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ORIGINAL RESEARCH

Associations of Tissue and Soluble LOX-1 with Human Abdominal Aortic Aneurysm

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BACKGROUND: Indication for prophylactic surgical abdominal aortic aneurysm (AAA) repair depends on the maximal aortic diameter. The lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is the major receptor for uptake of oxidized low-density lipoprotein cholesterol and is implicated in atherosclerosis. A soluble form of LOX-1 (sLOX-1) has been discussed as a novel biomarker in coronary artery disease and stroke. Herein, we assessed the regulation of aortic LOX-1 as well as the diagnostic and risk stratification potential of sLOX-1 in patients with AAA.

METHODS AND RESULTS: Serum sLOX-1 was assessed in a case–control study in AAA (n=104) and peripheral artery disease (n=104). sLOX-1 was not statistically different between AAA and peripheral artery disease but was higher in AAA (β=1.28, *P*=0.04) after adjusting for age, atherosclerosis, type 2 diabetes, prescription of statins, β-blockers, ACE inhibitors, and therapeutic anticoagulation. sLOX-1 was not associated with the aortic diameter, AAA volume, or the thickness of the intraluminal thrombus. Aortic LOX-1 mRNA expression tended to be higher in AAA when compared with disease, and expression was positively associated with cleaved caspase-3, smooth muscle actin, collagen, and macrophage content.

CONCLUSIONS: In AAA, sLOX-1 was differently affected by age, cardiometabolic diseases, and corresponding medical therapies. Comparison with nonatherosclerotic disease would be beneficial to further elucidate the diagnostic potential of sLOX-1, although it was not useful for risk stratification. Aneurysmal LOX-1 mRNA expression was increased and positively associated with smooth muscle cells and collagen content, suggesting that LOX-1 is eventually not deleterious in human AAA and could counteract AAA rupture.

Key Words: abdominal aortic aneurysm ■ LOX-1 ■ soluble LOX-1

Abdominal aortic aneurysms (AAAs) are defined by a dilation of the aortic diameter >30 mm.[1] Risk factors include family history of AAA, smoking, age >65 years, male sex, coronary artery disease (CAD), hypercholesterolemia, and hypertension.[1] Unexpected rupture of AAA without preceding symptoms is often the first clinical manifestation and a life-threatening condition.[2] Small AAAs (<50 mm) are monitored for their maximum aortic diameter, and elective surgery, either by open repair or endovascular therapy, is indicated at 55 mm.[3] To date, surgical therapy is the only treatment option for patients with AAA, and the decision relies on the maximum aortic diameter. Nonetheless, rupture at a diameter <50 mm is possible, and discovery of novel biomarkers that diagnose AAA, predict disease progression and fast-growing AAA has top priority in the field of research.[4] The majority of AAAs are covered by a nonocclusive intraluminal thrombus (ILT) that eventually affects vessel wall remodeling and rupture risk (eg, by secreting inflammatory cells or matrix-degrading

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CLINICAL PERSPECTIVE

What Is New?

- Serum soluble lectin-like oxidized low-density lipoprotein receptor-1(sLOX-1) was similar between patients with abdominal aortic aneurysm (AAA) and peripheral artery disease. sLOX-1 was differentially affected by cardiovascular risk factors and medical therapies in AAA and peripheral artery disease.
- Tissue LOX-1 expression was increased in aortic biopsies from patients with AAA, and LOX-1 expression was positively associated with aortic collagen and smooth muscle actin. These data suggest that LOX-1 is eventually not deleterious in human AAA and could counteract AAA rupture.

What Are the Clinical Implications?

- Serum concentrations of sLOX-1 could not discriminate AAA from patients with atherosclerotic peripheral artery disease.
- Serum sLOX-1 is not useful in predicting the AAA diameter and AAA volume.
- The role of tissue LOX-1 in stabilization of late-stage AAA should be analyzed in future studies.

Nonstandard Abbreviations and Acronyms

AOD	aortic-occlusive disease
CAS	carotid artery stenosis
ILT	intraluminal thrombus
oxLDL	oxidized LDL-cholesterol
sLOX-1	soluble lectin-like oxidized low-density lipoprotein receptor-1
r_s	Spearman's correlation coefficient
T2D	type 2 diabetes

enzymes). Formation of the ILT involves hemodynamics, the coagulation cascade, inflammatory cell activation, and cytokine/protease release.[5]

The lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) was originally identified as the major receptor for uptake of oxidized low-density lipoprotein cholesterol (oxLDL) in endothelial cells.[6] LOX-1 is also expressed by vascular smooth muscle cells, macrophages, and platelets.[7] Angiotensin II, oxLDL, proinflammatory cytokines, hypertension, diabetes, as well as pro-oxidative and mechanical stimuli can induce LOX-1 expression.[8] Upon activation, LOX-1 promotes atherosclerosis by affecting inflammation, apoptosis, and vascular remodeling.[7] LOX-1 is highly expressed in atherosclerotic plaques obtained from patients with carotid artery stenosis[9] and CAD,[10] but nothing is known about its gene expression in human AAA. Although AAA and atherosclerosis share common risk factors and mechanisms, both are considered as independent diseases with distinct pathomechanisms.[11,12]

Besides the membrane-bound LOX-1, a soluble form (sLOX-1) was identified. The release of sLOX-1 is stimulated under pathological conditions by proinflammatory C-reactive protein,[12,13] interleukin-18,[14] or oxLDL.[15] The cleavage from the membrane-bound LOX-1 is due to the activity of matrix-degrading enzymes,[16] known for their role in collagen and elastin degradation in AAA.[17] Previous studies provided evidence of sLOX-1 being a diagnostic and prognostic marker in stable CAD,[18,19] acute myocardial infarction,[20–23] ischemic and hemorrhagic stroke,[9,19] and acute aortic dissection.[19,24] However, the regulation of LOX-1 mRNA expression in aortic tissues from patients with AAA and the potential of sLOX-1 as a diagnostic marker has not been investigated so far. Analyzing aneurysmal tissue expression and corresponding circulating concentration is one possible approach to identify novel biomarkers.[25] Hence, we investigated the aneurysmal expression of LOX-1, a putative linkage to vessel wall degeneration along with the potential of sLOX-1 as a diagnostic and risk stratification marker in patients with AAA.

METHODS

The authors declare that all supporting data are available within the article and its online supplementary files.

Aortic Tissue Acquisition

A total of n=38 patients with diagnosed AAA undergoing elective open surgical repair were included in this consecutive study. Specimens were collected intraoperatively between March 2017 and October 2022 and were taken from the left anterior wall. The elective open surgical repair group included 4 patients, where 2 different specimens from the same AAA sac were obtained. Aortas in the control group were obtained from patients with aortic-occlusive disease (AOD, n=6) who underwent aortofemoral or aortobifemoral bypass surgery. Aortic segments were collected at the insertion site of the bypass at the abdominal aorta and were free of histopathological aneurysmatic dilations. All tissues were collected at least 10 minutes after removal, rinsed in ice-cold 1xDPBS, and cleaned from adjacent thrombus and blood clots. Tissue for RNA analysis was immediately frozen in liquid nitrogen.

Study Design and Study Population

This study included a case–control study that compared patients with AAA (cases) and peripheral artery disease (PAD) (controls). Initially, n=132 patients with PAD were included and were matched using propensity scores (see Statistical Analysis section) to n=104 patients with AAA based on age, sex, smoking, hypertension, CAD, carotid artery stenosis (CAS), and type 2 diabetes (T2D). Inclusion criteria for AAA were a diameter of the infrarenal aorta >40 mm, fast-growing AAA with >10 mm progress per year, or symptomatic AAA. Two patients received endovascular aortic repair due to iliac artery aneurysm but were also diagnosed for aortic dilation. Patients with PAD were surgically treated due to symptoms and angiographic signs of lower limb atherothrombosis. The clinical characteristics of patients with PAD and AAA enrolled for propensity score matching are shown in Table S1. The ethics committee of the Technische Universität Dresden approved this study (EK 151042017) and informed consent was obtained from every patient.

Clinical Variables

Serum low-density lipoprotein, high-density lipoprotein and total cholesterol, nonfasting glucose, and C-reactive protein concentrations were determined in the Institute for Clinical Chemistry and Laboratory Medicine at the Technische Universität Dresden using standard laboratory methods. Due to the lack of data on blood chemistry or medical therapies, the number of analyzed patients varies in each group. Cardiovascular risk factors, comorbidities, and prescribed medical therapies were evaluated retrospectively. Hypertension, T2D, and CAS were defined by a past documented history of diagnosis or treatment for these diseases. CAD was defined by a history of myocardial infarction, angina, or treatment for CAD. Smoking was defined as current or former smoker and sex was self-reported.

The mean aortic diameter was assessed by computed tomography angiography by measuring the outer adventitia to the outer adventitia diameter by a single observer. The thickness of the ILT was determined in the arterial phase on computed tomography scans after multiplanar reconstruction. The aorta was scanned in an axial position in 1-mm sections and the thickness of the ILT was measured at the largest distance from the inner surface of the lumen to the outer aortic wall. The AAA volume was measured using the automatic segmentation model of the IMPAX EE R20 software (Agfa HealthCare, Belgium) by 2 trained persons. The aorta was scanned with contrast media in the arterial phase using a slice thickness of 1 mm. The outer wall of the aneurysm or the true lumen was selected by hand every 6 mm in the transverse plane. Measurements were started cranial, at the beginning of the aneurysm, defined with a diameter >30 mm, until the end of the aneurysm or the aortic bifurcation. Afterwards nonrecognized areas were manually cropped. The volume of the AAA is given in mm^3 after subtracting the true lumen from the total AAA area. The ILT was included in the measurement of the total AAA volume but was not specifically marked for segmentation.

Blood Sampling and ELISA for Determination of sLOX-1 and oxLDL Concentrations

Non-fasting venous blood was collected between 2017 and 2022 in 7.5 mL S-Monovette Serum-Gel (Sarstedt, Germany) containing a clotting activator/gel. Blood samples were centrifuged at 1.500*g* for 12 minutes and the serum was stored in aliquots at –80°C. Serum LOX-1 (EHOLR1, Thermo Fisher Scientific, Germany) and plasma oxLDL (#10–1143-01, Mercodia, Uppsala, Sweden) were determined in duplicates by ELISA according to the manufacturer's instructions. If necessary, serum was diluted 1:2 or 1:5 and 2 internal controls were run on every plate. The intervariation coefficient was 14.2% for plates 1–5, and 20% for plates 6–7, respectively. The mean variation between all duplicates was 1±4%.

RNA Isolation, cDNA Synthesis, and Quantitative Polymerase Chain Reaction

Aortic segments were rinsed in ice-cold DPBS and cleaned from thrombus and attaching adventitial layer. Samples were dissected and shock frozen in liquid nitrogen. Tissue (30–50 mg) was homogenized in 1 mL TriFast (VWR, Germany) using a Precellys 24 homogenizer (VWR, Germany) and RNA was isolated according to the manufacturer's instructions. Afterwards, the RNA Clean and Concentrator Kit (R1016, Zymo Research, Germany) was used with an additional on column DNase I digestion to remove remaining DNA. Reverse transcription of mRNA into cDNA was performed with Multi Scribe Reverse Transcriptase (Thermo Fisher Scientific, Germany) using random hexamer primers according to the manufacturer's instructions. Quantification of mRNA expression was performed by real-time polymerase chain reaction with GoTaq qPCR Master Mix (Promega, Germany) and the Step One Plus Real-Time PCR System (Thermo Fisher Scientific). Data are presented as ΔC_T values. A geometric mean of RPL32, TBP, and B2M was used for cDNA content normalization. The used reference genes were selected from preliminary experiments and did not differ between the 2 groups. The use of a higher number of reference genes promotes a more accurate determination than relying on 1 reference gene.[26] To ensure comparability of data sets that were generated

between 2017 and 2020, the same internal control was run in every reverse transcription and quantitative polymerase chain reaction. Data on mRNA expression are presented in relation to this control (=1). Efficiency was checked for each pair of primers and was >90%. Primer sequences are summarized in Table S2.

Analysis of Aortic Elastin Degradation and Collagen Content by Elastica Van Gieson and Picro-Sirius Red Staining

Aortic wall segments were fixed in phosphate-buffered paraformaldehyde (4% PFA) for a maximum of 24 hours, dehydrated, decalcified, and embedded in paraffin. Serial sections (5 μm) were cut. Elastic fibers were stained with Elastica van Gieson and collagen by Picro-Sirius Red. Sections were photographed with the Axioscan slide scanner microscope (Carl Zeiss, Jena). Degradation of elastic laminae was classified into a score of 1–4[27] by 6 to 8 persons blinded to the experiment. These persons were trained before the final scoring. The average mean coefficient of variation between different participants was assessed after scoring 5 samples and was found to be 19.2%. The mean coefficient of variation between different specimen was analyzed after scoring the same samples 3 independent times and was 19.3%. Table S3 gives a detailed explanation on the scoring of elastin fiber degradation. Image J was used to quantify red, collagen-positive areas in the media, intima, and adventitia. Quantification was done by using a macro that measures the red color of the collagen fibers after subtraction of white, uncolored background. Adjacent adipose tissue was not included in the analysis. The mean of 2 to 3 slides was used and data are presented in percent of the total section area. The background of representative images was detected automatically and color composition was analyzed to equilibrate white balance of all images for representation. The background has been filled with white color. These changes were solely applied to enhance the visibility of slide scanner data in the manuscript context independent from the actual data analysis.

Immunohistochemistry for Staining α-Smooth Muscle Actin, Cleaved Caspase-3, CD68, and CD31

Aortic wall segments were fixed in phosphate-buffered 4% PFA, embedded in paraffin, and serial sections (5 μm) were cut. Antigen retrieval for α-smooth muscle actin, cleaved caspase-3, and CD68 was performed in citrate buffer containing 0.05% Tween 20 at pH 6.0. For CD31, 10 mmol/L Tris buffer containing 1 mmol/L EDTA and Tween 20 at pH 9.0 was used for antigen retrieval. Except for CD31, all sections were treated with proteinase K (20 μg/mL) in Tris-EDTA buffer. Endogenous peroxidase (S2023, Agilent) and unspecific binding sites were blocked (Protein block, X0909, Agilent). Primary antibodies with their corresponding concentrations are listed in Table S4. Secondary antibodies (Signal Stain Boost IHC Detection Reagent, Cell Signaling) were incubated for 1 hour at RT and AEC+ High Sensitivity Substrate Chromogen (K3461, Agilent) was used for color development in α-smooth muscle actin, cleaved caspase-3, and CD68 stained slides. CD31 was visualized by ImmPACT DAB Substrate, Peroxidase HRP (SK 4105, Vector Laboratories). Cell nuclei were counterstained with Mayer's Hemalum. Red or brown areas in the background of blue cell nuclei were quantified by Image J using a macro. The total intima, media, and adventitia area was manually cropped. Adjacent adipose tissue was excluded. Sections were analyzed after color deconvolution and segmenting channels for brown/red (antigens), thrombus (orange, undyed), and cell nuclei (blue). All measurement values were cross-checked if they visually matched. The mean of 2 to 3 sections per specimen was analyzed. Data are presented in percent of the total section area. Unspecific staining of atherosclerotic parts and the thrombus were excluded. The background of representative images was detected automatically and color composition analyzed to equilibrate white balance of all images for representation. The background has been filled with white color. These changes were solely applied to enhance the visibility of slide scanner data in the manuscript context independent from the actual data analysis. To exclude any unspecific binding, sections were incubated with the same concentration of the corresponding isotype control antibody (data not shown).

Quantification of Matrix Metalloproteinase-9 and Matrix Metalloproteinase-2 Activity and Expression of pro-Forms by Zymography

From all samples analyzed for LOX-1 expression, zymography was performed as described elsewhere.[28,29] An internal control (=1) was run on every gel and data are presented relative to this control to ensure comparability of data.

Statistical Analysis

Graph Pad Prism 9.0 (GraphPad Software, Inc., La Jolla, CA) and R Stats Package (Boston, MA) software were used for statistical analysis and $P \leq 0.05$ was considered as significant. Normality distribution was tested by the D'Agostino and Pearson test. Depending on normality testing, Mann–Whitney *U* or unpaired *t* test were used to compare AOD/PAD and

AAA. All data are shown as scatter dot plots; the horizontal line depicts the median or mean as indicated in the figure legends. Data are presented as scatter dot plots showing the median or mean with range or 95% CI as indicated in the figure legends or tables. Patients with AAA are shown as red circles and controls (AOD, PAD) as white circles. Comparison of 3 groups was done by Kruskal–Wallis and Dunn's post hoc test. Correlational analysis in non-Gaussian distributed data were done using Spearman's correlation, in Gaussian distributed data by Pearson's correlation, and is given as the correlation coefficient r_S/r_P. Differences in the distribution of cardiovascular risk factors and medical therapies across 2 independent groups (AOD, elective open surgical repair) were compared by Fisher's exact test. The null hypothesis (H_0) postulated that distributions of risk factors and medical therapies are independent of the outcome showing no differences between all groups. This study included a case–control study where patients in AAA were matched to PAD (1:1 ratio) by using propensity scores based on age, hypertension, sex, smoking status, CAD, CAS, and T2D. (R Core Team 2022, Vienna, Austria). Comparison of continuous variables was done by Mann–Whitney U test and categorical values were tested by Chi-square test. Differences in categorical values were tested by a binominal test. A penalized (elastic net) linear regression was used to identify medical therapies and cardiovascular risk factors with a potentially stronger influence on serum sLOX-1. The factors whose effects turned out to be stronger were further used as covariates in a multiple linear regression model with (logarithm of) sLOX-1 concentration as response.

RESULTS

Patient Selection, Aortic LOX-1 mRNA Expression, and Linkage to the AAA Diameter, Volume, and ILT Thickness

The clinical characteristics of the studied patients are shown in Table 1. The aortic diameter was higher (*P*=0.001) in AAA (63.67±14.41 mm) than in AOD controls (18.94±4.55 mm) and patients with AAA were older (*P*=0.01). The percentage of patients with prescription of angiotensin-converting enzyme inhibitors and diuretics was higher in AAA, whereas AOD received more acetylsalicylic acid. Aortic LOX-1 mRNA expression tended (*P*=0.06) to be higher in AAA than in controls. However, LOX-1 mRNA expression was not linked to the AAA diameter nor to the thickness of the ILT, but expression tended to be lower (*P*=0.06) in patients with a high AAA volume (Figure 1A through 1D).

Aortic LOX-1 mRNA, Aortic Wall Composition and Degradation

Histopathological vessel wall composition and degradation was characterized by elastic fiber degradation, collagen, α-smooth muscle, CD68, and cleaved caspase-3 content. Of these, elastin degradation and collagen and macrophage content were linked with the AAA diameter but none with the AAA volume (Figures S1 and S2A through S2H). Due to the descriptive nature of the present study, it might be speculated whether LOX-1 mRNA expression is a consequence of changes in AAA wall composition or is the underlying cause. Because all analysis was done in end-stage AAA, LOX-1 mRNA was set as the primary outcome variable. Expression of histopathological features was divided into low and high and LOX-1 mRNA was linked. LOX-1 mRNA expression was not statistically different in different stages of elastin fiber degradation and elastin mRNA did not change with LOX-1 mRNA expression (Figure 2A and S3A). Expression of LOX-1 mRNA was higher in AAA with high COL1A1 (*P*=0.006), COL3A1 (*P*=0.02) mRNA and aortic collagen expression (*P*=0.03) (Figure S3B through S3D). Interestingly, LOX-1 mRNA expression tended to be higher in samples having more cleaved caspase-3 (*P*=0.06) and was significantly increased with smooth muscle actin (*P*=0.02) positive areas (Figure 2B and 2C). No linkage with endothelial cell marker CD31 was present (Figure 2D).

Aortic LOX-1 mRNA, Inflammation and Activity of Matrix Metalloproteinases

Vascular LOX-1 expression was linked to inflammation,[7] which plays a key role in AAA.[30] Herein, LOX-1 mRNA expression was higher in samples with high CD68 positive staining, and C-C motif chemokine ligand 2 (CCL2) mRNA expression. Proinflammatory interleukin-6 (IL-6) was not associated with LOX-1 (Figure 3A through 3C). Activity of matrix degrading matrix metalloproteinase-9 (MMP9) and matrix metalloproteinase-2 (MMP2) are known to play a key role in matrix degeneration in AAA[31] and MMPs are involved in the shedding of sLOX-1 from the membrane-bound form.[32] However, LOX-1 mRNA expression was comparable in aortic samples with high and low MMP9 and MMP2 activity as it has been recently demonstrated in AngII-infused ApoE$^{-/-}$; LOX-1$^{-/-}$ mice[33] (Figure S4A through S4D). Furthermore, we tested whether serum sLOX-1 is linked with tissue LOX-1 mRNA expression and no linkages were found. However, tissue MMP9 activity tended to be higher (*P*=0.06) in patients with high sLOX-1 serum concentrations, suggesting that MMP9 could be involved in the generation of sLOX-1 (Figure S5A and S5B).

Table 1. Clinical Characteristics of Patients With AAA and AOD Controls

Tissue expression study				
	AOD	eAAA	*P* value	χ^2
Baseline demographics				
n, included	6	38		
Age, y, mean±SD	55.5±7.5	65.2±7.3	0.004	
Sex, M:F, % male	4:2, 66	34:4, 89	0.13	2.29
Aortic diameter, mm, mean±SD	18.94±4.55	63.67±14.41	<0.0001	
Cardiovascular risk factors				
LDL cholesterol, mmol/L, mean±SD, n	2.93±1.52 4	2.37±1.82 29	0.48	
Reference values <1.40 mmol/L for people with very high risk				
HDL cholesterol, mmol/L, median (range), n	1.23 (0.85–1.48) 4	1.13 (0.65–2.43) 29	0.88	
Reference values >0.90 mmol/L for men and >1.10 mmol/L for women				
Total cholesterol, mmol/L, median (range), n	3.02 (2.88–7.07) 4	4.47 (2.05–8.07) 29	0.19	
Reference values <4.00 mmol/L				
Triglycerides, mmol/L, median (range), n	1.57 (0.79–1.79) 5	1.74 (0.77–4.28) 31	0.28	
Reference values 0.35–1.70 mmol/L				
Blood glucose, mmol/L, median (range), n	5.50 (4.44–30.60) 4	5.35 (3.95–10.68) 31	0.74	
Reference value only for fasting glucose possible				
CRP, mg/L, median (range), n	4.05 (1.20–18.0) 6	3.25 (0.50–127.8) 38	0.73	
Reference values <5.0 mg/L				
Smoking, yes:no, %	5:1, 83	24:14, 63	0.65	
Hypertension, yes:no, %	4:2, 67	32:6, 84	0.29	
CAD, yes:no, %	3:3, 36	14:24, 39	0.62	
PAD, carotid artery stenosis, yes:no, %	6:0, 100	11:27, 29	0.0018	
T2D, yes:no, %	1:5, 17	5:33, 13	>0.99	
BMI, kg/m^2, median (range), n	22.40 (19.00–37.20) 6	27.00 (19.80–42.90) 38	0.18	
Pharmacological therapies				
Statins, yes:no, %	5:1, 84	25:13, 66	0.65	
ACE inhibitors, yes:no, %	0:6, 0	17:21, 45	0.07	
ARBs, yes:no, %	2:4, 23	13:25, 34	0.16	
CCB, yes:no, %	0:6, 0	15:23, 34	0.27	
ASA, yes:no, %	6:0, 100	21:17, 55	0.07	
β-blockers, yes:no, %	2:4, 33	17:21, 44	0.68	
Anticoagulation, yes:no, %	3:3, 53	9:29, 24	0.33	
Diuretics, yes:no, %	0:6, 0	16:22, 42	0.07	
T2D treatment, yes:no, %	1:5, 17	3:35, 8	0.46	
Insulin, yes:no, %	1:5, 17	3:35, 8	0.46	

Aortic tissues were obtained from patients undergoing elective open abdominal aortic aneurysm repair (eAAA) or surgery due to aortic-occlusive disease (AOD) where the aortic segment was obtained from the insertion site of the femoral or bifemoral bypass. For some patients, data on medical therapies were not available at the time of tissue collection and the number of analyzed patients (n) varies. Statistics: data are presented as median with range. Comparison of continous variables in AOD and eAAA was done by Mann–Whitney *U* test. Differences in the distribution of categorical outcome variables (cardiovascular risk factors, medical therapies) across the 2 independent groups were compared by the Fisher's exact test. ACE indicates angiotensin-converting enzyme; AOD, aortic-occlusive disease; ARB, angiotensin receptor blockers; ASA, acetylsalicylic acid; BMI, body mass index; CCB, calcium-channel blockers; CAD, coronary artery disease; CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PAD, peripheral artery disease; and T2D, type 2 diabetes.

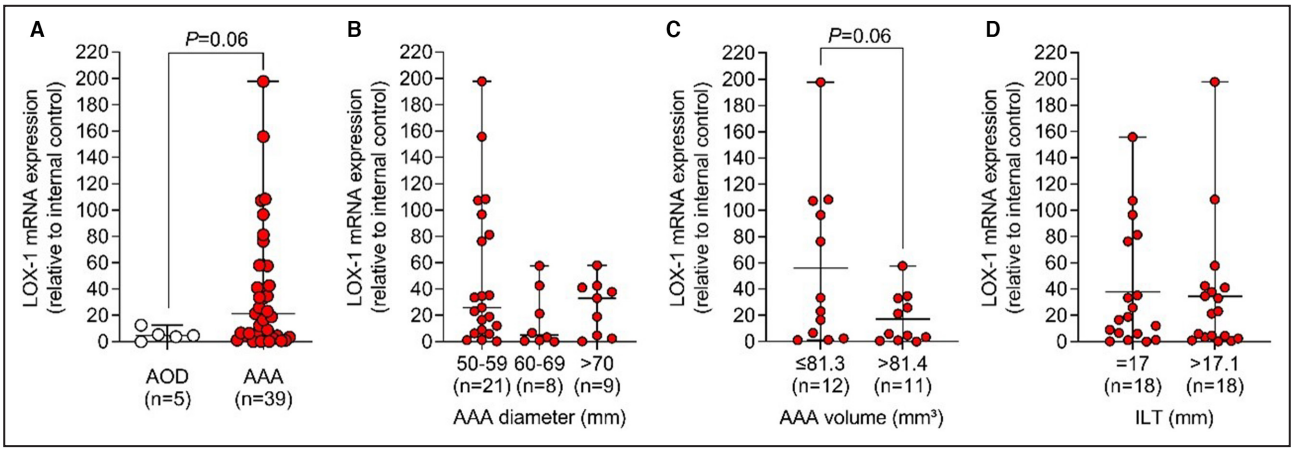


Figure 1. Aortic LOX-1 mRNA expression and relation to AAA diameter, volume, and thickness of the intraluminal thrombus. **A**, Aortic LOX-1 mRNA expression was analyzed by qPCR in aortic samples collected from patients with AAA (red circles) and aortic occlusive disease (AOD, white circles). Data were normalized to an internal control and are shown as scatter dot plots where the horizontal line depicts the median. **B**, The AAA diameter was divided into 3 groups (50–59, 60–69, and >70 mm) and LOX-1 mRNA expression was analyzed. The median of AAA volume (**C**) and thickness of the ILT (**D**) was calculated and LOX-1 mRNA expression analyzed. Statistics: data are shown as scatter dot plots where the horizontal line depicts the median (**A, B, D**) or mean (**C**) with range. Data were analyzed by Mann–Whitney *U* test (**A** and **D**), unpaired *t* test (**C**), or Kruskal–Wallis and Dunn's post hoc test (**B**). AAA indicates abdominal aortic aneurysm; AOD, aortic-occlusive disease; ILT, intraluminal thrombus; LOX-1, lectin-like oxidized low-density lipoprotein receptor-1; and qPCR, quantitative polymerase chain reaction.

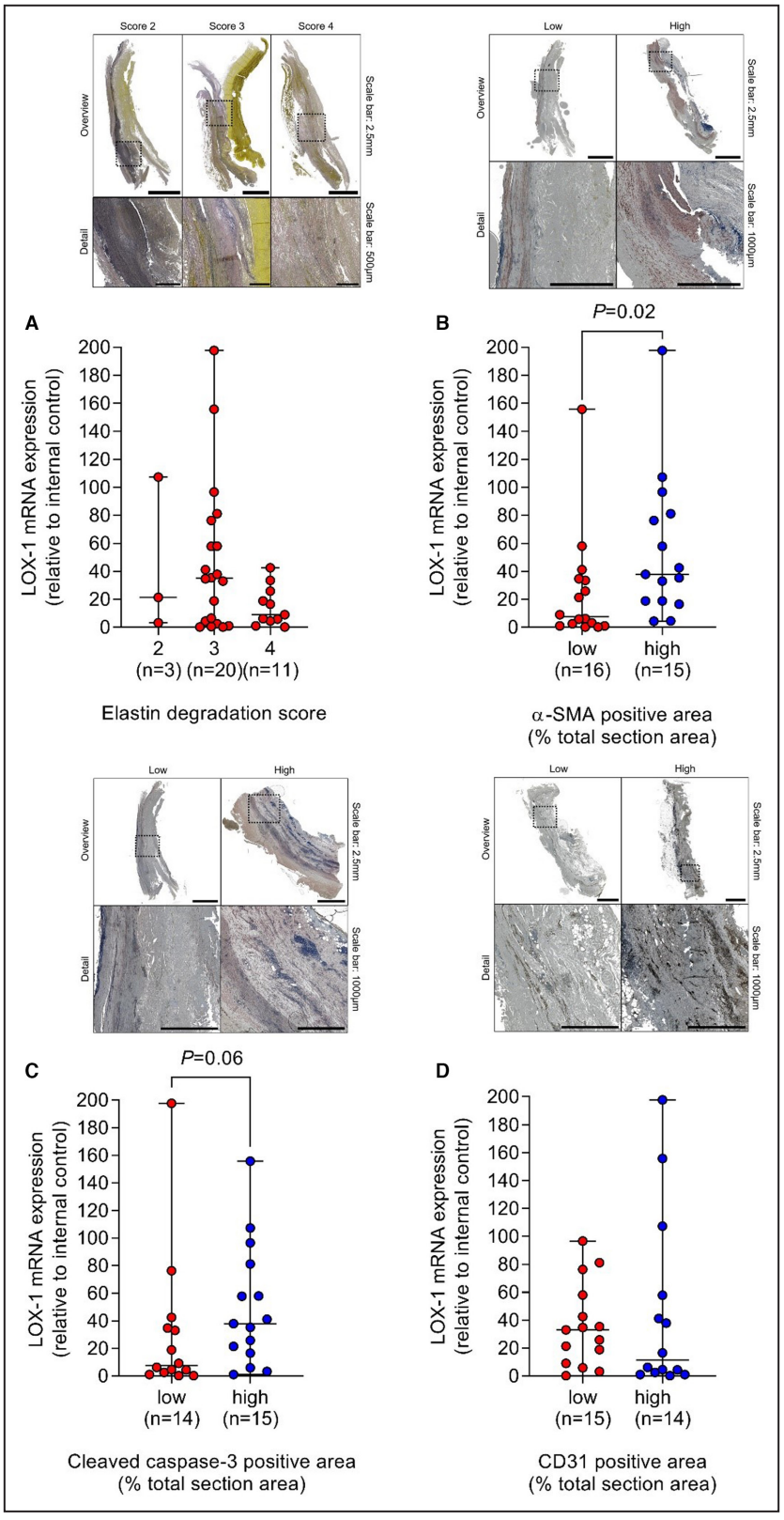
Patient Selection, Diagnostic Value of sLOX-1, and Associations with Aortic Diameter, AAA Volume, and Thickness of ILT

Serum sLOX-1 was analyzed in patients with AAA and in referral patients with PAD. The cohorts slightly differed in age, low-density lipoprotein cholesterol, the frequency of T2D, and in prescription of β-blockers and therapeutic anticoagulation (Table 2). Analysis of serum sLOX-1 revealed 1.12-fold higher concentrations in AAA (median: 52.47 versus 57.55 pg/mL, P=0.25) (Figure 4A). First, weighted linear regression was used to test effects of different variables on sLOX-1 in AAA and PAD separately. In AAA, prescription of β-blockers strongly influenced sLOX-1, whereas patients with CAS, prescription of statins, and angiotensin-receptor blockers had lower concentrations (Table S5). In patients with PAD, prescription of angiotensin-converting enzyme inhibitors, T2D and CAD influenced sLOX-1 while patients on statin admission and anticoagulation had reduced concentrations (Table S6). Based on effects of the weighted regression and differences in clinical characteristics, a multivariate regression model was chosen to adjust sLOX-1 to age, T2D, CAD, CAS, prescription of β-blockers, statins, angiotensin-converting enzyme inhibitors, and therapeutic anticoagulation. Correcting for these variables revealed an increase in sLOX-1 in AAA compared with PAD (β=1.29, P=0.04). Accordingly, an increase in age was associated with a lowering in sLOX-1 (β=0.98, P=0.007) (Table 3). Correlations with the AAA diameter, AAA volume, and the thickness of the ILT were tested to assess the risk stratification potential, but no associations were found (Figure 4B through 4D). In AAA, sLOX-1 was positively correlated with high triglycerides (r_S=0.21, P=0.03) and low high-density lipoprotein cholesterol, respectively (r_S=−0.39, P<0.0001) (Tables S7 and S8).

DISCUSSION

In the present study, we analyzed sLOX-1 concentrations in patients with AAA and assessed whether circulating sLOX-1 has a diagnostic potential and shows associations with the aortic diameter, the AAA volume, and the thickness of the ILT. Furthermore, the regulation of aortic LOX-1 tissue expression was analyzed to assess whether LOX-1 is linked to vessel wall degeneration or cellularity.

Serum LOX-1 is cleaved from the membrane-bound form by the action of proteases.[16] It has been shown that activated platelets, macrophages, adipocytes, and endothelial cells release sLOX-1.[19] Serum sLOX-1 concentrations were measured in a case–control study and were only slightly higher in AAA when compared with patients with PAD. An elevation in sLOX-1 was shown in obesity,[34] type 2 diabetes,[35] PAD,[36] CAD,[18] acute myocardial infarction,[20,37] and CAS.[15] In our study, cohorts were matched for age, sex, hypertension, smoking, CAD, CAS, and T2D. By this, effects on sLOX-1 either caused by atherosclerosis or other cardiometabolic disease could be mainly excluded. In AAA, sLOX-1 was increased in patients with prescription of β-blockers and slightly in CAD. On the other hand, patients with PAD with T2D, prescription of ACE



inhibitors, and CAD had elevated sLOX-1. These findings emphasize the differences in AAA and atherosclerotic disease and the known role of sLOX-1 in CAD.[20] sLOX-1 was higher in AAA after adjusting for age, T2D, prescription of β-blockers, and therapeutic anticoagulation. An inverse association between development of AAA and T2D[38] has been shown, while T2D is one of the strongest risk factors for PAD.[39] Most likely, T2D

Figure 2. Aortic LOX-1 mRNA expression and histopathological vessel wall degeneration in AAA.
Aortic LOX-1 mRNA expression was analyzed by qPCR, and data were normalized to an internal control. **A**, Elastin fibers were stained using Elastica van Gieson and degradation was scored by 8 persons blinded to the experiment. Score 2 represents the lowest observed elastin degradation, and score 4 the highest. **B**, α-smooth muscle cell (α-SMA) content was assessed by immunohistochemistry, and red-stained areas were quantified and set in relation to the total section area (%). **C**, Cleaved caspase-3 content was assessed by immunohistochemistry, and red-stained areas were quantified and set in relation to the total section area (%). **D**, CD31 content was assessed by immunohistochemistry, and brown-stained areas were quantified and set in relation to the total section area (%). Representative slides are shown above each figure. Expression values were divided into low (red circles) and high (blue circles), depending on the median of the data set. Statistics: data are shown as scatter dot plots where the horizontal line depicts the median with range. **A**, Kruskal–Wallis and Dunn's post hoc test. **B**–**D**, Data were analyzed by Mann–Whitney *U* test. LOX-1 indicates lectin-like oxidized low-density lipoprotein receptor-1; and qPCR, quantitative polymerase chain reaction.

and differences in prescription of β-blockers might have affected sLOX-1, leading to the observed findings. In addition, the increase in sLOX-1 in AAA could be also due to patients with PAD and without T2D. To date, nothing is known about sLOX-1 in nondiabetic patients with PAD.

Associations between the severity of disease and sLOX-1 have been demonstrated in CAD,[40–42] in acute myocardial infarction,[18,19] and in in-stent-restenosis after percutaneous coronary interventions.[41] In our study, no correlations of sLOX-1 with the AAA diameter, as the "criterion standard" for evaluation of rupture risk, the AAA volume, or thickness of the ILT were demonstrated. The AAA volume is largely affected by the vessel lumen, the blood flow, and the thickness of the ILT. Analysis of the AAA volume is used for analysis of peak wall stress and peak wall rupture index.[43] Compared with the AAA diameter, which reflects only a single dimension, 3-dimensional volume measurements may account for changes in AAA morphology.[44,45] In the present study, serum sLOX-1 was not correlated with the ILT, and a release of sLOX-1 from the ILT in AAA is therefore unlikely. An increase in sLOX-1 in coronary thrombi has been shown in patients with ST-segment–elevation myocardial infarction.[37]

It has been shown that statins increase sLOX-1 shedding in endothelial cells in vitro, mainly by affecting membrane cholesterol.[32] Several other compounds and medical therapies are known for their effects on membrane LOX-1 expression,[7] and this could have affected serum sLOX-1.

Studies have demonstrated that LOX-1 expression correlates with the soluble form and thus sLOX-1 represents membrane-bound LOX-1 expression and signaling.[15,16] In the present study, no linkages between sLOX-1 and aortic LOX-1 mRNA expression were demonstrated. However, due to the unavailability of specific antibodies for detection of LOX-1 protein in human AAA, we were only able to quantify LOX-1 mRNA expression. Furthermore, circulating sLOX-1 could be released from other diseased vessels or cells.[19]

Herein, sLOX-1 positively correlated with cardiovascular risk factors triglycerides and low high-density lipoprotein cholesterol, which could at least in part be explained by linkages with oxLDL and inflammation.[15]

Herein, aortic LOX-1 mRNA expression tended to be higher in patients undergoing elective open AAA repair when compared with AOD controls. Our findings were confirmed by a recently published study using human AAA and adjacent nonaneurysmal tissues as controls.[46] In this study, LOX-1 was primarily expressed by infiltrating macrophages.[46] In addition, an increased LOX-1 expression was also demonstrated in carotid artery plaques, whereas its expression was undetectable in aortas without atherosclerosis.[47] Because histopathological signs of atherosclerosis are frequently found in patients with AAA,[11] the increase could be due to atherosclerosis in AAAs. However, choosing AOD as controls, their small sample size and the differences in patient characteristics have to be considered. Eventually, differences in both diseases,[12] the higher age or differences in the prescription of angiotensin-receptor blockers and diuretics might have affected LOX-1 expression.

LOX-1 mRNA expression tended to be lower in samples with a larger AAA volume, whereas no associations with the AAA diameter were found. AngII infusion into ApoE$^{-/-}$; LOX-1$^{-/-}$ mice resulted in a more severe form of AAA, including higher rupture rates.[33] One possible explanation for the present data are changes in LOX-1 expressing cells in AAA with larger volumes (eg, endothelial cells that are replaced by the ILT[48] or a reduced number of smooth muscle cells in AAA).[49] In the present study, the AAA volume was not linked with the most relevant histopathological features. This could be due to the semiquantitative assessment of these parameters or the analysis of a small portion of the AAA sac compared with volume measurements that cover the whole AAA.

We could demonstrate that samples with a high smooth muscle actin and collagen content have a higher LOX-1 mRNA expression. Our results are strongly supported by data obtained from LOX-1$^{-/-}$ mice in an ApoE$^{-/-}$ background and AngII infusion. Aortae of these mice exhibit less increase in adventitial collagen and a thinner collagen layer. Moreover, fibrogenesis was attenuated and these mice showed less expression of smooth muscle and myofibroblast

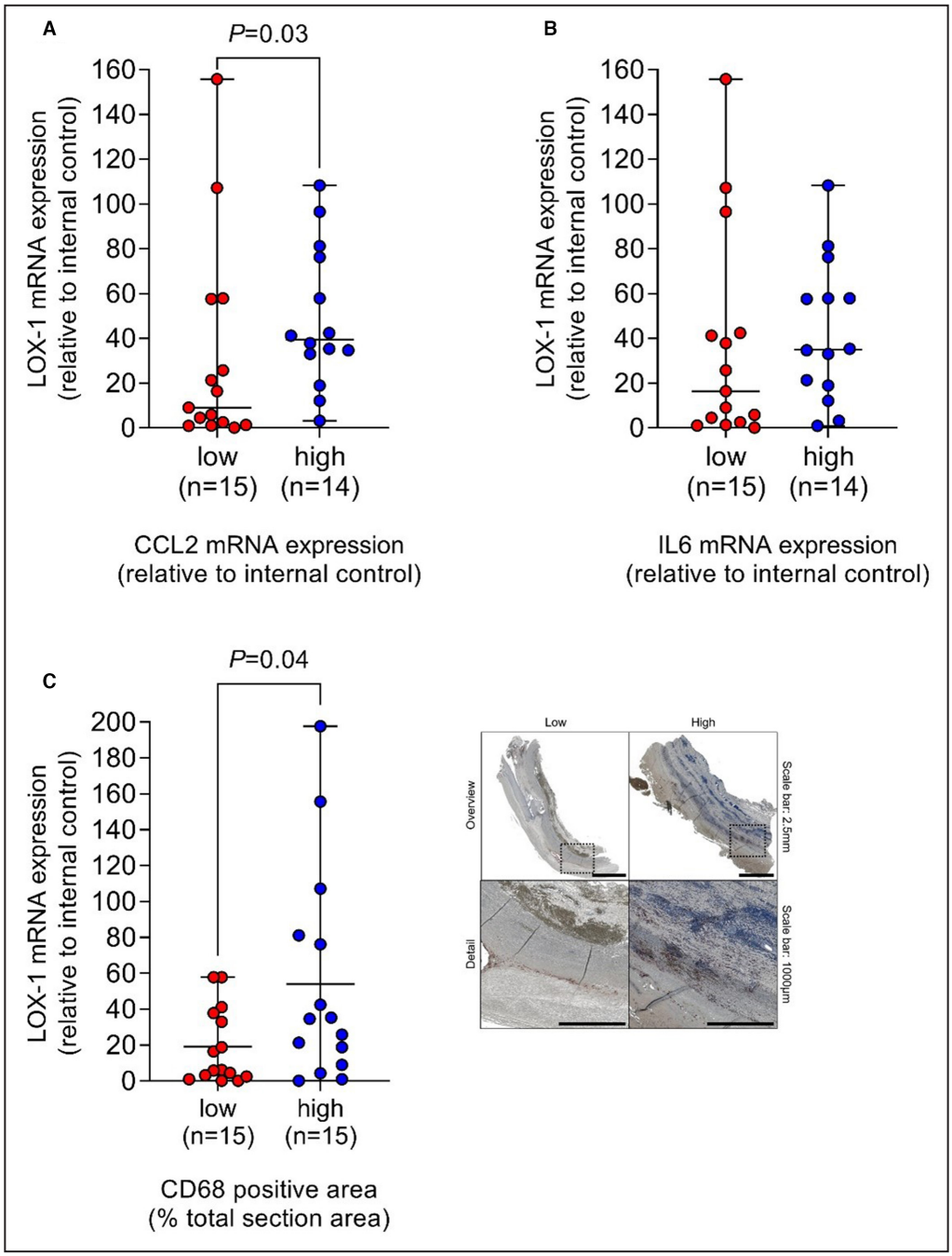


Figure 3. Aortic LOX-1 mRNA expression and inflammation in AAA.
Aortic mRNA expression of chemokine C-C motif ligand 2 (CCL2) and interleukin-6 (IL6) mRNA expression were analyzed by qPCR, and data are presented in relation to an internal control (=1). CD68 content was assessed by immunohistochemistry, and red-stained areas were quantified and set in relation to the total section area (%). CCL2 (**A**) and IL6 mRNA expression (**B**) and CD68-positive areas (**C**) were divided into low (red circles) and high (blue circles) depending on the median of the data set. Representative slides for CD68 immunohistochemistry are shown next the figure. Statistics: data are shown as scatter dot plots where the horizontal line depicts the median. Data were analyzed by Mann–Whitney *U* (**A** and **B**) or unpaired *t* test (**C**). AAA indicates abdominal aortic aneurysm; LOX-1, lectin-like oxidized low-density lipoprotein receptor-1; and qPCR, quantitative polymerase chain reaction.

markers in the aneurysmal wall.[33] Mechanistically, LOX-1 deletion abolished adventitial fibroblast proliferation.[33] In early carotid artery lesions, LOX-1 is found in luminal endothelial cells, whereas smooth muscle cells and macrophages express LOX-1 in advanced atherosclerotic lesions.[47] A breakdown in collagen is

Table 2. Clinical Characteristics of AAA and PAD Patients

Case-Control Study				
	PAD	AAA	*P* value	X^2
Baseline demographics				
n included	104	104		
sLOX-1, pg/mL, median with range, n	51.47 (2.40–771.3)	57.55 (3.10–595.1)	0.28	
Age, y, median with range	69.0 (48.0–90.0)	74.0 (51.0–89.0)	0.03	
Sex, M:F, % male	98:6, 94	101:3, 97	<0.0001	16.02
Aortic diameter, mm, median with 95% CI	22.2 (21.6; 23.0)	57.0 (55.9; 58.0)	<0.0001	
AAA volume, mm^3, median with 95% CI	nd	68.61 (54.54; 81.30)		
Thickness ILT, mm, median with 95% CI	nd	20.10 (17.30; 22.90)		
Cardiovascular risk factors				
LDL cholesterol, mmol/L, median with 95% CI, n	2.02 (1.78–2.19) 96	2.23 (2.08–2.61) 97	0.02	
Reference values <1.40 mmol/L for people with very-high risk				
HDL cholesterol, mmol/L, median with 95% CI, n	1.34 (1.21–1.43) 96	1.25 (1.11–1.30) 97	0.06	
Reference values >0.90 mmol/L for men and >1.10 mmol/L for women				
Total cholesterol, mmol/L, median with 95% CI, n	3.93 (3.61–4.06) 96	3.99 (3.77–4.36) 97	0.08	
Reference values <4.00 mmol/L				
Triglycerides, mmol/L, median with 95% CI, n	1.42 (1.26–1.58) 97	1.47 (1.37–1.67) 98	0.23	
Reference values 0.35–1.70 mmol/L				
Blood glucose, mmol/L, median with range, n	5.68 (5.34–5.95) 101	5.49 (5.31–5.81) 102	0.66	
Reference value only for fasting glucose possible				
CRP, mg/L, median with 95% CI, n	2.50 (1.70; 4.00) 104	2.60 (2.20; 3.30) 104	0.96	
Reference values <5.0 mg/L				
Smoking, yes:no, %	68:36, 65	55:49, 53	0.02	5.43
Hypertension, yes:no, %	103:1, 99	99:5, 95	0.09	2.75
CAD, yes:no, %	43:61, 41	34:70, 33	0.19	1.67
CAS, yes:no, %	27:83, 24	18:86, 17	0.70	0.14
PAD, yes:no, %	40:64, 100	20:82, 19	<0.0001	140.9
T2D, yes:no, %	33:64, 38	20:84, 20	0.003	8.87
BMI, kg/m^2, median with 95% CI, n	26.32 (25.26; 27.16) 104	27.25 (26.57; 28.09) 103	0.06	
Pharmacological therapies				
Statins, yes:no, %	90:14, 87	82:22, 79	0.15	2.15
ACE inhibitors, yes:no, %	46:58, 44	40:64, 38	0.39	0.71
ARBs, yes:no, %	36:66, 37	36:68, 35	0.77	0.08
CCB, yes:no, %	46:58, 44	39:65, 38	0.33	0.98
ASA, yes:no, %	78:26, 75	68:36, 65	0.13	2.29
β-blocker, yes:no, %	66:38, 63	49:55, 47	0.02	5.62
Anticoagulation, yes:no, %	38:66, 37	22:82, 21	0.01	2.45
Diuretics, yes:no, %	48:56, 46	35:69, 34	0.06	3.38
T2D treatment, yes:no, %	23:81, 22	19:85, 18	0.49	0.47
Insulin, yes:no, %	15:89, 14	7:97, 7	0.07	3.25

Serum from patients with AAA and PAD was collected in the pre-operative, non-fasting state.

Statistics: Patients in the PAD and AAA grouped were matched by propensity score method to account for baseline differences in covariates age, sex, smoking, hypertension, coronary artery disease (CAD), carotid artery stenosis (CAS) and type2 diabetes (T2D). Data are presented as median with range. Comparison of PAD and AAA was done by Mann-Whitney U test for continuous and by Chi-square test for continuous variables, respectively. For some patients, data on blood chemistry, cardiovascular risk factors and medical therapies were not documented and the number of analyzed patients (n) varies.

AAA indicates abdominal aortic aneurysm; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; ASA, acetylsalicylic acid; BMI, body mass index; CAD, coronary artery disease; CAS, carotid artery stenosis; CCB, calcium-channel blocker; CRP, C-reactive protein; HDL, high-density lipoprotein; ILT, intraluminal thrombus; LDL, low-density lipoprotein; OR, odds ratio; PAD, peripheral artery disease; SD, standard deviation; and T2D; type 2 diabetes.

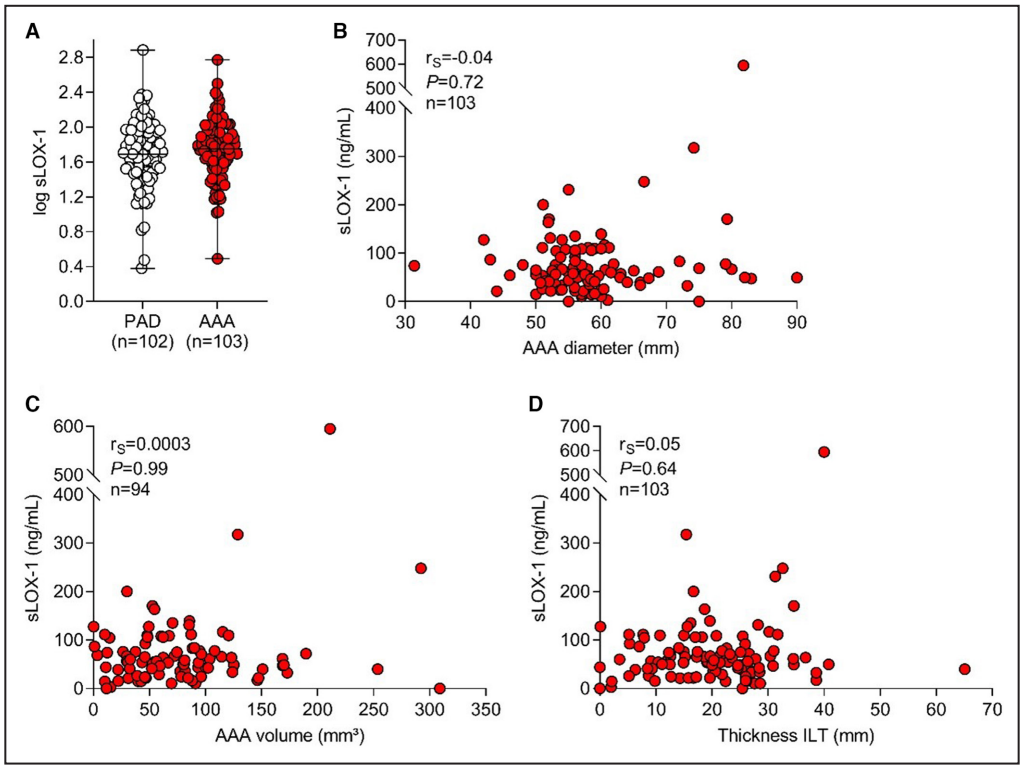


Figure 4. Diagnostic and risk stratification potential of serum sLOX-1 in patients with AAA.
A, Serum sLOX-1 was analyzed by ELISA in patients with AAA (red circles) or peripheral artery disease (PAD, with circles) in a case–control study. Data of sLOX-1 were log transformed and analyzed by using an unpaired *t* test. Two measurement values in the PAD and 3 in the AAA group, respectively, were excluded from the analysis because their concentrations were falling out of the range of the standard curve. **B–D**, The risk stratification potential of sLOX-1 was tested by using Spearman's correlation coefficient (r_S) between sLOX-1 and the AAA diameter, the AAA volume, and thickness of the intraluminal thrombus (ILT). For some patients, quantification of the AAA volume was not possible, and the number of analyzed samples is lower compared with the included patients. AAA indicates abdominal aortic aneurysm; and sLOX-1, soluble lectin-like oxidized low-density lipoprotein receptor-1.

typical in AAA,[17] and genetic deletion of LOX-1 reduced collagen accumulation in atherosclerotic Ldlr$^{-/-}$ mice.[50] An increased collagen can enhance the susceptibility to AAA rupture, whereas its loss can promote AAA formation. Therefore, an increase in collagen in late AAA might reflect the remodeling of the vessel wall, mainly caused by inflammation.[51] In addition, the aortic collagen content was linked to the AAA diameter, and associations between LOX-1 and collagen could depend on the aortic diameter. Compared with atherothrombosis, AAAs are characterized by a depletion in medial smooth muscle cells.[1] The association between LOX-1 and smooth muscle actin herein could be due to remaining smooth muscle cells or a co-staining of myofibroblasts[52] that are often found in the adventitia of inflammatory AAAs.[53]

Smooth muscle cell apoptosis is a hallmark in AAA.[54] A slight increase in LOX-1 mRNA was shown in samples with high apoptosis marker cleaved caspase-3. Previously published data showed that LOX-1 promotes apoptosis in macrophages[55] and vascular cells.[10,56] In human AAA, apoptosis was also observed in T-cells.[57] Herein, we could not delineate which cells express LOX-1 and cleaved caspase-3, that does not allow any conclusions about the cells undergoing apoptosis.

LOX-1 expression in vascular smooth muscle cells could be induced by proinflammatory cytokines[58] or oxLDL.[59] In the present study, LOX-1 mRNA expression was higher in AAA specimen with a high expression of macrophage marker CD68 and C-C motif chemokine ligand 2 (CCL2/MCP-1) mRNA expression emphasizing the well-established link between LOX-1 and inflammation[60] in AAA. It has been demonstrated recently that LOX-1 is primarily expressed by infiltrating macrophages in human AAA.[46]

CONCLUSIONS

In conclusion, the present study demonstrated that circulating sLOX-1 concentrations were not statistically different between patients with AAA and PAD.

Table 3. Soluble LOX-1 Concentrations and Clinical Confounder Variables

Variable	Estimate	CI (left; right)	*P* value
Disease (ref=PAD)	1.287	1.011;1.638	0.042
Age	0.982	0.970;0.995	0.007
T2D (ref=none)	1.210	0.931;1.572	0.156
CAD (ref=none)	1.204	0.928;1.562	0.164
CAS (ref=none)	0.800	0.599;1.068	0.131
β-blockers (ref=none)	1.268	0.994;1.619	0.058
Statins (ref=none)	0.760	0.559;1.033	0.082
ACE (ref=none)	1.258	0.993;1.592	0.058
Anticoagulation (ref=none)	0.870	0.676;1.121	0.283

Serum sLOX-1 concentrations were analyzed by ELISA and log transformed. Log sLOX-1 was set as the outcome variable, and effects of disease, age, T2D, CAD, CAS, prescription of β-blockers, statins, ACE inhibitors and therapeutic anticoagulation were adjusted by multivariate linear regression. The estimate shows the increase in sLOX-1 when the patient is diagnosed with AAA compared with PAD (ref=PAD). Estimates for T2D and medical therapies refer to the increase or decrease in sLOX-1 when the patient has the indicated disease or receives the treatment compared with patients without (ref=none). For age, the estimate refers to the decrease per 1 year of age. AAA indicates abdominal aortic aneurysm; ACE, angiotensin-converting enzyme; CAS, carotid artery stenosis; CAD, coronary artery disease; PAD, peripheral artery disease; ref, reference; sLOX-1, soluble lectin-like oxidized low-density lipoprotein receptor-1; and T2D, type 2 diabetes.

However, adjusting sLOX-1 for age, T2D, prescription of β-blockers, and therapeutic anticoagulation increased sLOX-1 in AAA, suggesting that other controls or a specific subgroup analysis is necessary. Serum sLOX-1 did not correlate with the aortic diameter, AAA volume, or the thickness of the ILT, implying that sLOX-1 is not a risk stratification marker in AAA. The increase in aneurysmal LOX-1 mRNA expression was connected to apoptosis, collagen, inflammation, and smooth muscle cells, suggesting that the role of circulating sLOX-1 has to be differentiated from those of membrane-bound LOX-1. Contrary to atherothrombosis and plaque rupture, LOX-1 may not promote the progression or rupture of human AAA. Further studies focusing on smooth muscle cells and collagen synthesis are needed. In perspective, the present findings contribute to a better understanding of LOX-1 in AAA and the development of novel therapeutic targets (eg, targeting adventitial thickening to counteract the increased risk of AAA rupture).

LIMITATIONS

The present study is an observational, descriptive study in a rather small cohort and does not allow drawing conclusions on causations and underlying mechanism of LOX-1 regulation, sLOX-1 release or its therapeutic potential. Establishing causal relationships between AAA and LOX-1 and sLOX-1 requires in vitro studies or preclinical AAA models. Moreover, analyzing a potential linkage between AAA rupture and LOX-1 expression requires, for example, the analysis of biomechanical properties such as peak wall stress and peak wall rupture index.[61] Furthermore, quantification of microvessels,[62] adventitial adipocyte aggregates,[63] and lymphocytic infiltrates[64] would have allowed obtaining more information on AAA progression. Herein, patients with PAD were used as referral controls and analyzing healthy, age and sex-matched people without T2D would allow to draw conclusions on the potential of sLOX-1 to discriminate AAA from the general population. The matching of patients in the case–control study was done for age, sex, smoking status, coronary heart disease, carotid artery stenosis, and T2D to minimize the differences between both groups. Despite this, differences in age and the prevalence of T2D exist, which could be due to the rather low number of patients compared with the high number of confounders. In addition, results have to be interpreted in the context of the rather high interassay variation of the ELISA. Analyzing LOX-1 protein and its localization within the AAA would have been more appropriate but requires the availability of specific antibodies detecting LOX-1 in human AAA tissues. Data for histopathological vessel wall degeneration were assessed semi-quantitatively and single-cell analysis would have given more appropriate information.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Tables S1–S8
Figures S1–S5

REFERENCES

1. Golledge J, Muller J, Daugherty A, Norman P. Abdominal aortic aneurysm: pathogenesis and implications for management. *Arterioscler Thromb Vasc Biol*. 2006;26:2605–2613. doi: 10.1161/01.ATV.0000245819.32762.cb
2. Davis FM, Rateri DL, Daugherty A. Abdominal aortic aneurysm: novel mechanisms and therapies. *Curr Opin Cardiol*. 2015;30:566–573. doi: 10.1097/HCO.0000000000000216
3. Hartl F, Reeps C, Wilhelm M, Ockert S, Zimmermann A, Eckstein HH. Open and endovascular repair of abdominal aortic aneurysms - clinical picture, evidence, results. *Dtsch Med Wochenschr*. 2012;137:1303–1308. doi: 10.1055/s-0032-1305055
4. Forsythe RO, Newby DE. Imaging biomarkers for abdominal aortic aneurysms. *Circ Cardiovasc Imaging*. 2019;12:e008917. doi: 10.1161/CIRCIMAGING.119.008917
5. Boyd AJ. Intraluminal thrombus: innocent bystander or factor in abdominal aortic aneurysm pathogenesis? *JVS Vasc Sci*. 2021;2:159–169. doi: 10.1016/j.jvssci.2021.02.001
6. Sawamura T, Kume N, Aoyama T, Moriwaki H, Hoshikawa H, Aiba Y, Tanaka T, Miwa S, Katsura Y, Kita T, et al. An endothelial receptor for oxidized low-density lipoprotein. *Nature*. 1997;386:73–77. doi: 10.1038/386073a0
7. Hofmann A, Brunssen C, Morawietz H. Contribution of lectin-like oxidized low-density lipoprotein receptor-1 and LOX-1 modulating compounds to vascular diseases. *Vasc Pharmacol*. 2018;107:1–11. doi: 10.1016/j.vph.2017.10.002
8. Hofmann A, Brunssen C, Poitz DM, Langbein H, Strasser RH, Henle T, Ravens U, Morawietz H. Lectin-like oxidized low-density lipoprotein receptor-1 promotes endothelial dysfunction in LDL receptor knockout background. *Atheroscler Suppl*. 2017;30:294–302. doi: 10.1016/j.atherosclerosissup.2017.05.020
9. Skarpengland T, Skjelland M, Kong XY, Skagen K, Holm S, Otterdal K, Dahl CP, Krohg-Sorensen K, Sagen EL, Bjerkeli V, et al. Increased levels of lectin-like oxidized low-density lipoprotein receptor-1 in ischemic stroke and transient ischemic attack. *J Am Heart Assoc*. 2018;7:7. doi: 10.1161/JAHA.117.006479
10. Li D, Mehta JL. Upregulation of endothelial receptor for oxidized LDL (LOX-1) by oxidized LDL and implications in apoptosis of human coronary artery endothelial cells. *Arterioscler Thromb Vasc Biol*. 2000;20:1116–1122. doi: 10.1161/01.ATV.20.4.1116
11. Golledge J, Norman PE. Atherosclerosis and abdominal aortic aneurysm: cause, response, or common risk factors? *Arterioscler Thromb Vasc Biol*. 2010;30:1075–1077. doi: 10.1161/ATVBAHA.110.206573
12. Biros E, Gäbel G, Moran CS, Schreurs C, Lindeman JH, Walker PJ, Nataatmadja M, West M, Holdt LM, Hinterseher I, et al. Differential gene expression in human abdominal aortic aneurysm and aortic occlusive disease. *Oncotarget*. 2015;6:12984–12996. doi: 10.18632/oncotarget.3848
13. Zhao XQ, Zhang MW, Wang F, Zhao YX, Li JJ, Wang XP, Bu PL, Yang JM, Liu XL, Zhang MX, et al. Crp enhances soluble LOX-1 release from macrophages by activating TNF-alpha converting enzyme. *J Lipid Res*. 2011;52:923–933. doi: 10.1194/jlr.M015156
14. Mitsuoka H, Kume N, Hayashida K, Inui-Hayashiada A, Aramaki Y, Toyohara M, Jinnai T, Nishi E, Kita T. Interleukin-18 stimulates release of soluble lectin-like oxidized LDL receptor-1 (sLOX-1). *Atherosclerosis*. 2009;202:176–182. doi: 10.1016/j.atherosclerosis.2008.04.002
15. Markstad H, Edsfeldt A, Yao Mattison I, Bengtsson E, Singh P, Cavalera M, Asciutto G, Bjorkbacka H, Fredrikson GN, Dias N, et al. High levels of soluble lectin like oxidized low-density lipoprotein receptor-1 are associated with carotid plaque inflammation and increased risk of ischemic stroke. *J Am Heart Assoc*. 2019;8:e009874. doi: 10.1161/JAHA.118.009874
16. Murase T, Kume N, Kataoka H, Minami M, Sawamura T, Masaki T, Kita T. Identification of soluble forms of lectin-like oxidized LDL receptor-1. *Arterioscler Thromb Vasc Biol*. 2000;20:715–720. doi: 10.1161/01.ATV.20.3.715
17. Abdul-Hussien H, Soekhoe RG, Weber E, von der Thusen JH, Kleemann R, Mulder A, van Bockel JH, Hanemaaijer R, Lindeman JH. Collagen degradation in the abdominal aneurysm: a conspiracy of matrix metalloproteinase and cysteine collagenases. *Am J Pathol*. 2007;170:809–817. doi: 10.2353/ajpath.2007.060522
18. Zhao ZW, Zhu XL, Luo YK, Lin CG, Chen LL. Circulating soluble lectin-like oxidized low-density lipoprotein receptor-1 levels are associated with angiographic coronary lesion complexity in patients with coronary artery disease. *Clin Cardiol*. 2011;34:172–177. doi: 10.1002/clc.20847
19. Hofmann A, Brunssen C, Wolk S, Reeps C, Morawietz H. Soluble LOX-1: a novel biomarker in patients with coronary artery disease, stroke, and acute aortic dissection? *J Am Heart Assoc*. 2020;9:e013803. doi: 10.1161/JAHA.119.013803
20. Hayashida K, Kume N, Murase T, Minami M, Nakagawa D, Inada T, Tanaka M, Ueda A, Kominami G, Kambara H, et al. Serum soluble lectin-like oxidized low-density lipoprotein receptor-1 levels are elevated in acute coronary syndrome: a novel marker for early diagnosis. *Circulation*. 2005;112:812–818. doi: 10.1161/CIRCULATIONAHA.104.468397
21. Kobayashi N, Takano M, Hata N, Kume N, Yamamoto M, Yokoyama S, Shinada T, Tomita K, Shirakabe A, Otsuka T, et al. Soluble lectin-like oxidized LDL receptor-1 (sLOX-1) as a valuable diagnostic marker for rupture of thin-cap fibroatheroma: verification by optical coherence tomography. *Int J Cardiol*. 2013;168:3217–3223. doi: 10.1016/j.ijcard.2013.04.110
22. Barreto J, Karathanasis SK, Remaley A, Sposito AC. Role of LOX-1 (lectin-like oxidized low-density lipoprotein receptor-1) as a cardiovascular risk predictor: mechanistic insight and potential clinical use. *Arterioscler Thromb Vasc Biol*. 2021;41:153–166. doi: 10.1161/ATVBAHA.120.315421
23. Kraler S, Wenzl FA, Georgiopoulos G, Obeid S, Liberale L, von Eckardstein A, Muller O, Mach F, Räber L, Losdat S, et al. Soluble lectin-like oxidized low-density lipoprotein receptor-1 predicts premature death in acute coronary syndromes. *Eur Heart J*. 2022;43:1849–1860. doi: 10.1093/eurheartj/ehac143
24. Kobayashi N, Hata N, Kume N, Yokoyama S, Takano M, Shinada T, Tomita K, Shirakabe A, Inami T, Seino Y, et al. Detection of acute aortic dissection by extremely high soluble lectin-like oxidized LDL receptor-1 (sLOX-1) and low troponin T levels in blood. *Int J Cardiol*. 2013;165:557–559. doi: 10.1016/j.ijcard.2012.09.001
25. Golledge J, Tsao PS, Dalman RL, Norman PE. Circulating markers of abdominal aortic aneurysm presence and progression. *Circulation*. 2008;118:2382–2392. doi: 10.1161/CIRCULATIONAHA.108.802074
26. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol*. 2002;3:research0034.1. doi: 10.1186/gb-2002-3-7-research0034
27. Wei H, Hu JH, Angelov SN, Fox K, Yan J, Enstrom R, Smith A, Dichek DA. Aortopathy in a mouse model of marfan syndrome is not mediated by altered transforming growth factor β signaling. *J Am Heart Assoc*. 2017;6:6. doi: 10.1161/JAHA.116.004968
28. Hofmann A, Muglich M, Wolk S, Khorzom Y, Sabarstinski P, Kopaliani I, Egorov D, Horn F, Brunssen C, Giebe S, et al. Induction of heme oxygenase-1 is linked to the severity of disease in human abdominal aortic aneurysm. *J Am Heart Assoc*. 2021;10:e022747. doi: 10.1161/JAHA.121.022747
29. Kopaliani I, Martin M, Zatschler B, Bortlik K, Muller B, Deussen A. Cell-specific and endothelium-dependent regulations of matrix metalloproteinase-2 in rat aorta. *Basic Res Cardiol*. 2014;109:419. doi: 10.1007/s00395-014-0419-8
30. Hellenthal FA, Buurman WA, Wodzig WK, Schurink GW. Biomarkers of abdominal aortic aneurysm progression. Part 2: inflammation. *Nat Rev Cardiol*. 2009;6:543–552. doi: 10.1038/nrcardio.2009.102
31. Klaus V, Tanios-Schmies F, Reeps C, Trenner M, Matevossian E, Eckstein HH, Pelisek J. Association of matrix metalloproteinase levels

with collagen degradation in the context of abdominal aortic aneurysm. *Eur J Vasc Endovasc Surg*. 2017;53:549–558. doi: 10.1016/j.ejvs.2016.12.030
32. Gioia M, Vindigni G, Testa B, Raniolo S, Fasciglione GF, Coletta M, Biocca S. Membrane cholesterol modulates LOX-1 shedding in endothelial cells. *PLoS One*. 2015;10:e0141270. doi: 10.1371/journal.pone.0141270
33. Takahashi K, Aono J, Nakao Y, Hamaguchi M, Suehiro C, Kurata M, Sakaue T, Kakino A, Sawamura T, Inoue K, et al. LOX-1 deficiency increases ruptured abdominal aortic aneurysm via thinning of adventitial collagen. *Hypertens Res*. 2023;46:63–74. doi: 10.1038/s41440-022-01093-x
34. Brinkley TE, Kume N, Mitsuoka H, Phares DA, Hagberg JM. Elevated soluble lectin-like oxidized LDL receptor-1 (sLOX-1) levels in obese postmenopausal women. *Obesity (Silver Spring)*. 2008;16:1454–1456.
35. Tan KC, Shiu SW, Wong Y, Leng L, Bucala R. Soluble lectin-like oxidized low density lipoprotein receptor-1 in type 2 diabetes mellitus. *J Lipid Res*. 2008;49:1438–1444. doi: 10.1194/jlr.M700551-JLR200
36. Fukui M, Tanaka M, Senmaru T, Nakanishi M, Mukai J, Ohki M, Asano M, Yamazaki M, Hasegawa G, Nakamura N. LOX-1 is a novel marker for peripheral artery disease in patients with type 2 diabetes. *Metabolism*. 2013;62:935–938. doi: 10.1016/j.metabol.2013.01.018
37. Lee AS, Wang YC, Chang SS, Lo PH, Chang CM, Lu J, Burns AR, Chen CH, Kakino A, Sawamura T, et al. Detection of a high ratio of soluble to membrane-bound LOX-1 in aspirated coronary thrombi from patients with ST-segment-elevation myocardial infarction. *J Am Heart Assoc*. 2020;9:e014008. doi: 10.1161/JAHA.119.014008
38. Raffort J, Lareyre F, Clément M, Hassen-Khodja R, Chinetti G, Mallat Z. Diabetes and aortic aneurysm: current state of the art. *Cardiovasc Res*. 2018;114:1702–1713. doi: 10.1093/cvr/cvy174
39. Thiruvoipati T, Kielhorn CE, Armstrong EJ. Peripheral artery disease in patients with diabetes: epidemiology, mechanisms, and outcomes. *World J Diabetes*. 2015;6:961–969. doi: 10.4239/wjd.v6.i7.961
40. Li B, Zhang LH, Yang XG, Liu XT, Ren YG. Serum sLOX-1 levels are associated with the presence and severity of angiographic coronary artery disease in patients with metabolic syndrome. *Clin Invest Med*. 2010;33:E398–E404. doi: 10.25011/cim.v33i6.14591
41. Pirillo A, Catapano AL. Soluble lectin-like oxidized low density lipoprotein receptor-1 as a biochemical marker for atherosclerosis-related diseases. *Dis Markers*. 2013;35:413–418. doi: 10.1155/2013/716325
42. Lubrano V, Del Turco S, Nicolini G, Di Cecco P, Basta G. Circulating levels of lectin-like oxidized low-density lipoprotein receptor-1 are associated with inflammatory markers. *Lipids*. 2008;43:945–950. doi: 10.1007/s11745-008-3227-9
43. Siika A, Lindquist Liljeqvist M, Hultgren R, Gasser TC, Roy J. Aortic lumen area is increased in ruptured abdominal aortic aneurysms and correlates to biomechanical rupture risk. *J Endovasc Ther*. 2018;25:750–756. doi: 10.1177/1526602818808292
44. Kitagawa A, Mastracci TM, von Allmen R, Powell JT. The role of diameter versus volume as the best prognostic measurement of abdominal aortic aneurysms. *J Vasc Surg*. 2013;58:258–265. doi: 10.1016/j.jvs.2013.05.001
45. Olson SL, Panthofer AM, Blackwelder W, Terrin ML, Curci JA, Baxter BT, Weaver FA, Matsumura JS. Role of volume in small abdominal aortic aneurysm surveillance. *J Vasc Surg*. 2022;75:1260–1267.e1263. doi: 10.1016/j.jvs.2021.09.046
46. Zhang H, Geng N, Sun L, Che X, Xiao Q, Tao Z, Chen L, Lyu Y, Shao Q, Pu J. Nuclear receptor Nur77 protects against abdominal aortic aneurysm by ameliorating inflammation via suppressing LOX-1. *J Am Heart Assoc*. 2021;10:e021707. doi: 10.1161/JAHA.121.021707
47. Kataoka H, Kume N, Miyamoto S, Minami M, Moriwaki H, Murase T, Sawamura T, Masaki T, Hashimoto N, Kita T. Expression of lectin like oxidized low-density lipoprotein receptor-1 in human atherosclerotic lesions. *Circulation*. 1999;99:3110–3117. doi: 10.1161/01.CIR.99.24.3110
48. Franck G, Dai J, Fifre A, Ngo S, Justine C, Michineau S, Allaire E, Gervais M. Reestablishment of the endothelial lining by endothelial cell therapy stabilizes experimental abdominal aortic aneurysms. *Circulation*. 2013;127:1877–1887. doi: 10.1161/CIRCULATIONAHA.113.001677
49. Quintana RA, Taylor WR. Cellular mechanisms of aortic aneurysm formation. *Circ Res*. 2019;124:607–618. doi: 10.1161/CIRCRESAHA.118.313187
50. Hu C, Dandapat A, Sun L, Chen J, Marwali MR, Romeo F, Sawamura T, Mehta JL. LOX-1 deletion decreases collagen accumulation in atherosclerotic plaque in low-density lipoprotein receptor knockout mice fed a high-cholesterol diet. *Cardiovasc Res*. 2008;79:287–293. doi: 10.1093/cvr/cvn110
51. Jana S, Hu M, Shen M, Kassiri Z. Extracellular matrix, regional heterogeneity of the aorta, and aortic aneurysm. *Exp Mol Med*. 2019;51:1–15. doi: 10.1038/s12276-019-0286-3
52. Sousa AM, Liu T, Guevara O, Stevens J, Fanburg BL, Gaestel M, Toksoz D, Kayyali US. Smooth muscle alpha-actin expression and myofibroblast differentiation by TGF-β are dependent upon MK2. *J Cell Biochem*. 2007;100:1581–1592. doi: 10.1002/jcb.21154
53. Sakata N, Nabeshima K, Iwasaki H, Tashiro T, Uesugi N, Nakashima O, Ito H, Kawanami T, Furuya K, Kojima M. Possible involvement of myofibroblast in the development of inflammatory aortic aneurysm. *Pathol Res Pract*. 2007;203:21–29. doi: 10.1016/j.prp.2006.08.008
54. Thompson RW, Liao S, Curci JA. Vascular smooth muscle cell apoptosis in abdominal aortic aneurysms. *Coron Artery Dis*. 1997;8:623–631. doi: 10.1097/00019501-199710000-00005
55. Guo R, Su Y, Liu B, Li S, Zhou S, Xu Y. Resveratrol suppresses oxidised low-density lipoprotein-induced macrophage apoptosis through inhibition of intracellular reactive oxygen species generation, LOX-1, and the p38MAPK pathway. *Cell Physiol Biochem*. 2014;34:603–616. doi: 10.1159/000363026
56. Chen J, Mehta JL, Haider N, Zhang X, Narula J, Li D. Role of caspases in Ox-LDL-induced apoptotic cascade in human coronary artery endothelial cells. *Circ Res*. 2004;94:370–376. doi: 10.1161/01.RES.0000113782.07824.BE
57. Chakraborty A, Li Y, Zhang C, Li Y, LeMaire SA, Shen YH. Programmed cell death in aortic aneurysm and dissection: a potential therapeutic target. *J Mol Cell Cardiol*. 2022;163:67–80. doi: 10.1016/j.yjmcc.2021.09.010
58. Hofnagel O, Luechtenborg B, Stolle K, Lorkowski S, Eschert H, Plenz G, Robenek H. Pro-inflammatory cytokines regulate LOX-1 expression in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol*. 2004;24:1789–1795. doi: 10.1161/01.ATV.0000140061.89096.2b
59. Viola M, Bartolini B, Vigetti D, Karousou E, Moretto P, Deleonibus S, Sawamura T, Wight TN, Hascall VC, De Luca G, et al. Oxidized low density lipoprotein (LDL) affects hyaluronan synthesis in human aortic smooth muscle cells. *J Biol Chem*. 2013;288:29595–29603. doi: 10.1074/jbc.M113.508341
60. Hofmann A, Brunssen C, Morawietz H. Contribution of lectin-like oxidized low-density lipoprotein receptor-1 and LOX-1 modulating compounds to vascular diseases. *Vasc Pharmacol*. 2017;107:1–11. doi: 10.1016/j.vph.2017.10.002
61. Zschäpitz D, Bohmann B, Lutz B, Eckstein H-H, Reeps C, Maegdefessel L, Gasser CT, Busch A. Rupture risk parameters upon biomechanical analysis independently change from vessel geometry during abdominal aortic aneurysm growth. *JVS Vasc Sci*. 2023;4:100093. doi: 10.1016/j.jvssci.2022.10.004
62. Gäbel G, Northoff BH, Weinzierl I, Ludwig S, Hinterseher I, Wilfert W, Teupser D, Doderer SA, Bergert H, Schönleben F, et al. Molecular fingerprint for terminal abdominal aortic aneurysm disease. *J Am Heart Assoc*. 2017;6:e006798. doi: 10.1161/JAHA.117.006798
63. Doderer SA, Gäbel G, Kokje VBC, Northoff BH, Holdt LM, Hamming JF, Lindeman JHN. Adventitial adipogenic degeneration is an unidentified contributor to aortic wall weakening in the abdominal aortic aneurysm. *J Vasc Surg*. 2018;67:1891–1900.e1894. doi: 10.1016/j.jvs.2017.05.088
64. Bruijn LE, van Stroe Gómez CG, Curci JA, Golledge J, Hamming JF, Jones GT, Lee R, Matic L, van Rhijn C, Vriens PW, et al. A histopathological classification scheme for abdominal aortic aneurysm disease. *JVS Vasc Sci*. 2021;2:260–273. doi: 10.1016/j.jvssci.2021.09.001

SUPPLEMENTAL MATERIAL

Associations of tissue and soluble LOX-1 with human abdominal aortic aneurysm

Short running title: sLOX-1 in AAA

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All authors do not have any conflict of interest.

Subject term list: Vascular disease, Aneurysm

SUPPLEMENTAL TABLES

Supplemental Table 1: Clinical characteristics of patients with PAD and AAA enrolled for propensity score matching. Abbreviations: CHD, Coronary heart disease; CAS, Carotid artery stenosis; PAD, Peripheral artery disease; T2D, Type 2 diabetes.

	PAD	**AAA**
n, enrolled	128	105
Age – years, median with range	68.0 (45.0 - 90.0)	74.0 (51.0 - 89.0)
Gender, m:f, % male	98:30, 77	101:4, 96
Smoking, yes:no – %	88:40, 69	57:48, 54
Hypertension, yes:no – %	128:0, 100	99:6, 94
CAD, yes:no – %	51:77, 40	33:72, 31
CAS, yes:no – %	25:103, 20	20:85, 19
PAD, yes:no – %	128:0, 100	18:87, 17
T2D, yes:no – %	47:81, 34	20:85, 19

Supplemental Table 2: Primers used for gene expression analysis by quantitative real-time PCR (qPCR).

Gene	**Primers**	**Sequence, 5'-3'**
B2M	Forward	GATGAGTATGCCTGCCGTGT
	Reverse	CATGATGCTGCTTACATGTCTCG
CCL2	Forward	GCTCAGCCAGATGCAATCA
	Reverse	TTTGCTTGTCCAGGTGGTC
COL1A1	Forward	AGACAGTGATTGAATACAAAACCA
	Reverse	GGAGTTTACAGGAAGCAGACA
COL3A1	Forward	CCTGAAGCTGATGGGGTCAA
	Reverse	TAGTCTCACAGCCTTGCGTG

ELN	Forward	TTTTATCCAGGGGCTGGTCTC
	Reverse	CGGGAACTGGCTTAAGAGGT
IL6	Forward	CCTGACCCAACCACAAATGC
	Reverse	ATCTGAGGTGCCCATGCTAC
LOX-1	Forward	CATTCAGCTTCCGAGCAAGGG
	Reverse	ACTCTCCATGGTGGTGCCTGG
RPL32	Forward	CACCGTCCCTTCTCTCTTCCT
	Reverse	TCTTGGGCTTCACAAGGGGT
TBP	Forward	CGCCGGCTGTTTAACTTCG
	Reverse	AGAGCATCTCCAGCACACTC

Abbreviations: B2M, beta-2-microglobulin; CCL2, C-C Motif Chemokine Ligand 2; COL1A1, Collagen, type I, alpha 1; COL3A1; Collagen, type III, alpha 1; ELN, Elastin; IL6, Interleukin-6; LOX-1, lectin-like oxidized low-density lipoprotein receptor-1; RPL32, ribosomal protein L32; TBP, TATA-box binding protein.

Supplemental Table 3: Characteristics of elastin degradation scoring in aortic walls.

Score	Features of the section
1	Large number of violet elastic laminae in the media. No gaps between the elastic laminae (continuous). No elastin breaks throughout the whole section.
2	Reduced number of elastic laminae. First, but only a low amount of breaks in elastic laminae. First larger gaps between the laminae.
3	Only spots of violet elastic fibers at different sites of slide, not continuous. Large elastic fiber breaks.
4	Little or no elastic fibers throughout the slide, no continuous laminae visible. Large areas of elastic fibers breaks.

Supplemental Table 4: Concentrations of primary antibodies used for immunohistochemistry.

Abbreviations: α-SMA, alpha-smooth muscle actin.

Protein	Supplier and order number	Concentration	Duration of incubation
α-SMA	A5228, Clone 1A4, Sigma Aldrich	2 µg/mL	1 h
Cleaved caspase-3	#9661, Cell Signaling	0.59 µg/mL	1 h
CD31	M0823, Clone JC70A, Agilent	4.1 mg/L	1 h
CD68	M0814, Clone KP1, Agilent	4 µg/mL	over night

Supplemental Table 5: Serum sLOX-1, cardiovascular risk factors and medical therapy in AAA. sLOX-1 concentrations were analyzed by ELISA and log transformed. Log sLOX-1 was set as the outcome variable and effects of comorbidities and medical therapies were analyzed by stepwise linear regression. Estimates show the increase or decrease in sLOX-1 when the patient is diagnosed with the indicated disease or receives the medical therapy compared to patients without (ref = none). **Abbreviations:** ACE, Angiotensin-converting enzyme; ARB, Angiotensin receptor blocker; ASA, Acetylsalicylic acid; CAS, Carotid artery stenosis; CHD, Coronary heart disease; LDL, Low-density lipoprotein.

	Estimate
Age	-0.0165
Aortic diameter	0.0057
LDL cholesterol	0.0612
CAD (ref = none)	0.0920
CAS (ref = none)	-0.5192
T2D (ref = none)	0.0925
Statins (ref = none)	-0.3494
ACE (ref = none)	0.0000
ARB (ref = none)	-0.2534
ASA (ref = none)	0.0863
β-blocker (ref = none)	0.5200
Anticoagulation (ref = none)	-0.2364

Supplemental Table 6: Serum sLOX-1, cardiovascular risk factors and medical therapy in PAD. sLOX-1 concentrations were analyzed by ELISA and log transformed. Log sLOX-1 was set as the outcome variable and effects of comorbidities and medical therapies were analyzed by stepwise linear regression. Estimates show the increase or decrease in sLOX-1 when the patient is diagnosed with the indicated disease or receives the medical therapy compared to patients without (ref = none). **Abbreviations:** ACE, Angiotensin-converting enzyme; ARB, Angiotensin receptor blocker; ASA, Acetylsalicylic acid; CAS, Carotid artery stenosis; CHD, Coronary heart disease; LDL, Low-density lipoprotein.

	Estimate
Age	-0.0171
Aortic diameter	-0.0233
LDL cholesterol	-0.1080
CAD (ref = none)	0.2100
CAS (ref = none)	-0.0138
T2D (ref = none)	0.2400
Statins (ref = none)	-0.1862
ACE (ref = none)	0.2661
ARB (ref = none)	0.0014
ASA (ref = none)	-0.0463
β-blocker (ref = none)	0.0000
Anticoagulation (ref = none)	-0.1249

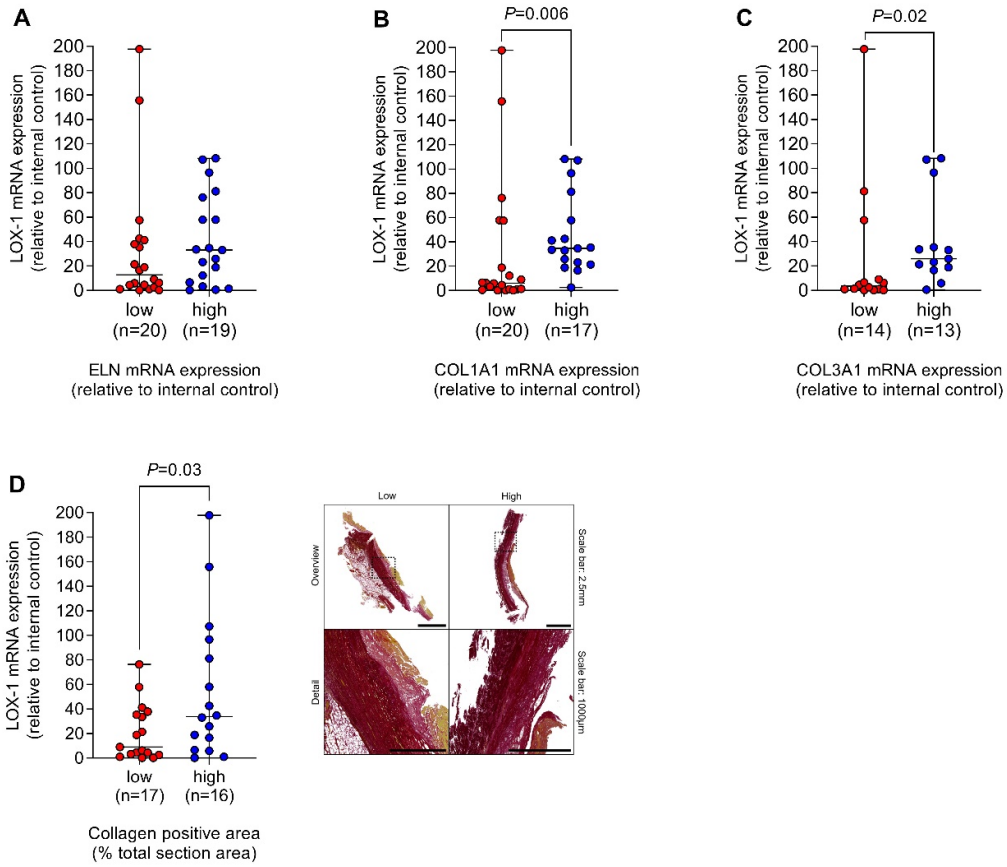
Supplemental Table 7: Correlations of sLOX-1 with cardiovascular risk factors in AAA. Serum sLOX-1 concentrations were analyzed by ELISA and log transformed. Correlations were tested by spearman's correlation (rs). **Abbreviations:** BMI, Body mass index; CRP, C-reactive protein; HDL, High-density lipoprotein; LDL, Low-density lipoprotein.

sLOX-1	CRP (mg/L)	LDL cholesterol (mmol/L)	HDL cholesterol (mmol/L)	Total cholesterol (mmol/L)	Triglycerides (mmol/L)	Glucose (mmol/L)	BMI (kg/m²)
Spearman's rs	0.07	0.18	-0.39	0.04	0.21	-0.05	-0.12
P-value	0.47	0.08	<0.0001	0.69	0.03	0.63	0.25
n	101	95	95	95	96	99	100

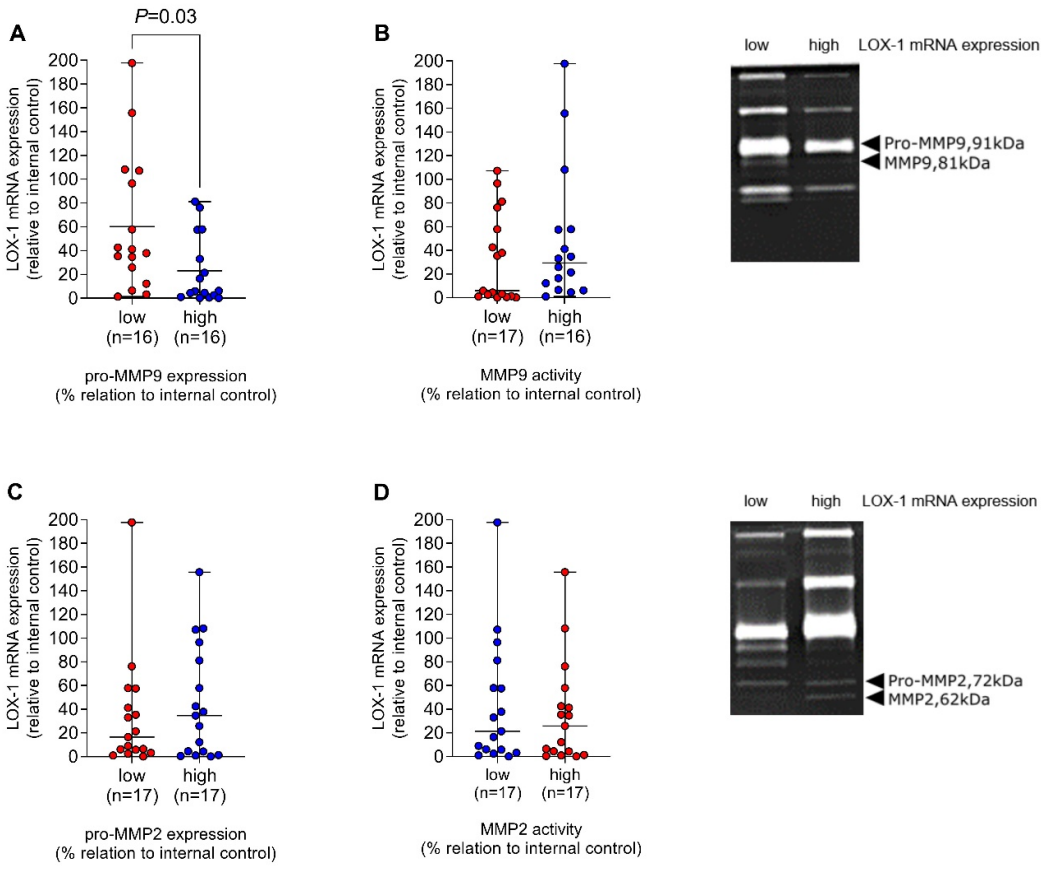
Supplemental Table 8: Correlations of sLOX-1 with cardiovascular risk factors in PAD. Serum sLOX-1 concentrations were analyzed by ELISA and log transformed. Correlations were tested by spearman's correlation (rs). **Abbreviations:** BMI, Body mass index; CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

sLOX-1	CRP (mg/L)	LDL cholesterol (mmol/L)	HDL cholesterol (mmol/L)	Total cholesterol (mmol/L)	Triglycerides (mmol/L)	Glucose (mmol/L)	BMI (kg/m²)
Spearman's rs	-0.09	-0.13	0.05	-0.06	0.06	0.05	0.16
P-value	0.35	0.19	0.63	0.56	0.53	0.62	0.09
n	102	94	94	94	95	99	102

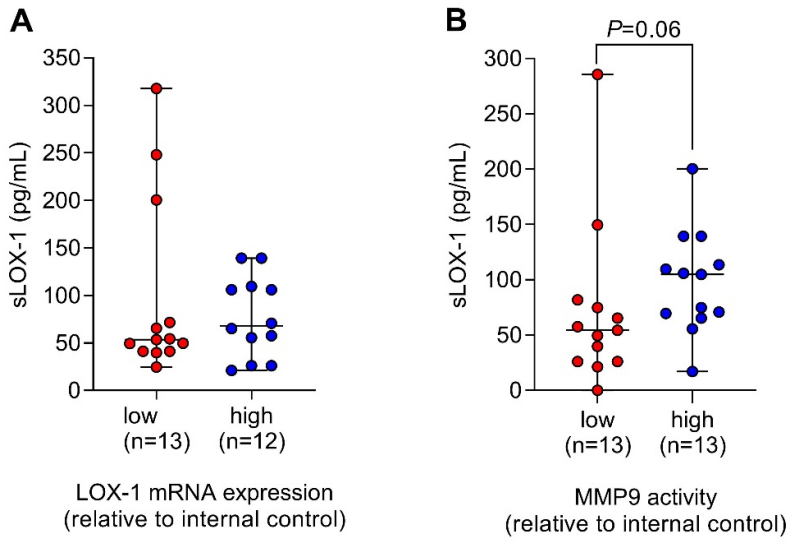
SUPPLEMENTAL FIGURES



Supplemental Figure 1: Aortic LOX-1, elastin and collagen expression in AAA. Aortic mRNA expression was analyzed by qPCR and data are presented in relation to an internal control (=1). Aortic **A,** mRNA expression of elastin (ELN), **B,** collagen, type I, alpha 1 (COL1A1) and **C,** mRNA expression of collagen, type III, alpha 1 (COL3A1). **D,** Collagen content was assessed by Picro-Sirius Red staining and red-stained areas were quantified and set in relation to the total section area (%). Expression was divided into low (red circles) and high (blue circles) depending on the median of the data set. **Statistics:** Data are shown as scatter dot plots where the horizontal line depicts the **A-C,** median or **D,** mean with range. Data were and analyzed by **A-C,** Mann-Whitney U test and **D,** unpaired t-test.

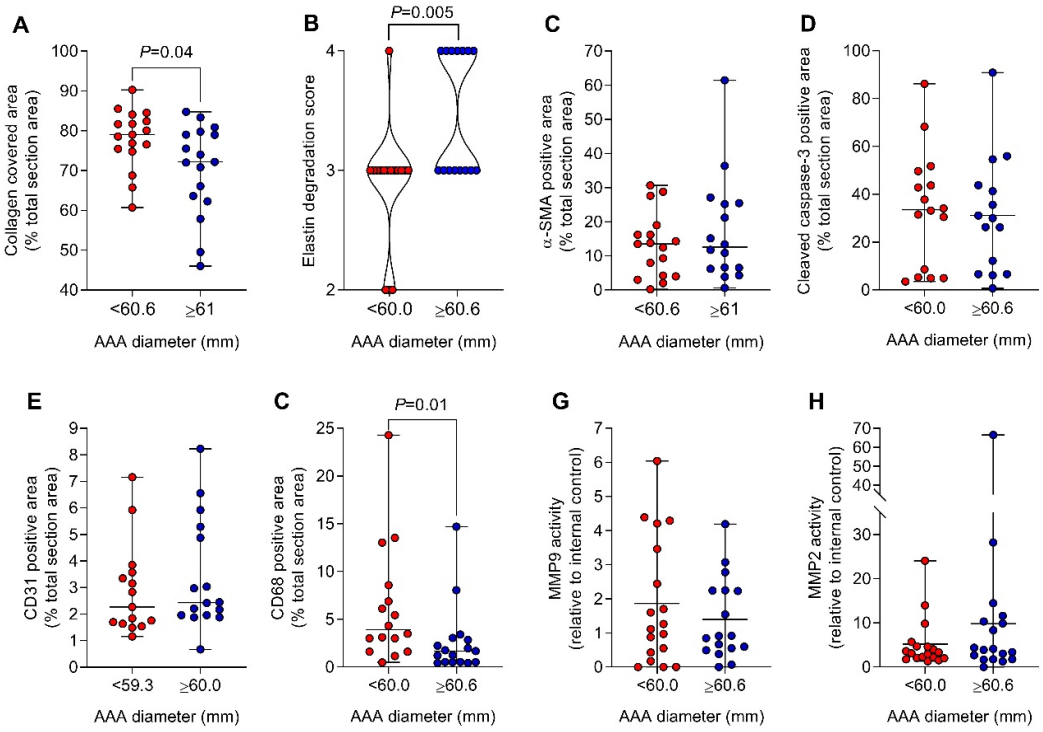


Supplemental Figure 2: Aortic LOX-1 mRNA expression and activity of matrix metalloproteases (MMP) in AAA. Aortic LOX-1 mRNA expression was analyzed by qPCR and data are presented in relation to an internal control (=1). **A, C,** Expression of pro-MMP9 and pro-MMP2 and **B, D,** activity of active enzymes (MMP9, MMP2) were analyzed using zymography and expression/activity was divided into low (red circles) and high (blue circles) depending on the median of the data set. Data are normalized to an internal control (=1). Representative zymograms are shown next to each figure. **Statistics:** Data are shown as scatter dot plots where the horizontal line depicts the **A,** mean or **B-D,** median with rangeData were analyzed by **B-D,** Mann-Whitney U or **A,** unpaired t-test.



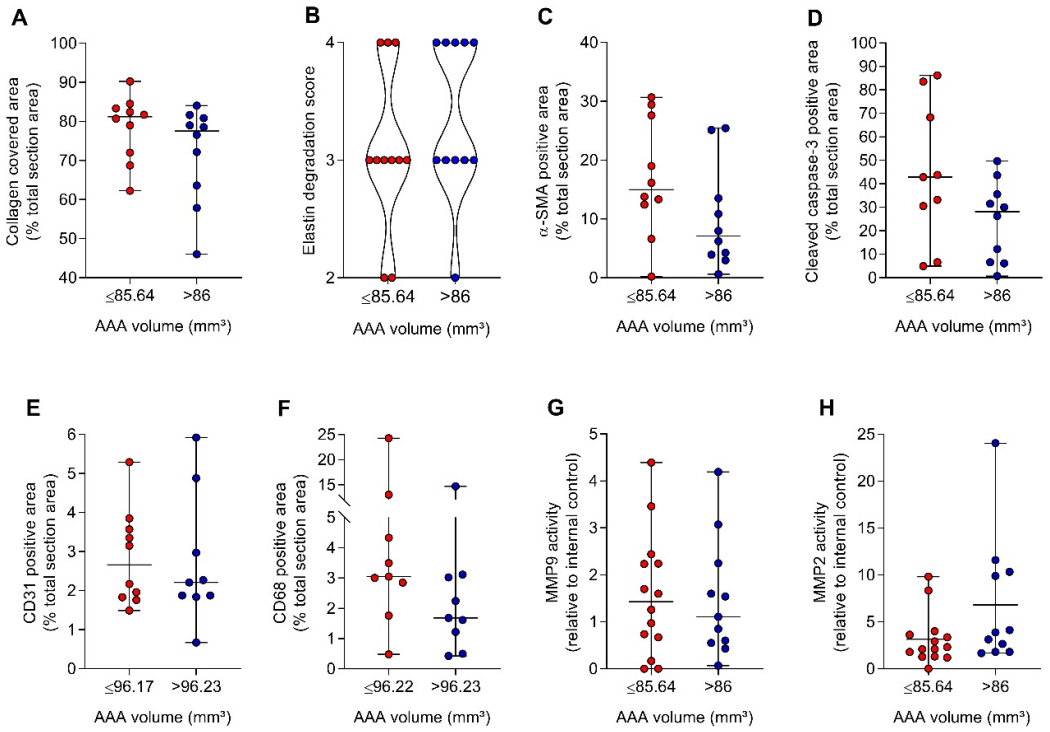
107

108 **Supplemental Figure 3: Serum sLOX-1, aortic LOX-1 mRNA expression and activity of**
109 **matrix metalloproteases (MMP) in AAA.** Aortic LOX-1 mRNA expression was analyzed by
110 qPCR and data are presented in relation to an internal control (=1). Activity of MMP9 was
111 analyzed by using zymography and data are normalized to an internal control (=1). Serum
112 sLOX-1 was analyzed by enzyme-linked immunosorbent assay (ELISA). **A, B,** LOX-1 mRNA
113 expression was divided into low (red circles) and high (blue circles) depending on the median
114 of the data set. One data point was excluded from the serum sLOX-1 because the concentration
115 exceeded the highest standard of the ELISA. **Statistics:** Data are shown as scatter dot plots
116 where the horizontal line depicts the median with range. Data were analyzed by Mann-Whitney
117 U test.



Supplementary Figure 4: AAA diameter and histopathological vessel wall degeneration in patients AAA. A, The AAA diameter was assessed by CT and was divided into two groups depending on the median and histopathological features were analyzed. **A,** Collagen content was assessed by Picro-Sirius Red staining and red-stained areas were quantified and set in relation to the total section area (%). **B,** Elastin fibers were stained using Elastica van Gieson and degradation was scored by eight persons blinded to the experiment. Score 2 represents the lowest observed elastin degradation, score 4 the highest. **C,** α-smooth muscle cell (α-SMA) content was assessed by immunohistochemistry and red-stained areas were quantified and set in relation to the total section area (%). **D,** Cleaved caspase-3 content was assessed by immunohistochemistry and red-stained areas were quantified and set in relation to the total section area (%). **E,** CD31 content was assessed by immunohistochemistry and brown-stained areas were quantified and set in relation to the total section area (%). **F,** CD68 content was assessed by immunohistochemistry and red-stained areas were quantified and set in relation to the total section area (%). Activity of **G,** MMP9 and **H,** MMP2 were analyzed using

zymography **Statistics:** Data are shown as scatter dot plots where the horizontal line depicts the **A, C, D, G,** mean or **B, E, F, H,** median with range. One data point was excluded from collagen in AAA diameter <60.6 mm due to technical reasons in the Picro-Sirius Red staining. Data were analyzed by **B, E, F, H,** Mann-Whitney U or **A, C, D, G,** unpaired t-test.



Supplementary Figure 5: AAA volume and histopathological vessel wall degeneration in AAA. A, The AAA volume was assessed by CT and was divided into two groups depending on the median and histopathological features were analyzed. **A,** Collagen content was assessed by Picro-Sirius Red staining and red-stained areas were quantified and set in relation to the total section area (%). **B,** Elastin fibers were stained using Elastica van Gieson and degradation was scored by eight persons blinded to the experiment. Score 2 represents the lowest observed elastin degradation, score 4 the highest. **C,** α-smooth muscle cell (α-SMA) content was assessed by immunohistochemistry and red-stained areas were quantified and set in relation to the total section area (%). **D,** Cleaved caspase-3 content was assessed by immunohistochemistry and red-stained areas were quantified and set in relation to the total section area (%). **E,** CD31 content was assessed by immunohistochemistry and brown-stained areas were quantified and set in relation to the total section area (%). **F,** CD68 content was assessed by immunohistochemistry and red-stained areas were quantified and set in relation to the total section area (%). Activity of **G,** MMP9 and **H,** MMP2 were analyzed using zymography

Statistics: Data are shown as scatter dot plots where the horizontal line depicts the **A, B, C, D, E, G** mean or **F, H,** median with range. One data point was excluded from collagen in AAA volume <85.6 mm^3 due to technical reasons in the Picro-Sirius Red staining. Data were analyzed by **F, H,** Mann-Whitney U or **A, B, C, D, E, G** unpaired t-test.