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High lipoprotein(a) is associated with major adverse limb events after femoral artery endarterectomy

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ABSTRACT

Backgrounds and aims: Elevated lipoprotein(a) (Lp[a]) has been identified as a causal risk factor for cardiovascular disease including peripheral arterial disease (PAD). Although Lp(a) is associated with the diagnosis of PAD, it remains elusive whether there is an association of Lp(a) with cardiovascular and limb events in patients with severe PAD.

Methods: Preoperative plasma Lp(a) levels were measured in 384 consecutive patients that underwent iliofemoral endarterectomy and were included in the Athero-Express biobank. Our primary objective was to assess the association of Lp(a) levels with Major Adverse Limb Events (MALE). Our secondary objective was to relate Lp(a) levels to Major Adverse Cardiovascular Events (MACE) and femoral plaque composition that was acquired from baseline surgery.

Results: During a median follow-up time of 5.6 years, a total of 225 MALE were recorded in 132 patients. Multivariable analysis, including history of peripheral intervention, age, diabetes mellitus, end stage renal disease and PAD disease stages, showed that Lp(a) was independently associated with first (HR of 1.36 (95% CI 1.02–1.82) $p = .036$) and recurrent MALE (HR 1.36 (95% CI 1.10–1.67) $p = .004$). A total of 99 MACE were recorded but Lp(a) levels were not associated with MACE.sLp(a) levels were significantly associated with a higher presence of smooth muscle cells in the femoral plaque, although this was not associated with MALE or MACE.

Conclusions: Plasma Lp(a) is independently associated with first and consecutive MALE after iliofemoral endarterectomy. Hence, in patients who undergo iliofemoral endarterectomy, Lp(a) could be considered as a biomarker to enhance risk stratification for future MALE.

1. Introduction

Patients with peripheral artery disease (PAD) are treated with lifestyle management and an appropriate medication regimen, such as antithrombotic or anticoagulant drugs in combination with lipid-lowering therapies, in order to reduce the risk of future cardiovascular events (CVE). In addition, patients with severe symptoms often require a vascular intervention to restore adequate perfusion. Although this will relieve most symptoms in the short term, the chronic nature of atherosclerosis will persist and the risk of future CVE remains extremely high [1]. Major Adverse Limb Events (MALE), a combination of lower limb amputation and peripheral vascular intervention, and Major Adverse Cardiovascular Events (MACE), a composite of non-fatal stroke/myocardial infarction and cardiovascular death, are two important categories of CVE that reflect more localized and systemic disease progression and are used as objective performance goals after

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revascularization [2].

Up to 42% and 13% of PAD patients will have a MALE and MACE within three years following a peripheral intervention, respectively, and consequently improvement of tertiary prevention is warranted to reduce this residual risk [3,4]. Patients at high risk for CVE may benefit from novel therapies such as dual antiplatelet therapy, addition of direct oral anticoagulants, PCSK9 inhibition, or colchicine therapy [5–7]. Moreover, insight into the individual risk of MALE could guide the preferred mode of intervention, or may substantiate treatment decisions when the efficacy of limb salvage is disputed. Unfortunately, early identification of these high-risk PAD patients is still lacking. Prediction models that incorporate clinical risk factors have so far been inconclusive with regards to individual risk, and are consequently not widely used in PAD [8,9]. In order to determine which PAD patients are at elevated risk, biomarkers associated with future CVE are needed.

Lipoprotein[a] (Lp(a)) is a polymorphic lipoprotein with much resemblance to low-density lipoprotein (LDL), with apolipoprotein(a) [apo(a)] covalently linked to ApoB100. From a biological and physiological point of view, Lp(a) exhibits several features that could render it a reliable biomarker. Independent of external factors like age, sex and fasting state, Lp(a) plasma levels are primarily genetically determined, which implies that plasma concentrations are fairly stable throughout life [10]. Lp(a) accumulates in the subendothelial space and interferes with fibrinolytic cascades, promotes expression of pro-inflammatory cytokines, enhances endothelial cell permeability, increases their proinflammatory phenotype and stimulates both smooth muscle cell migration and monocyte recruitment, all pivotal processes in atherosclerosis progression [10–13]. In carotid and coronary artery disease (CAD) Lp(a) has been shown to be a reliable marker for cardiovascular disease (CVD) progression [11,14,15].

With regards to the lower limbs, Lp(a) has primarily been investigated as a diagnostic marker for PAD [16]. Other studies revealed that higher levels of Lp(a) are associated with higher PAD-classifications, limb amputation, loss of patency and ankle-brachial-index (ABI) values [16–20]. The association of Lp(a) and MALE has not been investigated in surgical patients with severe PAD. Based on the association of Lp(a) in other cardiovascular arenas its involvement in processes contributing to progressive atherosclerosis, we hypothesize that high plasma levels of Lp(a) are associated with future MALE or MACE. This could improve identification of patients at increased risk for secondary CVE and could therefore enhance treatment strategies for these vulnerable patients.

In this study, we investigated the association of plasma Lp(a) levels with the risk of (recurrent) MALE and MACE in a cohort of patients undergoing iliofemoral endarterectomy.

2. Patients and methods

2.1. Study population

The Athero-Express (AE) (www.atheroexpress.nl) is an ongoing prospective biobank study (2002 – present) in which consecutive patients scheduled for carotid endarterectomy (CEA) or thromboendarterectomy (TEA) in two referral hospitals in the Netherlands (the St. Antonius Hospital Nieuwegein and the University Medical Center Utrecht) are included. The detailed protocol has been published before [21]. In short, pre-operative blood and perioperative atherosclerotic plaque samples are collected from all patients undergoing CEA or iliofemoral endarterectomy. All patients were medically treated according to the latest guidelines, either in collaboration with the general practitioner or specialists from (vascular) internal medicine [22]. Baseline patient characteristics were acquired by standardized pre-operative questionnaires and by examination of medical records. The first three consecutive years after the intervention, all patients received a questionnaire annually to collect follow-up data with regards to cardiovascular events and cardiovascular-related hospital admissions. These endpoints are verified by a medical professional with relevant correspondence of either the general practitioner or (referring) hospital.

For this study, all patients that underwent iliofemoral endarterectomy, with available lipid profile measurements, were included. Follow-up was extended by examination of medical records, and information about new peripheral procedures was recorded in more detail (side, target vessel, type of peripheral intervention).

The medical ethics committee of both hospitals approved the study, and all study participants gave informed consent in writing. The study was carried out in accordance with the Helsinki Declaration.

2.2. Laboratory measurements

Preoperative blood samples were collected during hospital admission, processed and stored at minus 80 °C until use. Lp(a) was measured in nanomole (nmol) per liter (L) by a particle-enhanced immunoturbidimetric assay (the Cobas c702 (Roche) and the LPA2 Tina-quant Lp(a) Gen.2 kit from Cobas (LPA2: CAN 8723)) in which Lp(a) agglutinates with latex particles coated with *anti*-Lp(a) antibodies. The precipitate is determined turbidimetrically at 800/660 nm. The measuring range of this assay was between 7 and 240 nmol/L.

Standard lipid profile measurements (cholesterol, triglycerides and HDL), were performed and LDL-c was calculated by using the Dahlen formula.

2.3. Atherosclerotic plaque assessment

For histological assessment of the atherosclerotic plaque, a standardized protocol was used that has previously been described in detail [23]. In short, plaques were stained with alpha-actin for smooth muscle cells (SMC), CD68 for macrophages, CD34 for microvessels, picrosirius red for collagen and lipid content, and hematoxylin-eosin and fibrin for intraplaque hemorrhage (IPH). The stainings were semi-quantitatively scored by two experienced independent observers as no/minor (0) and moderate/heavy [1]. Lipid content was estimated as a percentage of total plaque area and stratified into higher and lower than 10% and 40%. Intraplaque hemorrhage was rated as absent or present. Intra-observer and interobserver variability showed good reproducibility in an study performed previously (κ, 0.6–0.9) [24]. Finally, SMC and macrophage content were quantitatively scored using computerized analysis software AnalySIS 3.2 (Soft Imaging Systems GmbH, Münster, Germany). The content of SMCs and macrophages was expressed as the average percentage of positive staining of the plaque area from three representative areas of interest in the plaque, selected by an experienced technician at 40x magnification.

2.3.1. Outcomes

Our primary outcome of interest was MALE. MALE was defined as a composite of (new) infrainguinal (endo)vascular interventions that were performed due to a loss of patency or novel stenosis/occlusion in other ipsilateral segments. These included: percutaneous transluminal angiography (PTA), stent, drug coated balloon (DCB), drug coated stent (DCS), mechanical thrombectomy, atherectomy, thrombolytic (urokinase or alteplase) treatment, bypass surgery and major (above-the-ankle) amputations. Short-term reinterventions due to hemorrhagic bleeding of the patch, bypass or endovascular puncture site were excluded, as well as surgical site infections that required surgery. Diagnostic angiography with the intent of endovascular treatment, and failed endovascular procedures were defined as peripheral intervention, whereas a fully diagnostic angiography without the intent to treat was not. Objective loss of patency without subsequent intervention was not scored or included in the composite definition. The secondary endpoint of interest was MACE, a composite of non-fatal stroke and myocardial infarction, and death from all cardiovascular causes. Sudden death was categorized as cardiovascular death if no other explicit factors were found.

2.4. Statistical analysis

Quantitative data were expressed as mean (±standard deviation (SD)) or as median (interquartile range, (IQR)) as appropriate to their distribution, and were compared with Student *t*-test and a Mann-Whitney *U* test, respectively. Discrete data were presented as frequencies and percentages and were compared using chi-square of Fisher exact test. Comparison of baseline characteristics was performed for two groups stratified by outcome.

Lipoprotein(a) levels were transformed logarithmically for normalization and dichotomized (based on median values) for discrete analysis. Freedom from our primary endpoints was estimated using Kaplan-Meier survival analysis on dichotomized Lp(a) and included log-rank testing to calculate a statistical difference. Cox Proportional Hazard (PH) regression was used to calculate the hazard ratio (HR) with 95% confidence interval (CI) for the association between quantitative Lp(a) and the primary outcome during follow-up. Lp(a) was added to risk factors of several clinical models, that were derived from available literature, as to eliminate potential confounding and give an overview of the potential incremental value of Lp(a) in addition to these models. Missing data was imputed by predictive mean matching or were discarded when these exceeded 25%. By assessing the Schoenfeld residuals, the PH assumption was tested. When a time-dependent variable is present, a deterministic function of time will be included in the model for this variable.

For recurrent event analysis, three extensions of the Cox PH model were used. The Andersen-Gill (AG) and two variants of the Prentice-Williams-Peterson (PWP) models, namely the total-time (TT) model and gap-time (GP) model [25]. Akaike Information Criteria (AIC) were used to assess the goodness-of-fit and whether a risk factor should be used in a model. Stepwise Cox PH regression analysis was performed to see whether Lp(a) would be implemented in an automatically generated model free from potential investigator's bias. Univariable logistic regression was used to find whether Lp(a) levels were associated with the presence of these plaque characteristics. All P values were 2-tailed, with a value of $P < .05$ considered as statistically significant. Statistical analyses were performed with R version 4.0.4 inside an R Studio 1.4.1103 environment.

3. Results

3.1. Baseline characteristics

A total of 384 unique patients that underwent iliofemoral endarterectomy were included from the Athero-Express biobank. General baseline characteristics show that patients were predominantly male (73%), with a mean age of 69 (±SD 8.9) years and were slightly overweight (BMI 26 (±SD 4)) (Table 1). Intermittent claudication (IC), rest pain and ischemic wounds were indications for surgery in descending frequencies (58%, 26% and 16% respectively). About 41% of the patients had a history of an infrainguinal peripheral intervention before baseline surgery and 43% were previously diagnosed with CAD. The prevalence of diabetes mellitus (DM) was 27%. Median follow-up time in 384 patients was 5.6 years (IQR, 3.45–6.78); 146 patients died (all-cause mortality) during follow-up.

3.2. Baseline characteristics stratified by Lp(a)

Lipoprotein(a) levels ranged from 7 to 566 nmol/L with a median of 25.9 nmol/L (IQR 8.0, 128.3). For a comparison of Lp(a) and baseline characteristics, dichotomization of Lp(a) was performed based on the median (Table 1). Patients with higher levels of Lp(a) were more likely to have a statistically significant lower triglyceride level (1.7 mmol/L [IQR 1.2, 2.4]] *versus* 1.9 mmol/L [IQR 1.4, 2.5]); $p = 0.037$. LDL-C was not significantly different (1.1 mmol/L (±SD 0.4) *versus* 1.1 mmol/L (±SD 0.3)); $p = 0.65$.

No other significant differences in baseline characteristics were

Table 1
Baseline characteristics, overall and stratified by dichotomous Lp(a).

	Overall	Below median Lp(a)	Above median Lp(a)	*p*-value
N	384	192	192	
Age – y [a]	68.6 (8.9)	68.7 (9.1)	68.6 (8.7)	0.89
Gender – male (%)	281 (73.2)	138 (71.9)	143 (74.5)	0.65
BMI – kg/m^2 [a]	26.1 (4.0)	25.8 (4.1)	26.3 (3.9)	0.21
Smoking (%)	147 (38.9)	75 (39.3)	72 (38.5)	0.96
Fontaine stage				0.44
II	224 (58.3)	114 (59.4)	110 (57.3)	
III	99 (25.8)	52 (27.1)	47 (24.5)	
IV	61 (15.9)	26 (13.5)	35 (18.2)	
ABI	0.58 (0.2)	0.57 (0.2)	0.58 (0.2)	0.75
History of				
Peripheral intervention (%)	159 (41.4)	75 (39.1)	84 (43.8)	0.41
Coronary artery disease (%)	165 (43.1)	75 (39.3)	90 (46.9)	0.16
Stroke (%)	23 (6.6)	15 (8.5)	8 (4.6)	0.20
Hypertension (%)	270 (72.8)	131 (70.1)	139 (75.5)	0.28
Diabetes mellitus (%)	103 (26.8)	55 (28.6)	48 (25.0)	0.49
Medication				
Insulin (%)	32 (8.4)	15 (7.9)	17 (8.9)	0.85
Glucose inhibitors (%)	78 (20.4)	46 (24.1)	32 (16.7)	0.094
Anticoagulants (%)	55 (14.4)	27 (14.1)	28 (14.6)	1.00
Antiplatelets (%)	325 (85.1)	162 (84.8)	163 (85.3)	1.00
Lipid lowering drugs (%)	283 (73.9)	140 (73.3)	143 (74.5)	0.88
Statins (%)	281 (73.4)	139 (72.8)	142 (74.0)	0.88
Laboratory results				
eGFR, ml/min/1,73 m^2 [a]	80.6 (26.7)	81.3 (25.8)	80.0 (27.6)	0.59
Triglycerides, mmol/L [b]	1.8 [1.3, 2.4]	1.9 [1.4, 2.5]	1.7 [1.2, 2.4]	0.037
Lp(a), nmol/L [b]	25.9 [7.9, 128.3]	8.0 [7.0, 13.9]	128.4 [49.8, 201.5]	<0.001
LDL, mmol/L [a]	2.4 (0.9)	2.3 (0.9)	2.5 (0.8)	0.13
LDL corrected, nmol/L [a]	1.1 (0.4)	1.1 (0.4)	1.1 (0.3)	0.65
HDL, mmol/L [a]	4.4 (1.2)	4.3 (1.2)	4.5 (1.2)	0.23

[a] Parametric data expressed as mean (standard deviation).
[b] Non-parametric data expressed as median (interquartile range), MALE (Major Adverse Limb Events), BMI (Body Mass Index), ABI (Ankle Brachial Index), eGFR (Estimated Glomerular Filtration Rate), Lp(a) (Lipoprotein[a]), LDL (Low-density lipoprotein), HDL (High-density lipoprotein).

Table 2
Baseline characteristics stratified by MALE.

	No MALE	MALE	*p*-value
N	252	132	
Age – y [a]	69.8 (9.0)	66.6 (8.4)	0.001
Gender – male (%)	190 (75.4)	91 (68.9)	0.21
BMI – kg/m^2 [a]	25.9 (4.0)	26.4 (4.0)	0.23
Smoking (%)	92 (37.1)	55 (42.3)	0.38
Fontaine stage			0.31
II	154 (61.1)	70 (53.0)	
III	60 (23.8)	39 (29.5)	
IV	38 (15.1)	23 (17.4)	
ABI	0.56 (0.20)	0.61 (0.21)	0.064
History of			
Peripheral intervention (%)	98 (38.9)	61 (46.2)	0.20
Coronary artery disease (%)	105 (41.8)	60 (45.5)	0.57
Stroke (%)	19 (8.3)	4 (3.3)	0.11
Hypertension (%)	181 (74.2)	89 (70.1)	0.47
Diabetes mellitus (%)	64 (25.4)	39 (29.5)	0.45
Medication			
Insulin (%)	21 (8.4)	11 (8.3)	1.00
Glucose inhibitors (%)	49 (19.5)	29 (22.0)	0.67
Anticoagulants (%)	33 (13.1)	22 (16.7)	0.44
Antiplatelets (%)	212 (84.5)	113 (86.3)	0.75
Lipid lowering drugs (%)	182 (72.5)	101 (76.5)	0.47
Statins (%)	182 (72.5)	99 (75.0)	0.69
Laboratory results			
eGFR, ml/min/1,73 m^2 [a]	80.571 (26.013)	80.8 (27.9)	0.93
Triglycerides, mmol/L [b]	1.750 [1.290, 2.415]	1.8 [1.2, 2.5]	0.96
Lp(a), nmol/L [b]	19.4 [7.0, 97.4]	37.2 [10.3, 155.1]	0.017
LDL, mmol/L [a]	2.4 (0.92)	2.4 (0.83)	0.76
LDL corrected, nmol/L [a]	2.21 (0.92)	2.12 (0.87)	0.34
HDL, mmol/L [a]	1.1 (0.36)	1.1 (0.37)	0.67
Cholesterol, mmol/L [a]	4.4 (1.3)	4.3 (1.1)	0.57

[a] Parametric data expressed as mean (standard deviation).
[b] Non-parametric data expressed as median (interquartile range), MALE (Major Adverse Limb Events), BMI (Body Mass Index), ABI (Ankle Brachial Index), eGFR (Estimated Glomerular Filtration Rate), Lp(a) (Lipoprotein[a]), LDL (Low-density lipoprotein), HDL (High-density lipoprotein).

found, when stratified for high and low Lp(a) plasma levels.

3.3. Association of Lp(a) and MALE

A total of 132 patients had a first MALE with a median time of 381 days (IQR, 204–928). These MALE consisted of: amputations above [5] and amputations below the knee [5], bypass surgeries [28], redo-iliofemoral endarterectomies [12], thrombolyses [8] or endovascular interventions (73). Only seven patients recorded a first MALE after five years from inclusion.

Baseline characteristics when stratified for MALE are listed in Table 2. In short, patients with MALE were younger, more likely to have a history of peripheral intervention(s) and had a significantly higher plasma Lp(a): 19.4 nmol/L (IQR 7.0, 97.4) *versus* 37.2 nmol/L (IQR 10.3, 115); $p = 0.017$. History of CAD was equally prevalent in the group with MALE and in the group without MALE, at 41.8% and 45.5%, respectively ($p = 0.568$).

A Kaplan-Meier curve for first MALE stratified for low and high Lp(a) based on the median, demonstrated that the majority of first MALE took place within the first year after iliofemoral endarterectomy (Fig. 1). Analysis of Lp(a) quartile levels show that the lowest and the highest quartiles offer the greatest difference in hazard (Supplemental Fig. 1). Logrank test ($p = 0.039$) indicated that there is a statistical difference between the high and low Lp(a) groups. The association of Lp(a) and MALE was further investigated in multiple univariable analysis, where quantitative Lp(a) was found to be associated with MALE with a HR of 1.37 (95% CI, 1.0–1.8); $p = 0.030$ (Supplementary Table 1). Age (HR 0.98 (0.96–1); $p = 0.040$) and Fontaine stages were also associated with MALE. Of note, Fontaine stages were dependent on time and thus violated the PH assumption: meaning that the HR of Fontaine classification for MALE is declining over time, turning below 1 at 1.5 years after surgery.

In a multivariable model, which includes the risk factors history of peripheral interventions, age, Fontaine stages corrected by time, diabetes, and end stage renal disease (ESRD), Lp(a) was associated with

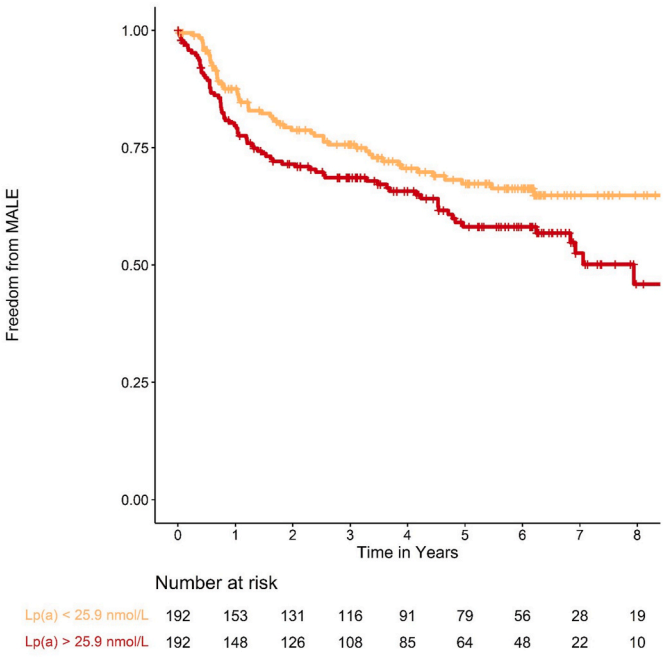

Fig. 1. Kaplan Meier curve for ipsilateral MALE stratified by quantiles of Lp(a). Freedom from major adverse limb events (MALE) in patients who underwent iliofemoral endarterectomy in relation to serum lipoprotein(a), below or above the median. Censoring includes all-cause death and loss to follow-up.

Table 3
Exemplary multivariable Cox PH model for first and recurrent major adverse limb events.

	Exemplary model for first MALE			Exemplary model for recurrent MALE		
Variable	HR	Conf. Int (95%)	*p*-value	HR	Conf. Int. (95%)	*p*-value
Lp(a)	**1.36**	**1.02–1.82**	**0.036**	**1.36**	**1.10–1.67**	**0.004**
History of peripheral intervention	1.28	0.90–1.82	0.178	1.17	0.89–1.55	0.28
Age	0.98	0.96–1.00	0.036	0.98	0.97–0.99	0.006
Diabetes mellitus	1.05	0.71–1.55	0.812	0.96	0.70–1.33	0.81
ESRD	14.80	3.30–66.31	<0.001	5.00	2.37–10.58	<0.001
Fontaine III <1.5Y	2.19	1.33–3.63	0.002	1.65	1.05–2.60	0.029
Fontaine IV < 1.5Y	1.89	1.02–3.53	0.044	1.74	0.98–3.05	0.056
Fontaine III >1.5Y	0.32	0.13–0.77	0.011	0.53	0.28–1.00	0.051
Fontaine IV > 1.5Y	0.47	0.18–1.25	0.131	0.65	0.26–1.59	0.34

HR (Hazard Ratio), Conf. Int. (Confidence Interval), MALE (Major Adverse Limb Events), Lp(a) (Lipoprotein[a]), ESRD (End Stage Renal Disease).
A multivariable Cox Proportional Hazard regression model to demonstrate risk factors and their relation to first and recurrent MALE by time. Prentice-Williams-Peterson Total-Time regression model is used for this multivariable recurrent event analysis. A Fontaine was corrected for time (below and above 1.5 years).

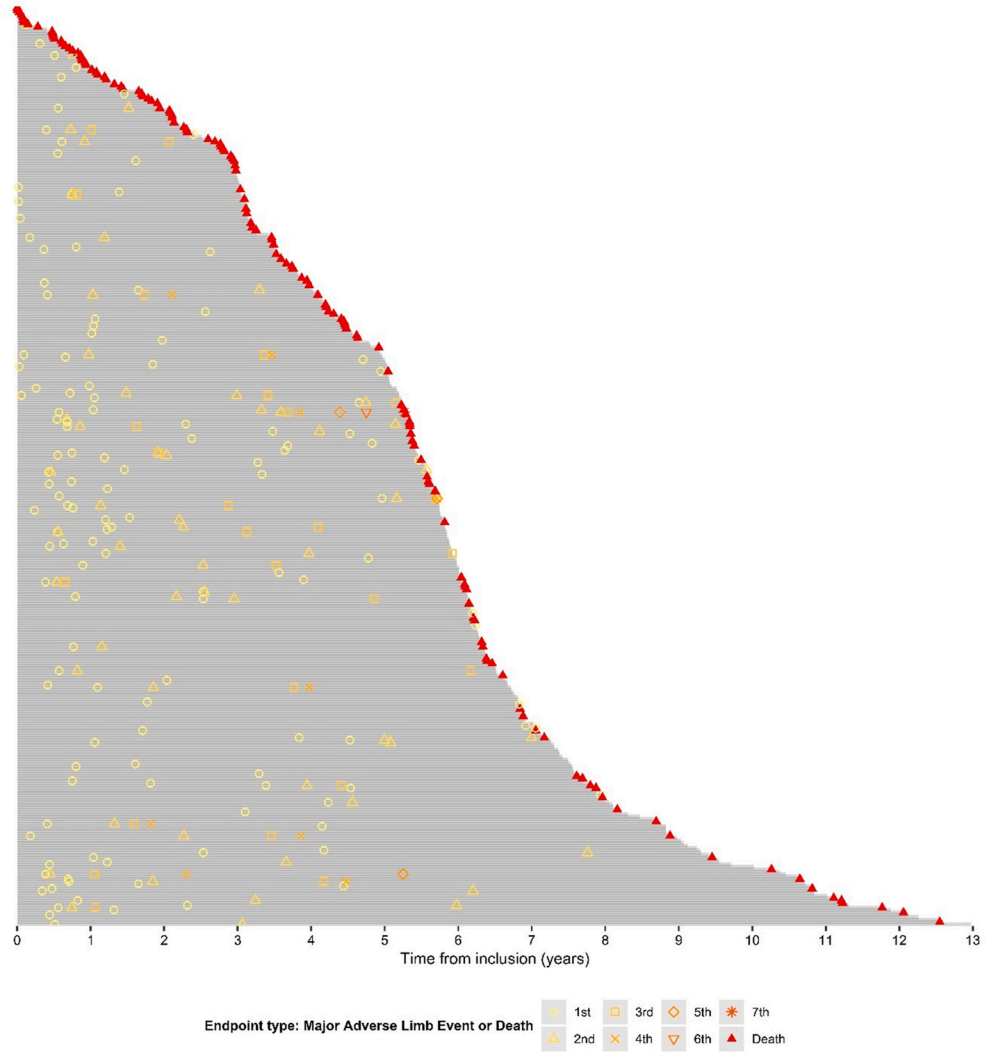

Fig. 2. A plot of recurrent major adverse limb events and terminal events.
On the y-axis, all participants are shown in ascending order for total follow-up length. For each participant, a horizontal grey line corresponds to length of follow-up. Consecutive major adverse limb events are shown on this line, with a gradient from yellow to red indicating the first till seventh major adverse limb events during follow-up. A red triangle at the end of follow-up indicates death. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

MALE with an HR of 1.36 (95% CI 1.02–1.82); $p = 0.036$ (Table 3). Furthermore, Lp(a) remained associated with MALE when risk factors of other existing PAD-related risk models were used (Supplementary Table 2) [9,26,27]. Likewise, automatic stepwise regression analysis selected Lp(a) as an independent factor in its multivariable model.

3.4. Association of Lp(a) and recurrent MALE

Having established that plasma Lp(a) levels were associated with the first MALE after iliofemoral endarterectomy, we investigated whether Lp(a) was also associated with recurrent MALE. A total of 225 MALE were recorded in 132 patients with successive frequencies for 2nd -7th MALE: 54, 26, 9, 3, 1 and 1. (Fig. 2).

We adopted the same risk factors as with the first MALE in a

multivariable multiple event model, showing that Lp(a) was also significantly associated with recurrent MALE with an HR of 1.36 (95% CI 1.10–1.67) $p = 0.004$ (Table 3). Other multivariable models offered a similar conclusion with corresponding HR of 1.30 (95% CI 1.06–1.61) $p = 0.014$ and 1.45 (95% CI 1.12–1.87) $p = 0.005$ (Supplementary Table 3). Stepwise regression analysis included Lp(a) alongside age, Fontaine stage, smoking status, history of CAD and eGFR (Supplementary Table 4).

3.5. Association of Lp(a) and MACE

A total of 99 patients had a MACE, with a median time of 1156 days (IQR 490–1985). Stratified for MACE, baseline characteristics were not significantly different (Supplementary Table 5). Furthermore, no association of Lp(a) and MACE was found in regression analysis HR 0.88 (95% CI 0.63–1.23); $p = 0.448$.

3.5.1. Association of Lp(a) and plaque characteristics

Since Lp(a) is involved in processes of atherosclerotic plaque progression, we investigated the association of Lp(a) with plaque characteristics (Supplementary Table 6). Lipoprotein(a) levels were positively associated with moderate/heavy staining of SMC: OR 1.85 (1.14–3.07); $p = 0.014$. A trend toward significance was observed between the association of Lp(a) with IPH (OR 1.49 (95% CI 0.99–2.26); $p = 0.06$). Univariable and multivariable Cox PH regression analysis indicated that plaque composition was not associated with (first or recurrent) MALE and MACE.

4. Discussion

In this study, we showed that in 384 unique patients who underwent iliofemoral endarterectomy, elevated levels of plasma Lp(a) were associated with an increased risk of first and recurrent MALE during a median follow-up of 5.6 years. The composite MALE has been frequently used as a relevant clinical endpoint in large clinical trials, and is considered as an objective performance goal as it provides a benchmark of symptoms in combination with failed patency or ongoing atherosclerotic disease in other arterial segments [2,5,28]. Since the incidence of MALE in patients with PAD is high, improvement of tertiary prevention would potentially benefit many patients, but increasing costs and elevated risk of adverse events (often attributed to this improved treatment) impede a roll-out of these add-on therapies for all PAD patients [29]. Additionally, enhanced knowledge about disease progression and need for (extensive) (endo-)vascular therapies could provide valuable information when limb salvage is questionable and objective substantiation is required before drastic measures such as amputation are undertaken. A risk-model, which could include Lp(a), could aid in the allocation of preventive and therapeutic applications.

The Athero-Express patients included in this study were predominantly male and the overall prevalence of CAD was high, which resembles other cohorts of Western European PAD patients [30]. Our results indicated that neither gender nor age, risk factors found to be related to Lp(a) levels in other research, were associated with Lp(a) [31,32]. The mortality rate observed in our study (38%) appears high, but is consistent with rates described in comparable studies; all-cause mortality after hospitalization for IC and Chronic Limb-Threatening Ischemia (CLTI) have been shown to be 31.6% and 57.5%, respectively [3].

With regards to our primary endpoints, the prospects of these patients are considered poor, as one-third of patients required a second intervention of the index limb within our follow-up time, and one in four patients experienced a MACE. This course of events is consistent with the findings of others, as 20% of CLTI-patients experience a MALE in the first year after surgery and this rate is about 35% at 5 years following open surgery of femoropopliteal lesions [33,34]. According to another study, MACE occurred in 20% of patients, three years after intervention [35].

Our analysis showed that Lp(a) is consistently and independently associated with MALE and recurrent MALE within median follow-up of 5.6 years. As the HR of Lp(a) is consistent with both first MALE and recurrent MALE, we believe that our analysis of recurrent data strengthens the evidence for the relationship of Lp(a) with MALE. To the best of our knowledge, we present the first study to analyze the association of Lp(a) in iliofemoral endarterectomy patients with (recurrent) MALE and therefore no direct comparison of our results and conclusions can be made.

In a retrospective study of 189 Japanese patients who underwent aortoiliac endovascular therapy, Lp(a) levels >40 mg/dL was associated with MALE [36]. However, differences in participants' race, treated vascular segment and mode of intervention prevent the extension of these conclusions to our patients. A Spanish prospective registry (FRENA) of stable out clinic patients concluded that in their PAD subgroup consisting of 528 patients, Lp(a) was associated with ischemic events, including lower limb amputation [18]. According to another study (41 limbs), Lp(a) levels >30 mg/dL was associated with restenosis at 6 months after infrainguinal PTA. However, the small study size and the perhaps short time frame are potential pitfalls that render the conclusions unsure [37]. In addition, a recent study investigating PAD patients concluded that Lp(a) levels >30 mg/dL was associated with the requirement for a peripheral artery operation, but a model with a cutoff point at 50 mg/dL was not [35]. However, most patients in this study were referred with an abdominal aortic aneurysm, and only a smaller proportion of patients was referred for lower limb PAD (CI and CLI). Unfortunately, no subgroup analysis based on these interventions, was performed. Furthermore, their outcome (revascularization of the lower extremities) did not include amputation. Although they briefly touched the subject of recurrent outcomes, no further regression analysis with regards to Lp(a) was performed with these data. The same study found no association of Lp(a) with MACE, which is in line with our results. However, since an association with Lp(a) and MACE has been found in major trials on other cardiovascular territories our results might have been influenced by a smaller sample size and a smaller event rate for MACE [38].

Lipoprotein(a) has been associated with arterial inflammation, thrombosis and progressive atherosclerosis, and thus we examined the association of Lp(a) with the composition of femoral atherosclerotic plaque [13]. Semi-quantitative analysis of 196 atherosclerotic femoral plaques demonstrated that moderate/heavy staining of SMC in the plaque was related to higher Lp(a) levels. This is in accordance with both human and animal studies, showing that Lp(a) is associated with proliferation of (vascular) SMC in the atherosclerotic lesion [39,40]. Since synthetic SMC present in atherosclerotic plaques contain a lower amount of alpha-smooth-muscle actin, the number of SMC might be underestimated. As this would proportionally be the case in all atherosclerotic plaques this would probably not influence the association with Lp(a) levels. We found that the association of Lp(a) and IPH had a trend towards significance. The association of Lp(a) with IPH cannot be substantiated by studies on plaque histology, although radiological IPH presence has been associated with Lp(a) in carotid plaques [41]. For the relationship of Lp(a) and IPH, several mechanisms have been suggested, including impairment of fibrinolysis due to the structural similarity of Lp(a) and plasminogen, the precursor of plasmin [42]. However, it remains unclear whether such interaction exists and whether this is relevant for lower limb PAD [43]. Although the increase of (semi-quantitative) SMC staining, as a substrate for progressive atherosclerosis, was associated with higher levels of Lp(a), the quantitative measurement was not and both characteristics were not related to (recurrent) MALE and MACE according to our analyses.

4.1. Strengths and limitations

The Athero-Express is a highly regarded biobank that has produced a

wealth of research. However, as a consequence of its broad inclusion period, preventive measures and therapeutic options have been improved over time and could potentially lead to a different prevalence of risk factors. Furthermore, the follow-up of early participants is potentially longer than that of more recent patients. However, we ensured that the minimum theoretical follow-up was 5 years and found no association between time of inclusion and Lp(a) levels and MALE.

The Athero-Express has a successful inclusion rate beyond 95%, limiting the chance of selection bias within both hospitals. Race is not formally registered, but our experience with these patients indicates that an overwhelming majority is of Caucasian descent. Since Lp(a) mass concentrations are dependent on race, we would like to emphasize that our conclusions are only appropriate to patients of similar descent and should not be applied to other races without further investigation [44–46].

The Lp(*a*)-levels of some samples exceeded the 240 nmol/L, the upper level of the measuring range of the essay used. By dilution, we confirmed these samples were indeed elevated beyond the calibration curve. Consequently, these corrected values were used in our analysis. On another note, the validity of the LDL-C values used in our analysis is open for debate, as these levels were calculated rather than measured.

With regards to our endpoint, some studies use the objective loss of patency, without correlation with symptoms, as a component of MALE. We believe that this constituent, without further consequences for treatment, is of lesser clinical relevance, although we understand that it could be considered pertinent in terms of disease progression. Due to heterogeneity of standard clinical follow-up beyond one year after surgery, diagnostic tests for loss of patency are not performed in the same way in all patients, resulting in selection bias. Given these arguments, we opted to exclude the loss of patency from our definition of MALE.

In this study, no attempt has been made to establish a definitive cutoff point for Lp(a), although several levels have been proposed as such [47,48]. Because we investigated a specific high risk subgroup, such cutoff point would offer little benefit to other populations and could potentially lead to an overestimation of the predictive efficacy of Lp(a). We believe that the use of quantitative Lp(a) is more transparent when looking for an independent association. Before Lp(a) can be used as a reliable biomarker for risk stratification and treatment allocation, future studies are required to create and validate a model that incorporates Lp(a) for the prediction of a clinically relevant outcome such as MALE.

On a similar note, we provided various statistical models in our analysis. It was not our intent to provide the best prediction model, but we sought to show the predictive performance of Lp(a) in relation with different, commonly used, risk factors (Supplementary Table III). The analysis of recurrent event data was performed with the same concept in mind. All three models treat recurring data differently and could potentially result in a significant association of Lp(a) in one model, but a non-significant in another. By including these approaches, we offered a transparent result which substantiates our conclusion.

4.2. Conclusions

This is the first study to demonstrate that Lp(a) is an independently associated with both first MALE and recurrent MALE after iliofemoral endarterectomy, in a population of Western-European patients with severe PAD. This identifies Lp(a) as a potential blood biomarker for subsequent lower limb events in high-risk patients, which can aid allocation of preventive and therapeutic treatments.

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Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Maarten C. Verwer: Study design, Data collection, Data analysis and interpretation, Drafting, the article and figures, Critical revisions, and final approval. **Farahnaz Waissi:** Data collection, Critical revisions, and final approval. **Joost M. Mekke:** Data collection, Critical revisions, and final approval. **Mirthe Dekker:** Critical revisions, and final approval. **Erik S.G. Stroes:** Critical revisions, and final approval. **Gert J. de Borst:** Drafting, the article and figures, Critical revisions, and final approval. **Jeffrey Kroon:** Critical revisions and final approval. **Constantijn E.V.B. Hazenberg:** Critical revisions, and final approval. **Dominique P.V. de Kleijn:** Study design, Drafting, the article and figures, Critical revisions, and final approval.

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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.2021.11.019.

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