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Plasma Lipoprotein Lipase Is Associated with Risk of Future Major Adverse Cardiovascular Events in Patients Following Carotid Endarterectomy

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WHAT THIS PAPER ADDS

Lipoprotein lipase (LPL) has been independently associated with primary acute myocardial infarction and coronary artery disease. The current study shows that LPL is also associated with secondary major adverse cardiovascular events. It was found to have varying hazards at different time point following carotid endarterectomy (CEA), which has not been described before. The study also demonstrated that higher LPL levels are associated with a more favourable plaque composition consisting of smaller lipid core and less macrophage burden. LPL is of particular interest currently due to possible new therapeutic strategies that target LPL activity via angiopoietin like 3, angiopoietin like 4, or apolipoprotein C3 (APOC3) to reduce the risk of cardiovascular disease.

Introduction: Carotid plaque intraplaque haemorrhage (IPH) is associated with future cardiovascular events. It was hypothesised that plasma proteins associated with carotid plaque IPH are also likely to be associated with major adverse cardiovascular events (MACE) after carotid endarterectomy (CEA).

Methods: In pre-operative blood samples from patients undergoing CEA within the Athero-Express biobank, proteins involved in cardiovascular disease were measured using three OLINK proteomics immunoassays. The association between proteins and IPH was analysed using logistic regression analyses. Subsequently, the association between the IPH associated plasma proteins and the three year post-operative risk of MACE (including stroke, myocardial infarction, or cardiovascular death) was analysed.

Results: Within the three year follow up, 130 patients (18.9%) of 688 symptomatic and asymptomatic patients undergoing CEA developed MACE. Six of 276 plasma proteins were found to be significantly associated with IPH, from which only lipoprotein lipase (LPL) was associated with the post-operative risk of MACE undergoing CEA. Within the 30 day peri-operative period, high plasma LPL was independently associated with an increased risk of MACE (adjusted hazard ratio [HR] per standard deviation [SD] 1.60, 1.10 — 2.30), $p = .014$). From 30 days to three years, however, high LPL was associated with a lower risk of MACE (adjusted HR per SD 0.80, 0.65 — 0.99, $p = .036$).

Conclusion: High LPL concentrations were found to be associated with a higher risk of MACE in the first 30 post-operative days but with a lower risk MACE between 30 days and three years, meaning that LPL has different hazards at different time points.

Keywords: Atherosclerosis, Carotid endarterectomy, Lipoprotein lipase, MACE, Proteomics
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INTRODUCTION

The selection of patients with carotid disease for carotid endarterectomy (CEA) relies on the net benefit balance between natural course risk *vs.* procedural risk to reduce the long term risk of ipsilateral stroke overall.[1] Despite improved secondary prevention measures consisting of cardiovascular risk management, patients who underwent CEA still have a residual risk of 13% of developing major adverse cardiovascular events (MACE), consisting of stroke, myocardial infarction, and cardiovascular death.[2] Identification of patients with a high risk of MACE is vital as these patients may possibly benefit from add on therapy such as Colchicine or PCSK-9 inhibitors.[3,4]

Intraplaque haemorrhage (IPH) assessed by histology of the excised carotid plaque is independently related to cardiovascular outcome following CEA.[5] IPH is thought to be caused by extravasation of erythrocytes from leaky intraplaque neovessels that are formed during neovascularisation.[6] Both increased neovascularisation and IPH are important features of plaque instability.[5,6] Since locally observed plaque characteristics, such as IPH and increased neovascularisation, are associated with an increased risk of secondary events in other vascular territories, this suggests that the plaque characteristics in one site may reflect the situation in other vascular sites.[5,7] An atherosclerotic plaque is, however, often not available for analysis. For this, non-invasive imaging techniques to examine plaque stability, often using IPH as a marker, can be used.[8] The presence of IPH on magnetic resonance imaging (MRI) has been associated with a higher risk of new cerebrovascular events. However, the widespread application of MRI in the clinical context for IPH assessment is hampered by heterogeneity in design and reporting standards, and by high costs.[8] A circulating blood marker that can identify patients at high risk of MACE after CEA would be easier to use in clinical practice, cheaper, lower the patient burden, and thus be extremely impactful.[9]

To identify a circulating biomarker, it was hypothesised that blood proteins associated with carotid plaque IPH are also associated with MACE after CEA. Therefore, in the present study, multiplex OLINK targeted proteomics immunoassays panels have been used to assess cardiovascular disease related proteins in plasma samples from patients undergoing CEA in the Athero-Express Biobank Study.[10] The aim was to identify (multiple) blood protein marker(s) that are associated with IPH and MACE. The primary focus was on the underlying pathology and not on creating an optimal risk prediction model using biomarkers, therefore extensive models for comparisons with clinical prediction models were not created.

MATERIALS AND METHODS

Study population

Data from the Athero-Express Biobank (www.atheroexpress.nl), an ongoing prospective study of patients undergoing CEA for asymptomatic or symptomatic atherosclerotic carotid stenosis, were used. A detailed description of the study design has been published previously.[10] In short, consecutive patients were recruited from the St. Antonius Hospital Nieuwegein, Nieuwegein, The Netherlands, and University Medical Centre Utrecht in Utrecht, The Netherlands, from 2002 onwards. During the longevity of the Athero-Express biobank the indication for surgery and the carotid endarterectomy itself were performed following recommendations by the ESVS.[1] Protein levels did not differ between year of surgery (operating room [OR]) years (Supplementary Figure S1). In addition, all patients were discussed pre-operatively and judged by a multidisciplinary vascular team. Patients that agreed to participate completed standardised baseline questionnaires about their medical history and medication use prior to surgery. Pre-operative blood samples were collected during hospital admission at baseline, processed, and stored at -80°C until further use. Plaque samples were obtained during the CEA and analysed as described below. For this study, all participants that underwent CEA, with available proteomics and follow up data, were included (Fig. 1). Initially, 700 carotid endarterectomy patients were randomly selected for the proteomics analysis, with the only criterion that the follow up data were completely available. The follow up data were carefully re-examined, after this initial selection, and this

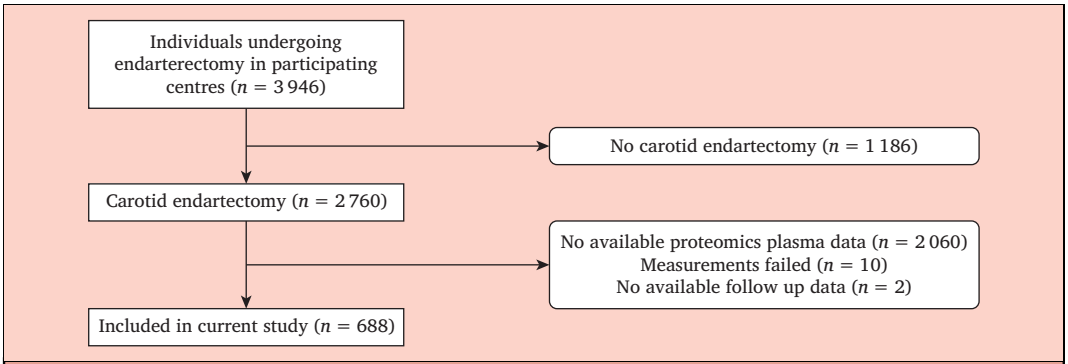

Figure 1. Flowchart of the number of individuals included in the analyses of the current study. A total of 1 186 patients were not included in the current study since they underwent iliofemoral endartectomy. In addition, 2 070 patients did not have available proteomics data and in 10 patients all measurements failed. Furthermore, two patients were dropped since they did not meet the. criteria of available follow up data.

resulted in two patients being dropped for having no available follow up data (Fig. 1). The current study was conducted in accordance with the Declaration of Helsinki and was approved by the ethical boards of both hospitals. The study received the proper ethical oversight. All patients provided written informed consent.

Laboratory measurements

The concentration of 276 proteins was determined in ethylenediaminetetraacetic acid plasma samples using the multiplex OLINK proteomics immunoassays Cardiovascular II panel, Cardiovascular III panel, and Cardiometabolic panel (OLINK Proteomics, Uppsala, Sweden). Each panel comprises 92 cardiovascular disease related biomarkers and enables the analysis of 96 samples simultaneously using only 1 μL of plasma. The high level of multiplexing is achieved by the proprietary Proximity Extension Assay (PEA) technology of OLINK. In PEA, matched pairs of oligonucleotide labelled antibodies bind pairwise to their target antigens.[11] On antibody binding, the matched oligonucleotides are brought into close proximity and a polymerase chain reaction target sequence is created, amplified, detected, and quantified using a DNA polymerase.[11] Raw biomarker values were converted to OLINK's arbitrary unit, normalised protein expression (NPX). NPX is a relative unit on a $\log^2$ scale. NPX values cannot be converted to an absolute protein concentration. All information regarding detection limits, assay performance and validation are available on the manufacturer's website (www.olink.com). For example, in the study data, for the Cardiovascular panel II that includes lipoprotein lipase (LPL) the intra- and interssay coefficient of variation was 5% and 13%, respectively. The abbreviations, full names, and respective OLINK multiplex panels of all measured proteins are described in Supplementary Table S1. Assays with less than 25% samples above the lower detection limit were excluded from further analysis, based on the recommendations of OLINK. This resulted in excluding nine from the total of 276 proteins. In addition, ten patients were dropped since the measurements of their blood specimens had failed.

Histopathological analysis of atherosclerotic plaque composition

Following CEA, the plaque samples were immediately transferred and processed according to a standardised protocol.[5,12] The 5 μm sections of the culprit lesion were subjected to histopathological examination. The semi-quantitative plaque characteristics were scored by a single experienced observer, who has been doing this since the beginning of the biobank, as no or minor and moderate or heavy staining according to pre-defined criteria using a microscope with 40 × magnification.[5] Haematoxylin and eosin (H&E) staining was used to obtain a general overview to assess any calcification(s) and the lipid core, picrosirius red and elastin von Gieson stainings were used to score collagen, alpha actin staining was used for smooth muscle cells, and CD68 staining for macrophages. For the presence of IPH using H&E and fibrin stainings, IPH was defined as haemorrhage within the plaque tissue and was rated as being absent or present. Intra- and interobserver variability has been examined previously and showed good reproducibility ($\kappa = 0.6 - 0.9$).[12]

Follow up analysis for major adverse cardiovascular events

Patients were followed up to three years after surgery for potential new vascular events. The composite endpoint MACE was defined at the start of the Athero-Express biobank in 2002 and included in the definition are non-fatal stroke (ischaemic or haemorrhagic), non-fatal myocardial infarction and vascular death, defined as death of presumed vascular origin (Supplementary Table S2). Clinical endpoints were assessed annually by means of patient questionnaires and validated by a review of medical records; if necessary, general practitioners or other treating physicians were contacted for follow up data.

Primary and secondary endpoints

The primary endpoint of this study was to identify (multiple) blood marker(s) associated with IPH and MACE, which includes testing the Cox proportional hazard assumption and making changes accordingly. The main secondary endpoint was to assess the association between the identified blood marker(s) and manually scored histological plaque characteristics.

Statistical analysis

Quantitative data were summarised as mean ± standard deviation (SD) or as median (interquartile range [IQR]) based on their distribution and were compared with the Student t test and a Mann—Whitney U test, respectively. Discrete data were presented as frequencies and percentages and were compared using the chi square or Fisher exact test.

First, univariable associations of each of the proteins included in the three OLINK panels and IPH were evaluated. These associations were adjusted for multiple testing. Since the majority of the investigated proteins were highly correlated, the effective number of independent tests for multiple testing correction were estimated using principal component analysis. The number of components to retain was determined by Cattell's scree test[13] and confirmed by the objective Cattell—Nelson—Gorsuch method.[14] For the protein data used in the present study, three components had to be retained, yielding a significance threshold of $0.05/3 = 0.0167$. Next, multivariable logistic regression was done correcting for confounders that were found to be associated with IPH.

Second, associations between each of the protein markers that were associated with IPH with the three year risk of MACE were evaluated using Cox proportional hazards models. These models make an important assumption, the proportional hazards assumption, which states that the hazard ratios (HRs) are constant over time. In other words, this means that the relative difference in hazard caused by

different values of a predictor remains the same over time. The assumption was checked for each protein marker using the Schoenfeld test (Supplementary Figure S2). If the assumption was violated, different time periods would be created for the risk of MACE in order to investigate the relationship between the protein marker and MACE.

Plasma concentrations measured by the OLINK panels were standardised to Z scores so that odds ratios (ORs) and HRs were expressed with SD with 95% confidence intervals (95% CI). Potential confounders for multivariable analysis were identified based on the association between dichotomised protein concentrations and baseline variables with $p < .10$ or based on multiple univariable analyses between baseline characteristics and the outcome variable (e.g., MACE) with $p < .10$. Subsequently, model reduction was done using the Akaike information criterion in a stepwise backward regression approach for each protein separately. Subsequently, the association between plasma proteins and plaque characteristics was investigated by univariable logistic regression to potentially unravel underlying biological mechanism. Note that this was investigated only for the plasma proteins that were found to be associated with IPH and MACE. *Post hoc* correlations between LPL with lipid profile were explored.

Across the baseline characteristics, the proportion of missing values was analysed (Supplementary Table S3). Missing values of confounders included in multivariable models were imputed using multivariable imputation by chained equations (mice package).[15] The statistical significance threshold was set at a two sided $p < .05$ across all analyses, if not stated otherwise. Analyses were performed using R (v4.0.5; The R Foundation for Statistical Computing).

Data and code availability

The data as used here are available through https://dataverse.nl/dataverse/umculab. All documented code can be found at https://github.com/CirculatoryHealth/AE_OLINK_LPL_and_MACE.

RESULTS

Baseline characteristics

A total of 688 patients who underwent CEA, with available OLINK proteomics and follow up data for MACE from the Athero-Express biobank, were included. General baseline characteristics showed that the study population had a mean age of 69.9 ± 9.1 years and that the majority was male (68.6%, Table 1). The majority had pre-procedural neurological symptoms (79.3%) and suffered from one or more cardiovascular comorbidities such as hypertension (68.9%), diabetes mellitus (23.7%), hypercholesterolaemia (59.7%), or history of coronary artery disease (CAD) (29.5%). The majority of patients used dual antiplatelet drugs (57.1%). Histological data regarding the presence of IPH was available for 549 patients. Baseline characteristics were stratified for IPH confirming earlier reports[5,16] that IPH was more likely to be present in plaque from men (Table 1). In addition, IPH was more likely to be present in patients with a significant contralateral carotid stenosis (Table 1).

Association with intraplaque haemorrhage

After quality control (see Materials and Methods), 268 proteins remained and were tested for an association with the presence of IPH in atherosclerotic plaques (Supplementary Table S4). A total of six proteins were significantly associated with the presence of IPH after correction for multiple testing using principal components (Table 2). After adjusting for confounders four proteins (platelet endothelial cell adhesion molecule [PECAM1], junctional adhesion molecule A [JAM-A], 2,4-dienoyl-CoA reductase [DECR1], and Integrin beta-1-binding protein [ITGB1BP2]) remained significantly associated with IPH (Table 2).

Association with the risk of major adverse cardiovascular events

With a median follow up of three years (IQR 2.12, 3.00), 130 of the 688 patients developed MACE, which consisted of 18.9% of the study population included in the current study.

For five of the six proteins the proportional hazard assumption was valid (Supplementary Figure S1). The uni- and multivariable associations with the three year risk of MACE for these five proteins are shown in Table 3. Only plasma JAM-A was univariably associated with the three year post-operative risk of MACE (HR 1.27, 95% CI 1.03 − 1.56, $p = .026$), but this association became insignificant after correction for confounders.

Violation of the proportional hazard assumption

The proportional hazard assumption between LPL and the post-operative risk of three year MACE was violated. Based on the Schoenfeld residuals (Supplementary Figure S1), the follow up period of three years was divided in two time periods: within 30 days (i.e., the peri-operative period) and from 30 days to three years. This was not done for the other five proteins, since the proportional hazard assumption was not violated for these proteins.

Major adverse cardiovascular events within 30 days after carotid endarterectomy

Higher LPL levels were significantly associated with a higher risk of MACE within the 30 day period in univariable analysis (HR 1.60, 95% CI 1.10 − 2.30, $p = .014$), which remained independently associated with MACE within 30 days after correcting for confounders including pre-procedural neurological symptoms in multivariable analysis (adjusted HR 1.80, 95% CI 1.20 − 2.67 $p = .004$, Fig. 2).

Major adverse cardiovascular events from 30 days to three years after carotid endarterectomy

Plasma LPL levels were not significantly associated with MACE from 30 days to three years in univariable analysis (HR 0.88, 95% CI 0.73 − 1.10, $p = .20$). However, in

Table 1. Baseline characteristics of the overall study population and stratification by intraplaque haemorrhage

	Overall (n= 688)	IPH (n = 339)	No IPH (n = 210)	p value
Age − y	69.9 ± 9.1)	69.7 ± 8.9)	69.8 ± 9.7)	.82
Men	472 (68.6)	246 (72.6)	126 (60.0)	.003
Systolic BP − mmHg	152 ± 25	155 ± 25	153 ± 26	.50
Diastolic BP − mmHg	82 ± 31	82 ± 13	80 ± 14	.18
Hypertension	474 (68.9)	236 (69.6)	135 (64.3)	.42
Diabetes mellitus	163 (23.7)	78 (23.0)	45 (21.4)	.74
Hypercholesterolaemia	411 (59.7)	200 (59.0)	121 (57.6)	.68
Total cholesterol − mmol/L	4.60 (3.80, 5.41)	4.60 (3.85, 5.37)	4.52 (3.60, 5.42)	.48
HDL − mmol/L	2.50 (2.00, 3.35)	2.50 (2.03, 3.23)	2.50 (1.89, 3.44)	.98
LDL − mmol/L	1.12 (0.90, 1.40)	1.09 (0.86, 1.36)	1.12 (0.90, 1.40)	.21
Triglycerides − mmol/L	1.30 (1.00, 1.93)	1.40 (1.01, 2.04)	1.30 (0.97, 1.69)	.075
BMI − kg/m^2	25.8 (24.0, 28.1)	25.81 (23.94, 28.35)	25.54 (23.81, 27.48)	.34
eGFR − ml/min/1.73 m^2	72.9 ± 20.9)	73.5 ± 21.3)	72.6 ± 19.6)	.63
Smoking status				.86
Current smoker	248 (36.0)	128 (37.8)	78 (37.1)	
Ex-smoker	333 (48.4)	162 (47.8)	97 (46.2)	
Never smoked	86 (12.5)	40 (11.8)	30 (14.3)	
History of coronary artery disease	202 (29.5)	103 (30.4)	54 (25.7)	.36
History of peripheral artery disease	117 (17.1)	273 (80.5)	181 (86.2)	.19
History of peripheral intervention(s)	117 (17.1)	65 (19.2)	29 (13.8)	.24
History of stroke or TIA	118 (17.2)	65 (19.2)	30 (14.3)	.11
Use of antihypertensive drugs	499 (72.5)	249 (73.5)	154 (73.3)	.53
Use of statins	532 (77.3)	252 (74.3)	165 (78.6)	.32
Use of antiplatelet drugs	595 (86.5)	299 (88.2)	185 (88.1)	.98
Use of dual antiplatelet drugs	393 (57.1)	206 (60.8)	131 (62.4)	.77
Use of oral anticoagulant drugs	75 (10.9)	39 (11.6)	20 (9.5)	.41
Ipsilateral pre-operative stenosis				.18
0–50%	6 (0.9)	3 (0.9)	0 (0.0)	
50–70%	63 (9.2)	26 (7.7)	22 (10.5)	
70–99%	599 (87.1)	301 (88.8)	178 (84.8)	
Significant contralateral carotid stenosis	229 (33.3)	124 (36.6)	65 (31.0)	.028
Pre-procedural neurological symptoms				.61
Asymptomatic	116 (16.9)	45 (13.3)	21 (10.0)	
Ocular	125 (18.2)	61 (18.0)	47 (22.4)	
Stroke	193 (28.1)	93 (27.4)	59 (28.1)	
TIA	227 (33.0)	124 (36.6)	75 (35.7)	

Data are presented as n (%). Parametric data are expressed as mean ± standard deviation, and non-parametric data as median (interquartile range). BP = blood pressure; HDL = high density lipoprotein; LDL = low density lipoprotein; BMI = body mass index; eGFR = estimated glomerular filtration rate; TIA = transient ischaemic attack.

multivariable analysis including confounders, higher LPL levels were significantly associated with a lower risk of MACE within the period from 30 days to three years after CEA (HR 0.80, 95% CI 0.65 − 0.99, p = .036, Fig. 3).

Associations with histological atherosclerotic plaque characteristics

The associations between the identified plasma marker LPL and histopathological features of plaque vulnerability were

Table 2. Associations between OLINK biomarkers and intraplaque haemorrhage, unadjusted and adjusted for confounders

Biomarker	Univariable models		Multivariable models*	
	OR (95% CI)	p value	OR (95% CI)	p value
PECAM1	0.74 (0.62–0.89)	.001	0.72 (0.59–0.86)	<.001
JAM-A	0.74 (0.62–0.89)	.001	0.71 (0.59–0.86)	<.001
DECR1	0.77 (0.65–0.92)	.004	0.74 (0.61–0.88)	.001
LPL	0.78 (0.62–0.93)	.006	0.85 (0.71–1.03)	.099
ITGB1BP2	0.68 (0.50–0.92)	.013	0.68 (0.48–0.92)	.015
IL1RL2	0.81 (0.68–0.97)	.016	0.85 (0.71–1.02)	.083

PECAM1 = platelet endothelial cell adhesion molecule; JAM-A = junctional adhesion molecule A; DECR1 = 2,4-dienoyl-CoA reductase; LPL = lipoprotein lipase; ITGB1BP2 = integrin beta-1-binding protein 2; IL1RL2 = interleukin-1 receptor-like 2; OR = odds ratio; CI = confidence interval.

* Multivariable models include the confounders gender and plasma triglycerides levels.

Table 3. Association with the three year post-operative risk of major adverse cardiovascular events, unadjusted and adjusted for confounders

Biomarker	Univariable models			Multivariable models		
	OR (95% CI)	*p* value	Events	OR (95% CI)	*p* value	Events
PECAM1*	1.09 (0.80–1.50)	.59	129	1.00 (0.85–1.17)	.98	129
JAM-A*	1.27 (1.03–1.56)	.026	129	1.07 (0.91–1.25)	.44	129
DECR1†	1.04 (0.92–1.19)	.52	129	1.02 (0.86–1.21)	.82	129
ITGB1BP2‡	0.92 (0.68–1.24)	.58	52	0.88 (0.64–1.22)	.45	52
IL1RL2§	0.68 (0.46–1.00)	.050	130	0.85 (0.72–1.02)	.082	130

PECAM1 = platelet endothelial cell adhesion molecule; JAM-A = junctional adhesion molecule A; DECR1 = 2,4-dienoyl-CoA reductase; LPL = lipoprotein lipase; ITGB1BP2 = integrin beta-1-binding protein 2; IL1RL2 = interleukin-1 receptor like 2; LDL, low density lipoprotein; HDL, high density lipoprotein; OR = odds ratio; CI = confidence interval.
* Multivariable analysis included the following confounders: age, HDL, history of peripheral intervention and ipsilateral carotid stenosis grade.
† Multivariable analysis included the following confounders: age, LDL, HDL, history of peripheral intervention and ipsilateral carotid stenosis grade.
‡ Multivariable analysis included the following confounders: age, LDL, HDL, history of peripheral intervention and Diabetes mellitus.
§ Multivariable analysis included the following confounders: age, LDL, HDL, history of peripheral intervention and kidney function and statin use.

investigated. LPL levels were inversely associated with a large lipid core (> 40%): OR 0.75 (0.63 — 0.90); $p = .002$; and moderate or heavy staining of macrophages: OR 0.81 (0.68 — 0.95); $p = .013$ (Fig. 4).

Post hoc correlations of lipoprotein lipase with lipid profile

LPL is responsible for the lipolysis of triglyceride rich lipoprotein, resulting in low plasma triglyceride levels and increased circulating high density lipoprotein (HDL) levels.[17] This is in accordance with the patient data showing that higher LPL levels are correlated with higher HDL levels ($R_s = 0.22$, $p < .001$) and with lower triglycerides levels ($R_s = -0.17$, $p < .001$), but not with low density lipoprotein levels ($R_s = 0.037$, $p = .44$) (Fig. 5A–C).

DISCUSSION

In this study, a targeted proteomic approach for the discovery of protein markers that are associated with IPH in carotid atherosclerosis and with the risk of MACE after CEA is reported. It was found that high levels of the proteins PECAM-1, JAM-A, DECR1, IL1RL2, LPL, and ITGB1BP2 were inversely associated with IPH. For PECAM-1, JAM-A, DECR1, IL1RL2, and ITGB1BP2 the proportional hazard assumption was valid. None of these five proteins was independently associated with the three year risk of MACE. In contrast, LPL had different hazards at different time points and the association with MACE was therefore examined for two time periods. LPL levels were associated with MACE in both time periods, but in opposite directions. That is, high LPL concentrations, were found to be associated with a higher risk of MACE in the first 30 post-operative days but with a lower risk MACE between 30 days and three years.

LPL is an extracellular enzyme with a major role in lipid homeostasis and is responsible for the lipolysis of triglyceride rich lipoprotein resulting in a more favourable

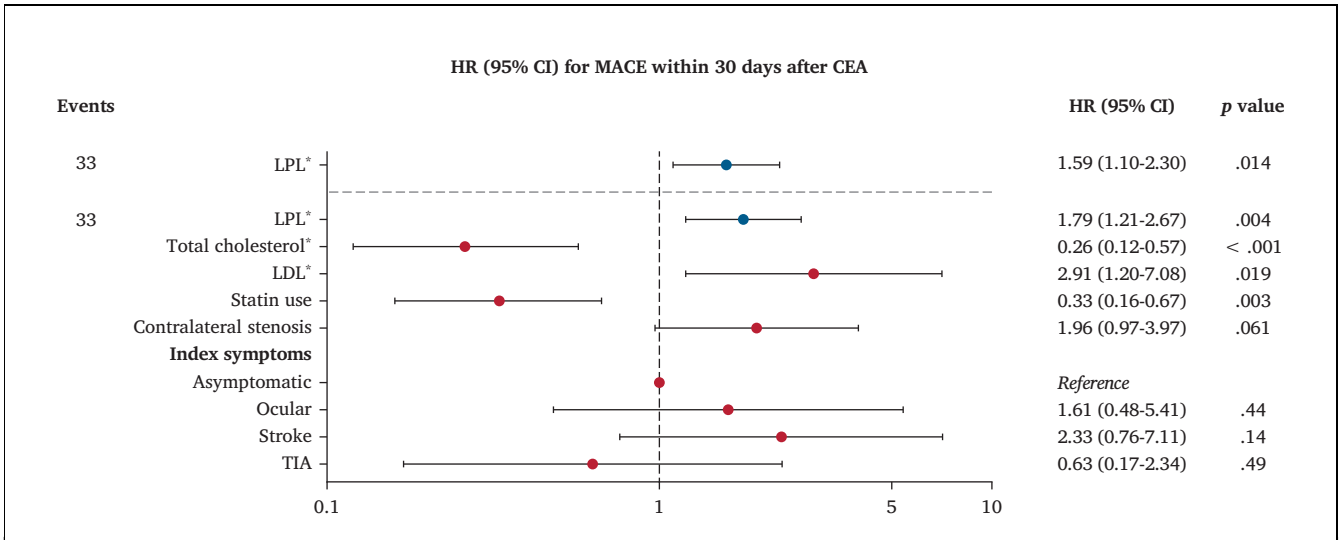

Figure 2. Univariable and multivariable associations between plasma lipoprotein lipase (LPL) (1 standard deviation [SD] increment) and major adverse cardiovascular events (MACE) within 30 days after surgery as derived from Cox regression analyses. Shown are hazard ratios (HR) and error bars corresponding to their 95% confidence intervals (CI). LDL = low density lipoprotein; TIA = transient ischaemic attack. *Variables were scaled to represent HR per 1 SD increment.

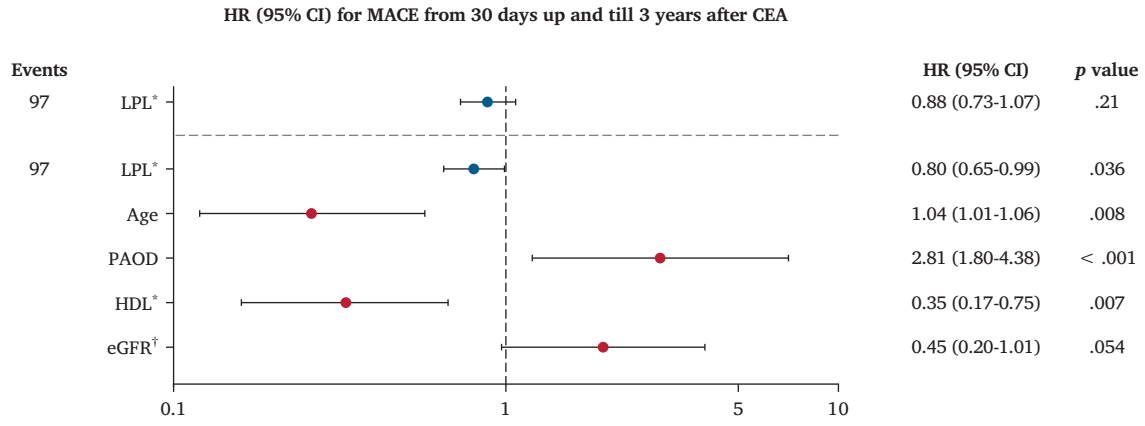

Figure 3. Univariable and multivariable associations between plasma lipoprotein lipase (LPL) (1 standard deviation [SD] increment) and major adverse cardiovascular events (MACE) from 30 days to three years after surgery, as derived from Cox regression analyses. Patients that experienced a peri-procedural event (MACE within 30 days after surgery) were excluded from this analysis. Shown are hazard ratios (HR) and error bars corresponding to their 95% confidence intervals (CI). PAOD = peripheral arterial occlusive disease; HDL = high density lipoprotein; eGFR = estimated glomerular filtration rate. *Variables were scaled to represent HR per 1 SD increment. †Variable was log transformed.

lipoprotein profile consisting of low plasma triglycerides levels and increased circulating HDL lipids.[17] This is supported by the results of a *post hoc* analysis between LPL and lipid test (Fig. 5A—C). The association between high LPL levels and a lower risk of MACE from 30 days to three years in the current study may be largely explained and driven by the anti-atherogenic function of LPL. Based on this finding, it is suspected that LPL and lipid homeostasis play a vital role in the development of both haemorrhagic strokes and ischaemic events such as ischaemic stroke and myocardial infarction both included in the composite MACE endpoint. Although the underlying pathophysiology and risk factors may differ, in elderly patients both haemorrhagic and ischaemic events share an atherosclerotic pathogenic origin.[18] Higher HDL levels were associated with a reduction in the risk of MACE after CEA after adjustment for confounding variables.[19] In addition, elevated fasting plasma triglycerides are increasingly recognised as important risk factors for ischaemic vascular disease.[20,21] This was particularly evident in a Mendelian randomisation study, in which genetically reduced low fasting triglyceride levels were found to be associated with lower all cause mortality rate.[22] Furthermore, in this particular Mendelian randomisation study, only known variants of the LPL were used, and survival was found to be increased based on the number of triglyceride lowering alleles.[22] Previous studies regarding LPL showed that high levels of LPL were independently associated with a lower risk of primary acute myocardial

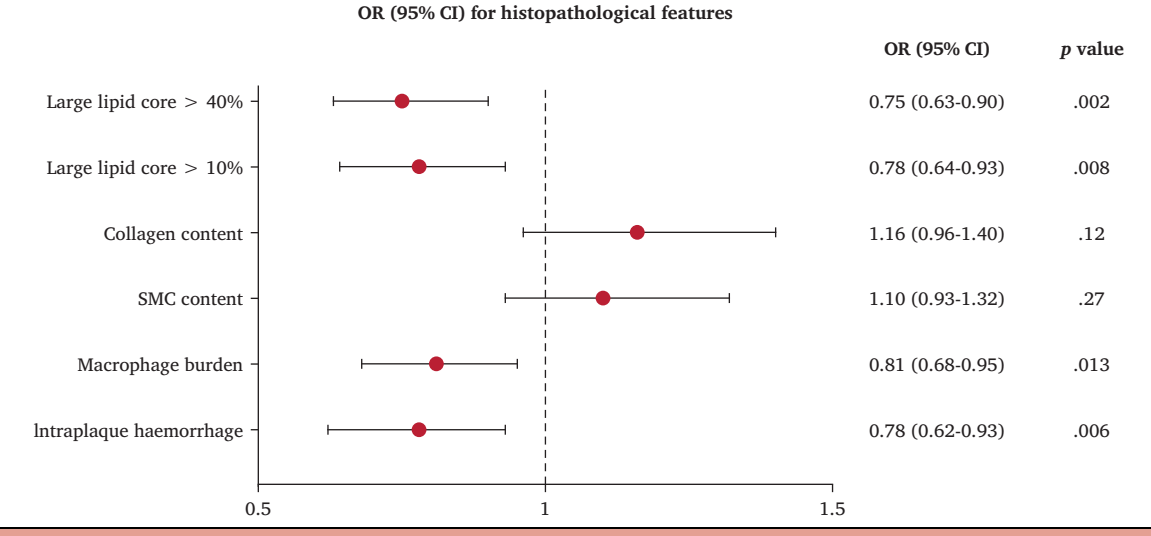

Figure 4. Association between lipoprotein lipase (LPL) with (1 standard deviation [SD] increment) and the individual (semi)-quantitative histopathological features (binary traits) as derived from logistic regression analyses. Shown are odds ratios (OR) with error bars corresponding to their 95% confidence interval (CI). LPL = lipoprotein lipase; SMC = smooth muscle cell.

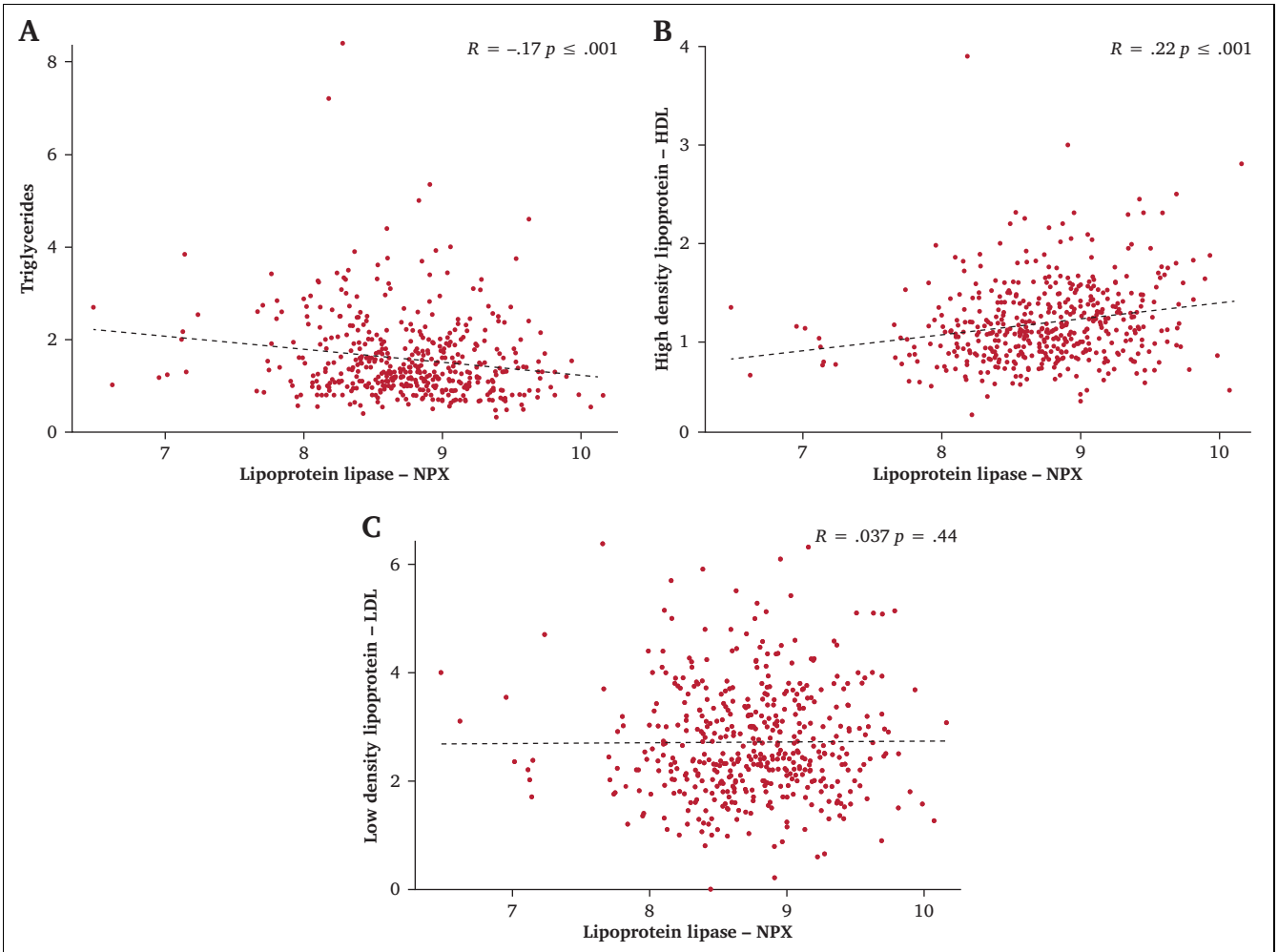

Figure 5. Scatterplots showing the association between lipoprotein lipase (LPL) and HDL and Triglycerides respectively. (A) Significant positive correlation between LPL levels (NPX) and HDL (mmol/L). (B) Significant negative correlation between LPL levels (NPX) and triglycerides (mmol/L). (C) LDL (mmol/L) was not correlated with LPL levels (NPX).

infarction and CAD,[23,24] which is in line with these results. In addition, this study demonstrated that higher LPL levels were not associated with features of a vulnerable carotid plaque but with a more favourable plaque composition consisting of a small lipid core (< 40%) and no to minor staining for macrophages compared with patients with lower LPL levels, which further underlines the anti-atherogenic effects of LPL.

In contrast, high LPL levels, measured in pre-operative plasma samples, were found to be associated with a higher risk of MACE shortly after CEA, which is new and puzzling and possibly related to surgery. The regulation of LPL is complex and tightly regulated by a number of proteins or drugs that either have a stabilising, inhibitory or activating effect on LPL, and thereby an anti-atherogenic or pro-atherogenic effect depending on their expression levels and micro-environment.[25] This might include the use of (dual) antiplatelet or anticoagulant drugs; however, an association between LPL levels and the use of antiplatelet, dual antiplatelet, and oral anticoagulant drugs was not found in the current study (Supplementary Table S5). However, heparin, which is used during CEA surgery, can drastically increase LPL activation.[26] This promotes the activation of protein kinase C,[27] a protein that plays an important role in thrombus formation, by regulating platelet activation.[28] In the cohort used in this study, heparin was never administrated before surgery. Therefore, it is hypothesised that especially after heparinisation during surgery, the risk of a thromboembolic MACE is raised in patients with high pre-surgical levels of LPL due to increased protein kinase C activation. This hypothesis, however, needs further validation in repeated measurement of LPL and protein kinase C activation before and after surgery. Since only pre-surgery blood samples in the Athero-Express biobank were collected, verification of this hypothesis needs other cohorts and post-surgery samples.

Due to the observational study design, a causal relationship between LPL and the other five plasma proteins (PECAM-1, JAM-A, DECR1, IL1RL2, and ITGB1BP2) and IPH cannot be proved. IPH is closely related to plaque neovascularisation, and it is often seen as a results of vascular leakage from intraplaque neovessels,[6] and these six proteins are involved in main pathways leading up to intraplaque angiogenesis, plaque progression and IPH. As mentioned earlier, LPL contributes to a more favourable lipoprotein profile, which at plaque level leads to a smaller

lipid core and limited intimal thickening. This reduces intraplaque hypoxia, which is a powerful inducer of plaque neovascularisation.[29] Thus, a possible explanation for the inverse association between LPL and IPH is that by reducing intimal thickening, LPL ultimately leads to less intraplaque bleeding by limiting plaque neovascularisation. PECAM-1 and JAM-A are highly expressed in vascular endothelial cells and have been implicated in key mechanisms involved in atherosclerosis such as preventing vascular leakage by maintaining the integrity of endothelial cells.[30] Both IL1RL2 and DECR1 have not been described in relation to neovascularisation or intraplaque haemorrhage, but their role has been described in processes closely related to atherosclerosis, playing a role in the local inflammatory response (IL1RL2),[31] or the energy metabolism of macrophages involved in atherosclerosis (DECR1).[32] ITGB1BP2 has not been described in the context of atherosclerosis.

Potential study limitations need to be addressed. First, due to the observational study design, the results cannot prove a causal relationship between LPL and the risk of MACE after CEA. Future studies are needed to further validate the value of this protein as biomarker. Second, owing to limited power, the analysis could not be stratified by gender, although it is known that IPH in men is associated with MACE but not in women.[16] Similar to this, pre-procedural symptoms were not stratified due to the low number of asymptomatic patients. In addition to this, the separate outcomes included in the composite endpoint MACE were not analysed due to low event numbers. Third, the majority of the patients included in Athero-Express are of European ancestries, meaning that generalisability to other ethnicities needs further validation. Last, the proteomics was done using samples from one time point, at baseline, immediately before CEA. Protein concentrations may change over time, and in the case of LPL, LPL activity and protein kinase C levels in post-heparin samples could not be analysed to further support the hypothesis of how high LPL is associated with a high risk of MACE in the 30 days after surgery. A major strength of this study is that an association between high LPL and the risk of secondary MACE in CEA patients was found; previous studies have only identified an association with primary CAD.[33] Furthermore, the current study contributes to the existing knowledge regarding atherosclerosis and LPL by analysing LPL inrelation to histology and secondary events. LPL is currently of particular interest because of possible new therapeutic strategies that involve targeting LPL activity via angiopoietin like 3 (ANGPTL3), angiopoietin like 4 (ANGPTL4), or apolipoprotein C3 (APOC3) and thereby reducing cardiovascular disease risk.[34,35] Additionally, the Athero-Express biobank is a highly regarded biobank that is unique of its kind due to the large number of CEA patients included with available plaque histology and proteomics data, which made it possible to analyse the relationship between plasma proteomics data and IPH and subsequently MACE.

In conclusion, high LPL concentrations were found to be associated with a higher risk of MACE in the first 30 days following surgery but a lower risk of MACE from 30 days to three years, meaning that LPL has different hazards at different time points. LPL in pre-operative plasma samples may therefore be considered as a predictor of higher perioperative risk of MACE and for a lower long term risk of MACE.

CONFLICT OF INTEREST

Dr Sander W. van der Laan has received Roche funding for unrelated work.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejvs.2023.01.035.

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