Eur J Vasc Endovasc Surg (2022) 63, 751–758

Combined Immunoglobulin Free Light Chains Are Novel Predictors of Cardiovascular Events in Patients With Abdominal Aortic Aneurysm

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WHAT THIS PAPER ADDS

This study shows the importance of adaptive immunity in human abdominal aortic aneurysm (AAA) by the release of combined free light chains (cFLCs) which could mediate some pathogenic mechanisms in the adventitial layer of AAA, rich in B cells. Increased plasma cFLC levels at baseline were associated with AAA presence, death, and cardiovascular events independently of other confounders. The data support the role of cFLCs as a pathogenic and/or prognostic marker of clinical outcomes in patients with AAA.

Objective: Abdominal aortic aneurysm (AAA) is characterised by the presence of B cells and immunoglobulins in the aortic wall, mainly in the adventitia. Kappa (κ) and lambda (λ) free light chains (FLCs) are produced from B cells during immunoglobulin synthesis. This study investigated the presence and prognostic value of combined FLCs (cFLCs or summed κ and λ) in patients with AAA.

Methods: cFLCs were analysed by a turbidimetric specific assay in tissue conditioned media from AAA samples ($n = 34$) compared with healthy aortas ($n = 34$) from France and in plasma samples from patients with AAA ($n = 434$) and age matched controls ($n = 104$) selected from the Viborg Vascular (VIVA) AAA screening trial in Denmark. *t* test, logistic regression, and Cox regression were used to test whether plasma cFLCs serve as a marker for AAA presence and whether cFLCs were predictive of death, major adverse cardiovascular events (MACE), or major adverse lower limb events (MALE).

Results: Increased cFLC levels were detected in the AAA adventitial layer compared with the AAA medial layer and healthy media layer (13.65 ± 3.17 *vs.* 6.57 ± 1.01 *vs.* 0.49 ± 0.09 mg/L, respectively, $p < .050$). The upper tertile of plasma cFLCs was independently associated with AAA presence after correcting for confounders (odds ratio [OR] 7.596, 95% confidence intervals [CI] 3.117 − 18.513; $p < .001$). Of 434 patients with AAA, 89 (20.5%) died, 104 (24.0%) suffered MACE, and 63 (14.5%) suffered MALE, during a five year follow up. In univariable analysis, the cFLC upper tertile was associated with a higher risk of death, MACE, and MALE ($p < .001$ for all). After adjustment for confounders, cFLCs remained an independent predictor of all cause mortality (hazard ratio [HR] 4.310, 95% CI 2.157 − 8.609; $p < .001$), MACE (HR 2.153, 95% CI 1.218 − 3.804; $p = .008$), or MALE (HR 3.442, 95% CI 1.548 − 7.652; $p = .002$) for those in the upper tertile.

Conclusion: Increased cFLCs are observed in adventitial tissue of patients with AAA, indicating local activation of B cells. Plasma cFLC levels are an independent predictor of death, MACE, and MALE in patients with AAA.

Keywords: Abdominal aortic aneurysm, Biomarkers, Immune response, Immunoglobulins, Mortality
Article history: Received 22 February 2021, Accepted 30 November 2021, Available online 2 March 2022

INTRODUCTION

Abdominal aortic aneurysm (AAA) is defined as a progressive dilatation of the aorta, which can be fatal when ruptured.[1] The only treatment to prevent rupture is open surgery or endovascular repair which is recommended when aortic size exceeds 5 − 5.5 cm. However, AAA progression is unpredictable, with periods of quiescence and periods of quick growth.[2] Moreover, patients with AAA have

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https://doi.org/10.1016/j.ejvs.2021.11.025

a higher risk of death mainly as a result of cardiovascular diseases (CVD).[3] In this respect, AAA and infrarenal aortic diameter are independent predictors of all cause cardiovascular death, and CV events.[4,5] The identification of novel determinants of AAA progression and cardiovascular events in patients with AAA is an unmet need.

On the other hand, efforts are being made to understand the pathological mechanisms underlying AAA to provide novel therapeutic targets. Dilatation in AAA is a consequence of extensive proteolysis, a main pathological mechanism of AAA.[6] Additionally, oxidative stress mainly because of red blood cell haemolysis[7] and adaptive[8] immune responses, along with vascular smooth muscle cell (VSMC) loss in the media layer, characterise the evolution of AAA. The presence of B and T cells in the adventitial layer was demonstrated more than three decades ago.[9] Moreover, these B cells are organised in ectopic lymphoid structures called tertiary lymphoid organs, able to mount an immune response against different antigens by releasing immunoglobulins (Igs). Increased amounts of Igs have been observed in AAA tissue, specifically in the adventitial layer.[10]

Igs are composed of two heavy chains and two light chains, among them kappa (κ) and lambda (λ). B cells along with plasmablasts produce excess light chains; those not bound to heavy chains are secreted into the circulation as polyclonal free light chains (FLCs).[11] Increased FLC levels have been observed as a result of excess antibody production by B cells in different autoimmune diseases or diminished renal clearance in chronic renal failure.[12] Furthermore, elevated combined FLCs (cFLCs, the sum of κ and λ levels) have been associated with increased all cause and CVD mortality.[13,14]

As the AAA wall is characterised by the presence of functional B cells able to produce Igs,[15] we hypothesised that under chronic immune conditions present in the AAA wall, an excess of FLCs could be synthesised and released. Thus, the presence and distribution of cFLCs in AAA tissue compared with healthy aortic tissue was analysed. Measuring polyclonal cFLCs as a marker of B cell activation can give new insight into the activity of the adaptive immune system in a variety of autoimmune diseases.[12] As AAA shares features of autoimmunity,[16] the concentration of cFLCs in plasma from patients with AAA was analysed, as well as the potential association between cFLCs levels and all cause mortality and CV events in the VIVA cohort (ClinicalTrials.gov NCT00662480).[17]

METHODS

To test the hypothesis, two different parallel studies were followed: one focusing on the tissue (Study 1) and the other focusing on the plasma (including Study 2A case/control and Study 2B prospective analysis of cases).

Study 1: Tissue conditioned media

Tissue conditioned media was obtained as described previously.[18] In brief, tissue samples were collected during surgical repair from consecutive patients with an AAA of aortic diameter $>$ 55 mm and dissected into luminal thrombus ($n = 17$) and wall (media and adventitial layer, $n = 34$). Healthy abdominal aortas ($n = 34$) were sampled from brain deceased organ donors and histologically analysed by a trained vascular surgeon (JBM) to ensure that these samples were not pathological (neither aneurysm nor atherosclerosis). All samples were obtained from 2006 to 2019 in France. The aortic tissue was washed and preserved in Ringer's lactate solution at 4°C until use, with a time frame between sampling and freezing always less than six hours. Then, tissue sections were cut into small pieces (5 mm^2) and incubated in RPMI 1640 medium free of proteins containing antibiotics/antimycotic (Gibco) for 24 hours at 37°C (6 mL/g of wet tissue). The conditioned media (supernatants containing proteins released by the tissue samples) was obtained after centrifugation (3000 g for 10 minutes at 20°C) and kept at −80°C until further processing. Ethical committee advice and patient informed consent were obtained (RESAA and AMETHYST studies, CPP Paris-Cochin n° 2095, 1930 and 1931, INSERM Institutional Review Board, IRB0000388). Healthy abdominal aortas were obtained with the authorisation of the French Biomedicine Agency (PFS 09—007, BBMRI network, BB-0033—00029).

Study 2: Plasma

This observational study was conducted in the frame of a population based image screening trial for AAA in Danish men aged 65—74 years between October 2008 and October 2010 (VIVA, ClinicalTrials.gov NCT00662480). The protocol design has been reported in detail elsewhere. Briefly, the VIVA AAA cohort included cases of AAA diagnosed by population based screening in the VIVA trial, which randomised more than 50,000 men aged 65—74 1:1 to vascular screening for AAA, peripheral arterial disease (PAD) and hypertension, or control. Cases with AAA ($n = 615$) were recommended 40 mg simvastatin and low dose aspirin and offered AAA repair ($n = 102$) if their AAA was 55 mm or more in diameter, and annual follow up if smaller. Blood samples were taken when the patient attended a study consultation for information and initiation of CVD prevention. For this study, 434 (71%) men with AAA had blood samples available. No other exclusion criteria were used apart from cases in which blood sampling could not be performed for logistic reasons at some trial consultations, blood sampling failure or samples which were incorrectly labelled making the merge with other data impossible, or when samples were exhausted. The study was conducted and reported in adherence to the STROBE recommendations. All subjects gave informed consent, and the local ethics committee of the Viborg Hospital approved the study, which was performed in accordance with the Helsinki Declaration.

Designs and outcome measures

Study 2A. A sex and age case control design using cases of AAA (aortic diameter $>$ 30 mm) and healthy controls (aortic diameter $<$ 30 mm) was used to evaluate the association

between plasma levels of cFLCs and AAA. Fasting blood samples were obtained at diagnosis from 434 patients with AAA and 104 age matched controls free of AAA who were recruited from the original cohort at baseline after the screening tests. Ankle systolic blood pressure (ABI) was calculated as the mean of two recorded ankle arterial blood pressure measurements divided by the brachial systolic blood pressure. The ABI of the limb with the lowest measured ABI was used for analysis. Glomerular filtration rate was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) cystatin C equation,[19] and classified into estimated glomerular filtration rate (eGFR) above and below 90 mL/min. CVD included hospital recorded stroke or transient ischaemic event, acute myocardial infarction, angina pectoris, and PAD.

Study 2B. Additionally, a prospective cohort design was used to investigate the potential association between cFLCs levels and death and CV events in the patients with AAA included in Study 2A. The potential association between cFLCs and AAA rupture could not be tested because of the low number of events ($n = 2$). Patients with AAA were followed by annual follow up scans to check for progression to operation threshold sise, and by nationwide registers regarding death, MACE (including percutaneous coronary intervention, stroke, acute myocardial infarction, angina, and coronary revascularisation), and MALE (including acute lower limb ischaemia, revascularisation because of intermittent claudication or critical chronical lower limb ischaemia or major amputation). MACE and MALE were defined based on previously defined ICD codes (Supplementary material). Beside an annual follow up scan by the study nurses of 30—50 mm AAAs, five year follow up on all randomised men was performed by retrieving data from nationwide registries for the publication of the main results of the trial,[17] consisting among others of the Central Personal Registry in which vital status is recorded, the National Patient Registry in which it is mandatory to record all admissions to hospital and attendance at outpatient clinics to obtain reimbursement, and the Danish Vascular Registry, a national quality database in which all vascular procedures are recorded including indication. All data are linked together at individual level by a unique personal number given to each inhabitant and required to live in Denmark. Consequently, follow up was 100%. Validation studies of the registries have shown high internal validity.[20]

Biochemical analysis

κ and λ light chains, as well as cystatin C, were measured with commercial assays (LK016.OPT and LK018.OPT, and LK048.0PT, The Binding Site) on the OPTILITE turbidimeter (The Binding Site) following the manufacturer's recommendations. Intra- and interprecision coefficients of variation were 2.8% and 2.1% and 4.1% and 3.7% for κ and λ, respectively, and 1.6% and 3.2% for cystatin C. Data are presented as the sum of κ and λ levels (cFLCs). High sensitivity CRP (hs-CRP), as a general marker of inflammation and one of the most studied markers of cardiovascular risk, was measured at the same time as FLCs analysis, on an Architect c8000 analyser according to the manufacturer's instruction (Abbott Laboratories). Samples were coded and analysts did not have access to the identity of the samples.

Statistics

Normality was tested using Shapiro-Wilk test, q-q plots, and histograms. Missing data were not replaced by artificial interpolated or imputed data. Differences between AAA tissues and healthy aortas were assessed by ANOVA followed by Tukey multiple comparison test. Differences in cFLCs between cases with AAA and controls were assessed by the chi square and *t* tests. When the sampling occurred, the hypothesis about an association between AAA and FLCs had not yet been formulated. A paper reported data on FLCs in healthy controls.[21] Therefore, it was assumed that if the mean of FLC is 28 and standard deviation is ± 10, the smallest detectable difference between AAA and healthy controls with the available sample size is 10% at 5% significance level and 80% power, suggesting a robust study concerning the comparison between controls and AAAs.

Potential confounders were identified in univariable analyses by a p value $< .10$. Logistic regression analysis was used to analyse whether cFLCs were independently associated with AAA. Receiver operating characteristic (ROC) curve analysis was performed to discriminate between patients with AAA and control subjects, and the predictive potential was compared with the ROC curve analysis of C reactive protein by R statistics. Finally, multivariable Cox proportional hazard models were constructed to study whether patients with AAA at the upper tertile of cFLCs at baseline had an increased risk of death, MACE, and MALE. SPSS 21.0 and STATA/SE 13.1 for Windows were used as statistical packages.

RESULTS

Free light chains in abdominal aortic aneurysm tissue conditioned media

Levels of cFLCs in the conditioned media of different layers of AAA wall (media and adventitia) and healthy aortic wall were analysed using a turbidimetric assay specific for light chains not bound to heavy chains.[22] Tissue conditioned media from the adventitial AAA displayed an increase of cFLCs compared with the medial AAA (13.65 ± 3.17 *vs.* 6.57 ± 1.01 mg/L, $p < .05$) or the healthy wall (0.49 ± 0.09 mg/L, $p < .0001$). In addition, cFLCs were also detected in AAA luminal thrombus conditioned media (7.27 ± 1.5 mg/L).

Free light chains in plasma of patients with abdominal aortic aneurysm

Systemic cFLCs concentrations in plasma of patients with AAA and controls were measured (Table 1). An increase in cFLCs levels was observed in the plasma of patients with AAA compared with controls (45.8 ± 1.5 *vs.* 33.2 ± 2.0 mg/L, $p < .001$, Fig. 1A). The upper tertile of cFLCs was independently associated with AAA presence after

Table 1. Clinical characteristics of 434 patients with abdominal aortic aneurysm (AAA) and 104 healthy controls with aortic diameter < 30 mm

	All – *n*	Controls (*n* = 104)	AAA (*n* = 434)	*p* value
Age – y	538	69.9 ± 2.8	69.9 ± 2.7	.94
Diastolic blood pressure – mmHg	535	81.9 ± 9.9	87.4 ± 12.2	<.001
Systolic blood pressure – mmHg	535	147.3 ± 18.2	155.1 ± 21.7	<.001
Body mass index – kg/m^2	529	25.9 ± 3.0	27.3 ± 3.5	<.001
Ankle brachial index	535	1.1 ± 0.1	0.9 ± 0.2	<.001
Aortic diameter – mm	538	18.2 ± 2.9	40.6 ± 11.7	<.001
Familial predisposition	535	7 (6.9)	13 (2.9)	.13
Diabetes mellitus	538	14 (13.5)	45 (10.4)	.36
Smoking	538	13 (12.5)	178 (41)	<.001
Previous cardiovascular disease – %	538	9 (8.7)	92 (21.2)	.003
Previous stroke or TIA	538	2 (1.9)	17 (3.9)	.33
Previous acute myocardial infarction	538	1 (1.0)	27 (6.2)	.030
Previous angina pectoris	538	3 (2.9)	42 (9.7)	.025
Previous peripheral arterial disease	538	1 (1.0)	6 (1.4)	.73
Statins	533	38 (35.6)	222 (51.5)	.004
Low dose aspirin	533	27 (26)	200 (46.3)	<.001
Beta blockers	535	22 (21.2)	128 (29.6)	.087
ACE inhibitors	535	20 (19.2)	113 (26.1)	.14
eGFR < 90 mL/min	538	26 (24.0)	151 (34.9)	.024
Serological parameters				
Median Hs-CRP (IQR) – mg/L	538	1.60 (0.70, 3.67)	3.00 (1.5, 6.43)	<.001
Cholesterol – mmol/L	529	4.8 ± 1.2	4.9 ± 0.9	.68

Data are presented as *n* (%) or mean ± standard deviation, unless stated otherwise. TIA = transient ischaemic attack; ACE = angiotensin converting enzyme; eGFR = estimated glomerular filtration rate; Hs-CRP = high sensitive C reactive protein; IQR = interquartile range.

correcting for potential confounders (OR 7.596, 95% CI 3.117 – 18.513; $p < .001$, Table 2). As expected, smoking, diastolic blood pressure, body mass index, and ABI (OR 3.334, 95% CI 1.594–6.970; OR 1.047, 95% CI 1.020 – 1.075; OR 1.203, 95% CI 1.090 – 1.328; OR 0.003, 95% CI 0.000 – 0.034, respectively; $p < .001$ for all) were also independently associated with AAA presence. ROC curve analyses showed that cFLCs levels were statistically significantly better predictors of AAA presence (AUC 0.771, 95% CI 0.719 – 0.822; $p < .001$; Fig. 1B) than hs-CRP levels (AUC 0.640, 95% CI 0.576 – 0.704; difference in area 0.131;0.0427, Z = 3.0676, $p = .002$).

As cFLCs have been previously associated with mortality and have been suggested as a marker of CV risk,[22] it was tested whether cFLCs were predictive of death, MACE, or MALE in this AAA cohort. In patients with AAA followed for five years, 89 (20.5%) died, 22 after AAA repair; 104 (24.0%) suffered from MACE, 14 after AAA repair; and 63 (14.5%) suffered from MALE, six after AAA repair. Interestingly, crude analysis showed that the upper tertile of cFLCs was associated with both total mortality and CV events (MACE and/or MALE) (Fig. 2). After multivariable analysis, cFLCs in the upper tertile remained an independent predictor of all cause mortality (HR 4.310, 95% CI 2.157 – 8.609; $p < .001$), MACE (HR 2.153, 95% CI 1.218 – 3.804; $p = .008$), or MALE (HR 3.442, 95% CI 1.548 – 7.652; $p = .002$) independently of confounding factors (Table 3). As expected, age and diabetes were associated with mortality, MACE and MALE (age HR 1.126, 95% CI 1.035 – 1.225, $p = .006$; HR 1.066, 95% CI 0.991 – 1.148, $p = .086$; HR 1.139, 95% CI 1.035 – 1.254, $p = .008$; diabetes HR 2.294, 95% CI 1.205 – 4.366, $p = .011$; HR 1.817, 95% CI 1.039 – 3.177, $p = .036$; HR 1.900, 95% CI 0.905 – 3.989, $p = .090$), while higher ABI values were inversely associated with mortality and MACE and MALE (ABI HR 0.246, 95% CI 0.068 – 0.887, $p = .032$; HR 0.099, 95% CI 0.033 – 0.295, $p < .001$; HR 0.190, 95% CI 0.044 – 0.816, $p = .025$). In contrast, previous CV disease was also inversely associated with MACE and MALE (HR 0.454, 95% CI 0.264 – 0.783, $p = .004$; HR 0.474, 95% CI 0.229 – 0.979, $p = .044$), probably because of therapeutic intervention.

DISCUSSION

AAA is a pathological and degenerative process of the aortic wall involving mediators of both systemic and local origin. This interrelationship between these compartments is supported by the retention of blood cells (e.g., neutrophils, platelets), blood borne proteases (e.g., plasmin[ogen]), and high abundant plasma proteins (complement C3, Igs[23]) in the intraluminal thrombus of AAA. In this work, high cFLCs levels were detected in luminal thrombus tissue conditioned media, probably associated with retention by intraluminal thrombus from the circulation (because of the scarce presence of B cells in the intraluminal thrombus). In contrast, B lymphocytes and Igs are abundantly present in tertiary lymphoid organs of AAA wall, mainly in the adventitial layer. In this study, the highest levels of cFLCs were observed in AAA adventitial tissue conditioned media, suggesting local production of FLCs and further supporting

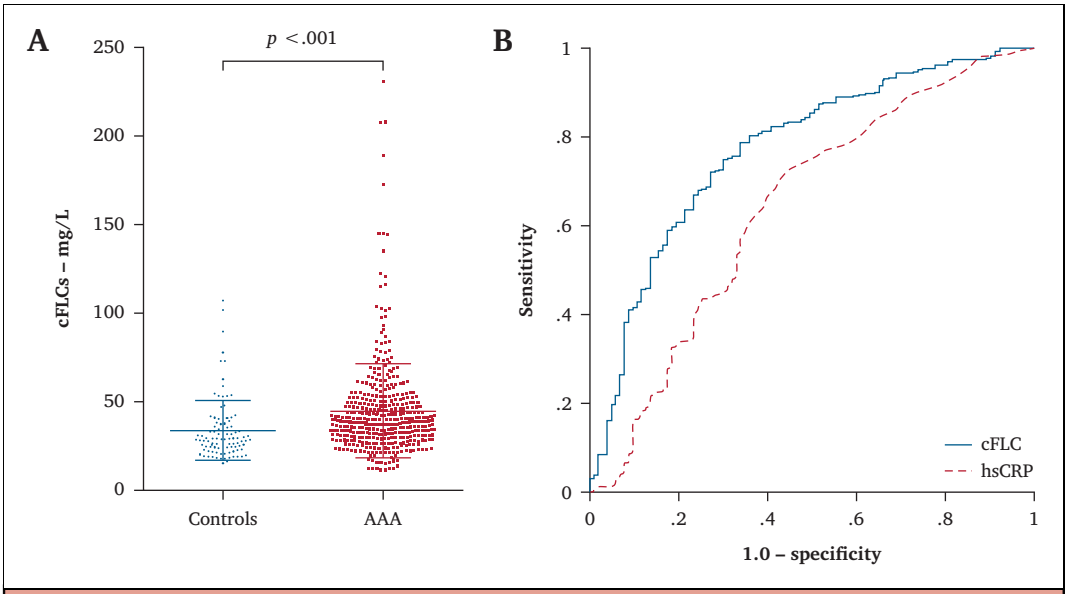

Figure 1. (A) Combined kappa and lambda free light chains (cFLCs) concentration in plasma of 434 patients with abdominal aortic aneurysm (AAA) and 104 controls and (B) receiver operating characteristic (ROC) curve of cFLCs and high sensitive C reactive protein (hs-CRP) to discriminate patients with AAA ($n = 434$) from control subjects ($n = 104$).

that B cells are activated in the AAA wall. The functional consequences of B cell activation in AAA have been demonstrated previously in experimental AAA models. In this respect, mature B cell deficient mice were protected from AAA[24] while reconstitution with IgG antibodies restored susceptibility to AAA in those mice.[25] Similarly, depletion of B cells with an anti-CD20 antibody prevented the development of elastase induced AAA.[26] FLCs have demonstrated pathogenic effects on immune cells.[27,28] However, the receptor(s) or effector pathways involved in FLCs actions, as well as the potential antigens, remain to be determined. FLCs could be an attractive target for novel therapies[29] in different diseases where immune inflammatory responses play a key pathogenic role. Further studies should test the impact of FLCs modulation on the pathogenesis of AAA.

The non-clonal/polyclonal increase of cFLCs in plasma has been observed in different immune diseases where B cell

Table 2. Multivariable logistic regression analysis of the tertiles of combined kappa and lambda free light chains (cFLCs) as categorical independent risk factor for abdominal aortic aneurysm (AAA) presence in 434 patients with AAA and 104 controls using the lowest tertile as reference. Potential confounders were identified in univariable analyses by a p value < .10

	Adjusted OR (95% CI)	p value
Univariable model		
Lowest cFLCs tertile, ref.		<.001
Medium cFLCs tertile	3.834 (2.329–6.310)	<.001
Upper cFLCs tertile	6.460 (3.661–11.400)	<.001
Multivariable model		
Lowest cFLCs tertile, ref.		<.001
Medium cFLCs tertile	3.492 (1.851–6.589)	<.001
Upper cFLCs tertile	7.596 (3.117–18.513)	<.001
Current smoking	3.334 (1.594–6.970)	.001
Previous CVD	1.503 (0.569–3.969)	.41
Use of beta blockers	0.749 (0.363–1.545)	.43
Use of low dose aspirin	1.657 (0.806–3.405)	.17
Use of statins	1.207 (0.610–2.388)	.59
Diastolic blood pressure – mmHg	1.047 (1.020–1.075)	.001
Body mass index – kg/m^2	1.203 (1.090–1.328)	<.001
ABI	0.003 (0.000–0.034)	<.001
eGFR < 90 mL/min	0.962 (0.473–1.956)	.91
Hs-CRP – mg/L	0.997 (0.979–1.015)	.72

OR = odds ratio; CI = confidence interval; ref. = reference; CVD = cardiovascular disease; ABI = ankle brachial index; eGFR = estimated glomerular filtration rate; Hs-CRP = high sensitive C reactive protein.

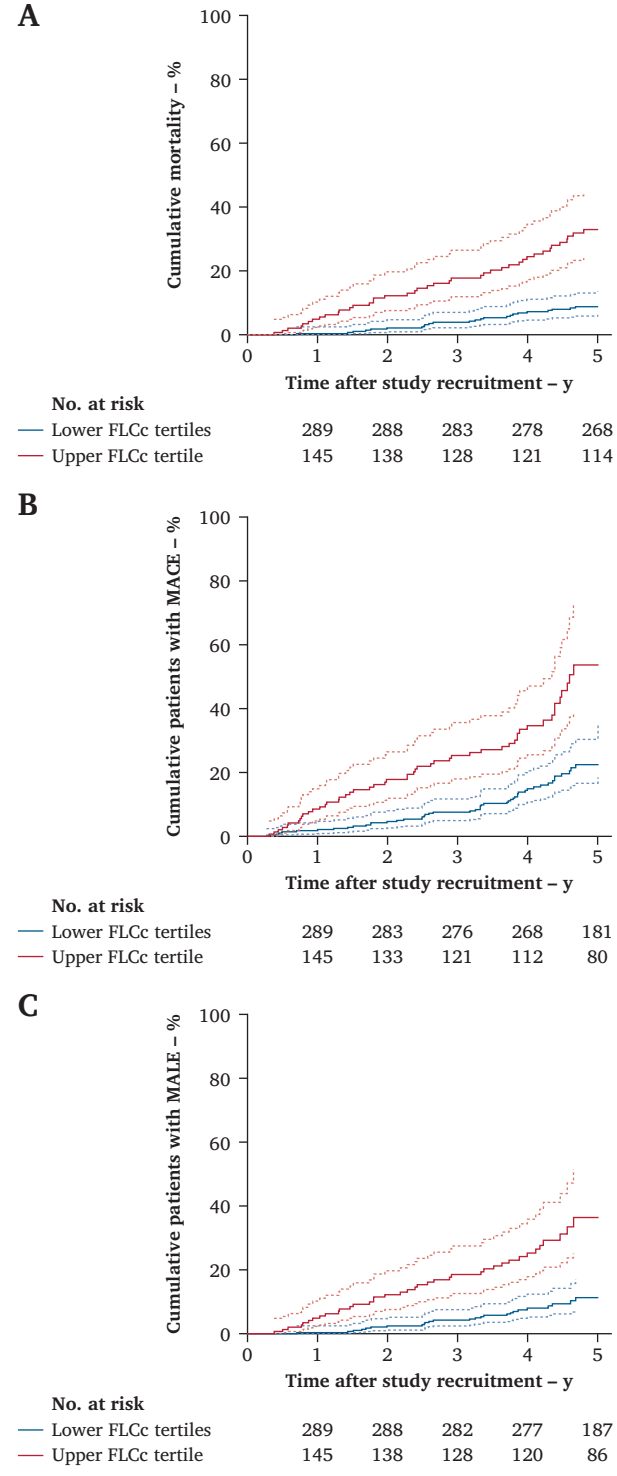

Figure 2. Cumulative Kaplan–Meier estimates with 95% confidence intervals of (A) total mortality, (B) major adverse cardiovascular events (MACE), and (C) major adverse limb events (MALE) according to tertiles of combined kappa and lambda free light chains (cFLCs) in patients with abdominal aortic aneurysm (AAA).

activation is pathophysiologically involved, ranging from inflammatory bowel disease to rheumatoid arthritis or heart failure (HF).[30] Accordingly, it has been shown that high cFLCs levels are observed in patients with AAA when compared with controls. The potential cause/source of high cFLCs in AAA is unknown. Different CV risk factors and comorbidities have also been associated with high cFLCs. In the present study, the association between cFLCs and AAA presence remained statistically significant after correcting for different potential confounding factors. The increase of cFLCs in AAA plasma could be linked to a global inflammatory burden but as its association with AAA is independent of CRP, it could be related to a potential B cell adaptive immune response. However, as paired cFLCs data from tissue and plasma of the same patients were not available, the potential mechanism(s)/sources behind the observed association between cFLCs and AAA presence cannot be ascertained in this observational study. In this sense, several biomarkers have been associated with AAA presence (including CRP),[31] although at present, none is used for AAA diagnosis. It was shown that cFLCs were superior in terms of sensitivity in detecting AAA compared with CRP. However, the AUC of cFLCs (0.77) remains far from that required for clinical use, so these data should not be interpreted in terms of clinical applicability but rather in terms of cFLCs as a new potential pathogenic marker of AAA.

Previously, high cFLCs concentrations have been related to total mortality in the general population.[13] cFLCs were an independent predictor of death in patients recently hospitalised with decompensated HF.[32] Moreover, the upper quartile of cFLCs was associated with a composite endpoint of re-hospitalisation or death in patients with acute HF.[33] In agreement, in this work, it has been shown that patients with AAA in the upper tertile of cFLCs have a higher all cause mortality risk, independent of different risk factors and comorbidities. Unfortunately, in this analysis HF could not be included as a covariable as it covers a composite of several heart diseases, with poor validity in the registries. cFLCs were also correlated with disease activity in various autoimmune disorders including systemic lupus erythematosus and RA,[34,35] and were predictive of future need for percutaneous coronary intervention in patients with STEMI.[36] Moreover, the association between high cFLC levels and MACE and/or MALE in patients with high CVD risk such as AAA was demonstrated. The potential causes of these associations are unknown but were independent of other CV risk factors, renal function, and/or CRP. Importantly, the association between cFLCs and CV events was also independent of aortic diameter, suggesting an unknown specific role of cFLCs in AAA progression to clinical events. Whether or not cFLCs could be included as a risk stratification tool in patients with AAA deserves further study.

Strengths and limitations

Regarding the analysis of cFLCs in plasma of patients with AAA, the strength of this study lies in the population based design in the VIVA trial, which has a high attendance rate yielding a very small risk of selection bias; however, for

Table 3. Multivariable Cox regression analysis of the upper combined kappa and lambda free light chains (cFLCs) tertile as independent risk factor for overall mortality, major adverse cardiovascular event (MACE), or major adverse limb effect (MALE) in abdominal aortic aneurysm (AAA) disease in 434 patients with AAA and 104 controls using the lower tertiles as reference

	Overall mortality		MACE		MALE	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Univariable analysis						
Lowest cFLCs tertile, ref.		<.001		<.001		<.001
Medium cFLCs tertile	1.647 (0.874−3.102)	.12	1.368 (0.791−2.365)	.26	1.898 (0.838−4.295)	.12
Upper cFLCs tertile	3.684 (2.089−6.495)	<.001	2.730 (1.670−4.465)	<.001	4.831 (2.334−9.997)	<.001
Multivariable analysis						
Lowest cFLCs tertile, ref.		<.001		.014		.003
Medium cFLCs tertile	2.106 (1.004−4.418)	.050	1.257 (0.695−2.272)	.45	1.586 (0.670−3.758)	.29
Upper cFLCs tertile	4.310 (2.157−8.609)	<.001	2.153 (1.218−3.804)	.008	3.442 (1.548−7.652)	.002
Age − y	1.126 (1.035−1.225)	.006	1.066 (0.991−1.148)	.086	1.139 (1.035−1.254)	.008
Family predisposition	0.733 (0.227−2.367)	.60	1.149 (0.493−2.682)	.75	0.770 (0.183−3.234)	.72
Diabetes mellitus	2.294 (1.205−4.366)	.011	1.817 (1.039−3.177)	.036	1.900 (0.905−3.989)	.090
Hypertension	0.706 (0.431−1.157)	.17	0.926 (0.591−1.450)	.74	0.606 (0.342−1.072)	.085
Previous cardiovascular disease	0.776 (0.425−1.418)	.41	0.454 (0.264−0.783)	.004	0.474 (0.229-0.979)	.044
Use of low dose aspirin	0.711 (0.414−1.220)	.22	1.021 (0.638−1.634)	.93	0.782 (0.420−1.454)	.44
Use of ACE inhibitors	1.417 (0.823−2.440)	.21	1.359 (0.843−2.191)	.21	2.165 (1.176−3.986)	.013
Use of beta blockers	1.457 (0.830−2.556)	.19	1.918 (1.196−3.076)	.007	1.761 (0.945−3.282)	.075
Ankle brachial index	0.246 (0.068−0.887)	.032	0.099 (0.033−0.295)	<.001	0.190 (0.044−0.816)	.025
AAA diameter − mm	1.000 (0.980−1.020)	.99	0.986 (0.966−1.005)	.15	0.994 (0.970−1.017)	.59
eGFR < 90 mL/min	1.015 (0.613−1.681)	.95	1.359 (0.864−2.135)	.18	1.306 (0.736−2.317)	.36
Hs-CRP − mg/L	1.010 (1.000−1.020)	.049	1.006 (0.994−1.018)	.34	1.008 (0.997−1.020)	.16

MACE = major adverse cardiovascular events; MALE = major adverse lower limb events; ref. = reference; HR = hazard ratio; CI = confidence interval; eGFR = estimated glomerular filtration rate; Hs-CRP = high sensitive C reactive protein.

practical reasons, not all diagnosed cases had samples taken, leaving a risk of selection bias. These were mainly cases referred for surgical evaluation before blood sampling in the trial could be arranged, and cases with large AAA associated with higher mortality and CVD morbidity compared with smaller AAA. However, this potential bias most probably shifts the reported associations towards the null hypothesis, implying an underestimation of the associations, consequently information bias is unlikely. In addition, a systematic approach was used to identify confounders, but in the end, residual confounding by nature is always a risk in observational studies. Finally, the present study was performed in a single plasma cohort and further validation in additional cohorts is needed to confirm the results.

In conclusion, increased cFLCs have been observed in AAA tissue, mainly in the adventitial layer, indicating participation of local activated B cells. High plasma cFLCs levels are independently associated with AAA presence and all cause mortality, MACE, and MALE, suggesting the potential prognostic value of cFLCs in the clinical outcomes of patients with AAA.

ACKNOWLEDGEMENTS

We want to thank Ignacio Mahillo at IIS-FJD for statistical advice and all the Biochemistry staff at Quironsalud Pozuelo for their help and collaboration. We also want to thank Dario Gomez, Juan Rodriguez, and Nuno Barbosa (The Binding Site) for providing us with the kits.

CONFLICT OF INTEREST

None.

FUNDING

This study was funded by the Spanish MINECO (PID2019−106814RB-I00 and PGC2018−097019-B-I00), la Caixa Foundation (HR17−00247), CAM (S2017/BMD-3673), and Fondo de Investigaciones Sanitarias ISCiii-FEDER (PI19/00128).

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejvs.2021.11.025.

REFERENCES

1 Owens DK, Davidson KW, Krist AH, Barry MJ, Cabana M, Caughey AB, et al. Screening for Abdominal Aortic Aneurysm: US Preventive Services Task Force Recommendation Statement. *JAMA* 2019;**322**:2211−8.
2 Limet R, Sakalihassan N, Albert A. Determination of the expansion rate and incidence of rupture of abdominal aortic aneurysms. *J Vasc Surg* 1991;**14**:540−8.
3 Cornuz J, Sidoti Pinto C, Tevaearai H, Egger M. Risk factors for asymptomatic abdominal aortic aneurysm: systematic review and meta-analysis of population-based screening studies. *Eur J Public Health* 2004;**14**:343−9.
4 Brady AR, Fowkes FG, Thompson SG, Powell JT. Aortic aneurysm diameter and risk of cardiovascular mortality. *Arterioscler Thromb Vasc Biol* 2001;**21**:1203−7.

5 Norman PE, Muller J, Golledge J. The cardiovascular and prognostic significance of the infrarenal aortic diameter. *J Vasc Surg* 2011;**54**:1817–20.

6 Sakalihasan N, Michel JB, Katsargyris A, Kuivaniemi H, Defraigne JO, Nchimi A, et al. Abdominal aortic aneurysms. *Nat Rev Dis Primers* 2018;**4**:34.

7 Michel JB, Martin-Ventura JL. Red blood cells and hemoglobin in human atherosclerosis and related arterial diseases. *Int J Mol Sci* 2020;**21**:6756.

8 Dutertre CA, Clement M, Morvan M, Schakel K, Castier Y, Alsac JM, et al. Deciphering the stromal and hematopoietic cell network of the adventitia from non-aneurysmal and aneurysmal human aorta. *PLoS One* 2014;**9**:e89983.

9 Koch AE, Haines GK, Rizzo RJ, Radosevich JA, Pope RM, Robinson PG, et al. Human abdominal aortic aneurysms. Immunophenotypic analysis suggesting an immune-mediated response. *Am J Pathol* 1990;**137**:1199–213.

10 Walton LJ, Powell JT, Parums DV. Unrestricted usage of immunoglobulin heavy chain genes in B cells infiltrating the wall of atherosclerotic abdominal aortic aneurysms. *Atherosclerosis* 1997;**135**:65–71.

11 Napodano C, Pocino K, Rigante D, Stefanile A, Gulli F, Marino M, et al. Free light chains and autoimmunity. *Autoimmun Rev* 2019;**18**:484–92.

12 Hutchison CA, Landgren O. Polyclonal immunoglobulin free light chains as a potential biomarker of immune stimulation and inflammation. *Clin Chem* 2011;**57**:1387–9.

13 Dispenzieri A, Katzmann JA, Kyle RA, Larson DR, Therneau TM, Colby CL, et al. Use of nonclonal serum immunoglobulin free light chains to predict overall survival in the general population. *Mayo Clin Proc* 2012;**87**:517–23.

14 Anandram S, Assi LK, Lovatt T, Parkes J, Taylor J, Macwhannell A, et al. Elevated, combined serum free light chain levels and increased mortality: a 5-year follow-up, UK study. *J Clin Pathol* 2012;**65**:1036–42.

15 Guedj K, Khallou-Laschet J, Clement M, Morvan M, Delbosc S, Gaston AT, et al. Inflammatory micro-environmental cues of human atherothrombotic arteries confer to vascular smooth muscle cells the capacity to trigger lymphoid neogenesis. *PLoS One* 2014;**9**:e116295.

16 Gregory AK, Yin NX, Capella J, Xia S, Newman KM, Tilson MD. Features of autoimmunity in the abdominal aortic aneurysm. *Arch Surg* 1996;**131**:85–8.

17 Lindholt JS, Søgaard R. Population screening and intervention for vascular disease in Danish men (VIVA): a randomised controlled trial. *Lancet* 2017;**390**:2256–65.

18 Fontaine V, Touat Z, Mtairag el M, Vranckx R, Louedec L, Houard X, et al. Role of leukocyte elastase in preventing cellular re-colonization of the mural thrombus. *Am J Pathol* 2004;**164**:2077–87.

19 Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med* 2012;**367**:20–9.

20 Schmidt M, Schmidt SA, Sandegaard JL, Ehrenstein V, Pedersen L, Sørensen HT. The Danish National Patient Registry: a review of content, data quality, and research potential. *Clin Epidemiol* 2015;**7**:449–90.

21 Bradwell AR, Carr-Smith HD, Mead GP, Tang LX, Showell PJ, Drayson MT, et al. Highly sensitive, automated immunoassay for immunoglobulin free light chains in serum and urine. *Clin Chem* 2001;**47**:673–80.

22 Basile U, La Rosa G, Napodano C, Pocino K, Cappannoli L, Gulli F, et al. Free light chains a novel biomarker of cardiovascular disease. A pilot study. *Eur Rev Med Pharmacol Sci* 2019;**23**:2563–9.

23 Martinez-Pinna R, Madrigal-Matute J, Tarin C, Burillo E, Esteban-Salan M, Pastor-Vargas C, et al. Proteomic analysis of intraluminal thrombus highlights complement activation in human abdominal aortic aneurysms. *Arterioscler Thromb Vasc Biol* 2013;**33**:2013–20.

24 Zhou HF, Yan H, Stover CM, Fernandez TM, Rodriguez de Cordoba S, Song WC, et al. Antibody directs properdin-dependent activation of the complement alternative pathway in a mouse model of abdominal aortic aneurysm. *Proc Natl Acad Sci U S A* 2012;**109**:E415–22.

25 Furusho A, Aoki H, Ohno-Urabe S, Nishihara M, Hirakata S, Nishida N, et al. Involvement of B cells, immunoglobulins, and syk in the pathogenesis of abdominal aortic aneurysm. *J Am Heart Assoc* 2018;**7**:e007750.

26 Schaheen B, Downs EA, Serbulea V, Almenara CC, Spinosa M, Su G, et al. B-cell depletion promotes aortic infiltration of immunosuppressive cells and is protective of experimental aortic aneurysm. *Arterioscler Thromb Vasc Biol* 2016;**36**:2191–202.

27 Redegeld FA, van der Heijden MW, Kool M, Heijdra BM, Garssen J, Kraneveld AD, et al. Immunoglobulin-free light chains elicit immediate hypersensitivity-like responses. *Nat Med* 2002;**8**:694–701.

28 Braber S, Thio M, Blokhuis BR, Henricks PA, Koelink PJ, Groot Kormelink T, et al. An association between neutrophils and immunoglobulin free light chains in the pathogenesis of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2012;**185**:817–24.

29 Brebner JA, Stockley RA. Polyclonal free light chains: a biomarker of inflammatory disease or treatment target? *F1000 Med Rep* 2013;**5**:4.

30 Esparvarinha M, Nickho H, Mohammadi H, Aghebati-Maleki L, Abdolalizadeh J, Majidi J. The role of free kappa and lambda light chains in the pathogenesis and treatment of inflammatory diseases. *Biomed Pharmacother* 2017;**91**:632–44.

31 Wang Y, Shen G, Wang H, Yao Y, Sun Q, Jing B, et al. Association of high sensitivity C-reactive protein and abdominal aortic aneurysm: a meta-analysis and systematic review. *Curr Med Res Opin* 2017;**33**:2145–52.

32 Jackson CE, Haig C, Welsh P, Dalzell JR, Tsorlalis IK, McConnachie A, et al. Combined free light chains are novel predictors of prognosis in heart failure. *JACC Heart Fail* 2015;**3**:618–25.

33 Shantsila E, Wrigley B, Lip GY. Free light chains in patients with acute heart failure secondary to atherosclerotic coronary artery disease. *Am J Cardiol* 2014;**114**:1243–8.

34 Aggarwal R, Sequeira W, Kokebie R, Mikolaitis RA, Fogg L, Finnegan A, et al. Serum free light chains as biomarkers for systemic lupus erythematosus disease activity. *Arthritis Care Res (Hoboken)* 2011;**63**:891–8.

35 Kormelink TG, Tekstra J, Thurlings RM, Boumans MH, Vos K, Tak PP, et al. Decrease in immunoglobulin free light chains in patients with rheumatoid arthritis upon rituximab (anti-CD20) treatment correlates with decrease in disease activity. *Ann Rheum Dis* 2010;**69**:2137–44.

36 Shantsila E, Tapp LD, Lip GY. Free light chains in patients with acute coronary syndromes: Relationships to inflammation and renal function. *Int J Cardiol* 2015;**185**:322–7.