as a potentially understudied yet important risk factor of ASCVD initiation and accelerated disease progression. FPLC denotes fast protein liquid chromatography, and FU follow-up.

limitations inherent to any observational study, including residual confounding. Furthermore, although regression models accounting for the time elapsed between symptom onset and biomarker measurements yielded consistent results, future studies are warranted to assess whether changes in LDL charge occur acutely and are also determined by pre-hospital delays. Indeed, experimental data on why and how shifts in LDL charge occur are scarce; thus, preclinical efforts disentangling the mechanistic basis of altered LDL electronegativity should be continued incessantly, aiming toward the future goal to assess clinical applicability of this novel marker, including possible therapeutic avenues. Finally, the generalizability of our results to patients of other ethnicities and/or stable coronary disease warrants further study.

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Data availability statement

The data underlying this study will be made available to other researchers upon reasonable request to the corresponding authors, subject to institutional and ethical committee approvals.

Clinical trial number

ClinicalTrials.gov Identifier: NCT01000701.

CRediT authorship contribution statement

Simon Kraler: Conceptualization, Funding acquisition, Formal analysis, Project administration, Data curation, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. **Florian A. Wenzl:** Writing – review & editing. **Jody Vykoukal:** Formal analysis, Data curation, Investigation, Methodology, Resources, Writing – review & editing. **Johannes F. Fahrmann:** Funding acquisition, Formal analysis, Data curation, Investigation, Methodology, Resources, Writing – review & editing. **Ming-Yi Shen:** Funding acquisition, Formal analysis, Data curation, Methodology, Resources. **Der-Yuan Chen:** Funding acquisition, Methodology, Resources. **Kuan-Cheng Chang:** Funding acquisition, Methodology, Resources. **Ching-Kun Chang:** Funding acquisition, Methodology, Resources. **Arnold von Eckardstein:** Writing – review & editing. **Lorenz Räber:** Writing – review & editing. **François Mach:** Writing – review & editing. **David Nanchen:** Writing – review & editing. **Christian M. Matter:** Writing – review & editing. **Luca Liberale:** Writing – review & editing. **Giovanni G. Camici:** Writing – review & editing. **Alexander Akhmedov:** Funding acquisition, Formal analysis, Conceptualization, Supervision, Project administration, Writing – review & editing. **Chu-Huang Chen:** Funding acquisition, Formal analysis, Conceptualization, Project administration, Supervision, Writing – review & editing. **Thomas F. Lüscher:** Funding acquisition, Conceptualization, Project administration, Supervision, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: SK also received funding from the Swiss Heart Foundation, the Novartis Foundation for Medical-biological Research, and the Theodor-Ida-Herzog-Egli Foundation, and equipment and materials from Roche Diagnostics outside the submitted work. LR received funding from Abbott, Biotronik, Boston Scientific, Heartflow, Sanofi, and Regeneron, and declares consulting fees from Abbott, Amgen, AstraZeneca, Canon, Medtronic, NovoNordisk, Occlutech, Sanofi, and Vifor, payment or honoraria from Abbott and Occlutech, and travel support from AstraZeneca. FM has received research grants to the institution from Amgen, AstraZeneca, Boston Scientific, Biotronik, Eli Lilly, Medtronic, MSD, and St. Jude Medical, including speaker and/or consultant fees. AvE

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2023.05.014.

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