Aortic aneurysms and markers of platelet activation, hemostasis, and endothelial disruption in people living with HIV

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Introduction: People living with HIV (PLWH) are at twice the risk of developing cardiovascular diseases and have more than four times higher odds of aortic aneurysm (AA) than the uninfected population. However, biomarkers of AA in PLWH are yet to be discovered. We aimed to investigate whether circulating biomarkers reflecting platelet activation, hemostasis and endothelial disruption, i.e. sCD40L, D-dimer, syndecan-1, and thrombomodulin, were associated with AA in PLWH.

Methods: Five hundred seventy one PLWH from the Copenhagen Comorbidity in HIV Infection (COCOMO) study $\geq$40 years of age with an available contrast-enhanced CT scan as well as available biomarker analyses were included. The biomarkers were analyzed on thawed plasma. For each biomarker, we defined high level as a concentration in the upper quartile and low level as a concentration below the upper quartile. For D-dimer, the cut-off was defined as the lower limit of detection. Using unadjusted and adjusted logistic and linear regression models, we analyzed associations between AA and sCD40L, D-dimer, syndecan-1, and thrombomodulin, respectively in PLWH.

Results: PLWH had median (IQR) age 52 years (47-60), 88% were male, median (IQR) time since HIV diagnosis was 15 years (8-23), and 565 (99%) were currently on antiretroviral treatment. High level of sCD40L was associated with lower odds of AA in both unadjusted (odds ratio, OR, 0.23 (95% CI 0.07-0.77; P=0.017)) and adjusted models (adjusted OR, aOR, 0.23 (95% CI 0.07-0.78; P=0.019)). Detectable level of D-dimer was associated with higher odds of AA in both unadjusted (OR 2.76 (95% CI 1.34-5.67; P=0.006)) and adjusted models (aOR 2.22 (95% CI 1.02-4.85; P=0.045)).

Conclusions: SCD40L was associated with lower odds of AA whereas D-dimer was independently associated with higher odds of AA in PLWH. This calls for further investigations into specific biomarkers to aid early diagnosis of AA in PLWH.

KEYWORDS

HIV, PLWH, aortic aneurysm, sCD40L, D-dimer (DD), syndecan-1, thrombomodulin (TM)

1 Introduction

As the median age of people living with HIV (PLWH) increases (1), so does the burden of age-related comorbidities (2). The risk of incident cardiovascular diseases (CVD) in PLWH is twice that of uninfected individuals (3), with myocardial infarction, stroke, and coronary artery disease being the most frequent manifestations (4). In a previous study by our group, HIV infection was associated with a 4.5 increased odds ratio (OR) of having aortic aneurysms (AA) compared to uninfected controls (5). AA is a rare but potentially severe manifestation of CVD, as the mortality rate of a ruptured abdominal AA may be as high as 80% (6). The European Society of Cardiology recommends screening of populations with a high risk of abdominal AA (7), because most AA are asymptomatic and diagnosed incidentally (8).

The initial phase of the pathogenesis of AA is not fully understood, but studies have suggested endothelial cell injury to contribute (9, 10). Associations have been found between AA, in the majority of studies in patients undergoing surgery for AA, and a marker of platelet activation, soluble CD40L (sCD40L) (11, 12), a marker of hemostasis, D-dimer (13–17), and the markers of endothelial disruption, syndecan-1 (18–20) and soluble thrombomodulin (21, 22), respectively. Importantly, all of these biomarkers have been reported to be elevated in PLWH compared to uninfected controls (23–28).

Possible associations between these biomarkers and AA have yet to be established but could aid in the early identification of high-risk patients. So far, no studies on the association between AA and markers of platelet activation, hemostasis, and endothelial disruption, respectively, in PLWH have been published. Thus, we investigated whether sCD40L, D-dimer, syndecan-1, and thrombomodulin (the biomarkers) are associated with AA in a large cohort of well-treated PLWH. We hypothesized that each of the biomarkers would be independently associated with AA in PLWH.

2 Materials and methods

2.1 Study design and population

The Copenhagen Comorbidity in HIV Infection (COCOMO) study is a non-interventional cohort study that aims to assess non-AIDS comorbidities in PLWH. Inclusion criteria were age above 18 years and a positive HIV test. Between March 2015 and December 2016, 1099 PLWH living in the greater Copenhagen area were included. Procedures for recruitment and collection of data have previously been described elsewhere (29). A combined thoracic and abdominal contrast-enhanced computed tomography (CT) scan was offered to all COCOMO participants. In this study, we included COCOMO participants who were ≥40 years of age, had a contrast-enhanced CT performed, and had biomarkers measured in plasma.

Written informed consent was obtained from all participants. Ethical approval was obtained by the Regional Ethical Committee of Copenhagen (COCOMO: H-8-2014-004). The study was carried out in accordance with the Declaration of Helsinki.

2.2 Clinical characteristics and self-reported outcome

A physical examination including measurement of blood pressure and anthropometrics was performed by trained clinical staff. Venous blood was collected non-fasting. Blood for plasma samples (EDTA anti-coagulated) was stored on ice until centrifugation in a cold centrifuge at 4°C, and cryovials were transferred to liquid nitrogen within 72 hours (29). Extensive questionnaires included questions regarding medication, medical history, and smoking. From medical records, HIV-specific data such as transmission mode, duration of HIV infection, type of antiretroviral therapy, and hepatitis B and C status were obtained. Hepatitis B virus co-infection was defined as positive hepatitis B virus surface-antigen and hepatitis C virus co-infection as positive hepatitis C virus RNA. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg and/or current use of antihypertensive medication according to Joint National Committee guidelines (30).

2.3 CT examinations and aortic analyses

Contrast-enhanced thoracic and abdominal CT examinations including contrast-enhanced CT angiography were performed using a 320-detector CT scanner (Aquilion ONE, ViSION Edition, Canon Medical Systems, Otawara, Japan) at Rigshospitalet University Hospital, Copenhagen, Denmark.

Aortic analyses were performed by two trained examiners on contrast-enhanced CT images. Maximal and minimal inner aortic diameter was measured at seven anatomical points of the aorta, in four of which maximal outer diameter of the aorta was measured as

Abbreviations: PLWH, People living with HIV; CVD, Cardiovascular diseases; AA, Aortic aneurysms; sCD40L, Soluble CD40 ligand; FEU, Fibrin Equivalent Units; ET-1, Endothelin-1; OR, Odds ratio; aOR, Adjusted odds ratio; CI, Confidence interval; SD, Standard deviation.

well (5). According to the European Society of Cardiology guidelines, aortic aneurysms were defined as ≥50% increase in aortic diameter compared to the expected normal diameter or an infrarenal diameter of ≥30 mm (7). This resulted in the definition of AA as diameter of ascending aorta ≥45 mm and/or diameter of descending aorta ≥35 mm and/or diameter of suprarenal aorta ≥30 mm and/or diameter of the infrarenal aorta ≥30 mm (31).

2.4 Markers of platelet activation, hemostasis, and endothelial disruption

Plasma concentrations of sCD40L, soluble syndecan-1, and thrombomodulin, were analyzed on thawed plasma using Luminex® Human Discovery Assays (R&D Systems, UK, Europe) in a 1:2 dilution, according to the manufacturer's instructions, at the Department of Clinical Immunology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark. Plasma concentrations of D-dimer were measured as routine biochemistry on fresh blood samples at Department of Clinical Biochemistry, Copenhagen University Hospital, Herlev, Copenhagen, Denmark (29).

2.5 Statistics

Continuous data were reported using means and standard deviations for normal deviates and medians with interquartile ranges for variables not normally distributed, as appropriate. For categorical data, frequency counts and percentages of subjects within each category were reported. Logistic regression analyses were applied where the dependent variable was binary and linear regression where the dependent variable was continuous.

As the primary outcome, we investigated the association between AA and high levels of sCD40L, syndecan-1, and thrombomodulin and detectable level of D-dimer one at a time using simple and multivariable logistic regression. For each biomarker, we created a dichotomous variable defining high level of the biomarker as a concentration in the upper quartile and low level as a concentration below the upper quartile. We used the third quartile as cut-off in the dichotomous variables for sCD40L, syndecan-1 and thrombomodulin to make the effect estimates more easily interpretable. For D-dimer, the cut-off was defined as the lower limit of detection (290 ng/mL (Fibrin Equivalent Units, FEU)), creating a dichotomous variable with detectable versus undetectable as most of the measurements were below the lower limit of detection. We created two predefined models; model 1 adjusted for age (per decade) and sex and model 2 adjusted for traditional risk factors that were significant in previous analyses on AA in PLWH (5) (age, sex, BMI category, hypertension, and smoking (current/previous/never)). Furthermore, in sensitivity analyses, we investigated the associations between AA, sCD40L, and (one at a time) use of aspirin, use of statins, weekly alcohol intake, and hypertension. As a sensitivity analysis, we included all four biomarkers into a single analysis with AA.

Additionally, continuous variables were used in pre-specified, secondary analyses to make sure any linear associations between exposure and outcome would not be overlooked. We adjusted for the same predefined models 1 and 2. Moreover, in sensitivity analyses, we investigated associations between aortic diameter (maximum outer diameter of ascending, descending, suprarenal, and infrarenal aorta) and high versus low levels of the biomarkers. Lastly, in sensitivity analyses, we examined associations between aortic wall thickness (maximal outer minus maximal inner diameter of ascending, descending, suprarenal, and infrarenal aorta) and high versus low levels of the biomarkers.

P-values <0.05 were considered statistically significant, and all P-values were two-sided. R (version 4.1.0 (2021-05-18)) was used for all statistical analyses (32).

3 Results

We included 571 participants aged 40 years or older with available contrast-enhanced CT and biomarker measurements. As reported previously, 43 aneurysms were found in 39 PLWH (6.8%). The median (IQR) age of PLWH was 52 years (47-60) and 88% were male (Table 1). The median (IQR) time since HIV diagnosis was 15 years (8-23) and 565 (99%) were currently on antiretroviral therapy (Table 1). Participant characteristics, HIV-specific variables, and biomarkers are listed in Table 1.

TABLE 1 Participant characteristics, HIV-specific risk factors, and concentrations of the biomarkers.

Variable	PLWH (N=571)
Characteristics	
Age, median [IQR]	51.6 [47.0-59.8]
Male sex, n (%)	503 (88.1%)
BMI, mean (SD)	24.8 (3.5)
BMI classification, n (%)	
Underweight	14 (2.5%)
Normal weight	301 (52.7%)
Pre-obesity	211 (37.0%)
Obesity	43 (7.5%)
Smoking status, n (%)	
Never smoker	188 (32.9%)
Current smoker	152 (26.6%)
Previous smoker	219 (38.4%)
Hypertension, n (%)	263 (46.1%)
Platelets, ×10⁹/L, mean (SD)	226.8 (57.5)
HIV-specific risk factors	

(Continued)

TABLE 1 Continued

Variable	PLWH (N=571)
Transmission mode, n (%)	
MSM	415 (72.7%)
Heterosexual	116 (20.3%)
IDU	6 (1.1%)
Other	28 (5%)
Current CD4+, cells/µL, median [IQR]	680 [520-870]
<200	7 (1.2%)
200-349	34 (6.0%)
350-499	79 (13.8%)
≥500	446 (78.1%)
CD4+ nadir <200, n (%)	235 (41.2%)
CD4+/CD8+-ratio, median [IQR]	0.813 [0.578-1.13]
Time since HIV diagnosis, median [IQR]	15.1 [7.74-22.6]
Time on ART, median [IQR]	12.8 [5.94-17.8]
Currently on ART, n (%)	565 (98.9%)
Viral load >50	23 (4.0%)
Hepatitis B-virus co-infection, n (%)	18 (3.2%)
Hepatitis C-virus co-infection, n (%)	33 (5.8%)
Concentrations of the biomarkers	
Syndecan-1, pg/mL, median [IQR]	2070 [1801-2413]
Thrombomodulin, pg/mL, median [IQR]	6598 [5541-7823]
sCD40L, pg/mL, median [IQR]	184 [118-243]
D-dimer, ng/mL (FEU), median [IQR]	290 [290-290]

ART, antiretroviral therapy; IDU, injecting drug use; MSM, men who have sex with men; FEU, fibrin equivalent units.

3.1 Aortic aneurysms and the association with markers of platelet activation, hemostasis, and endothelial disruption

We found high levels of sCD40L to be associated with lower odds of AA; univariably with OR 0.23 (95% CI 0.07-0.77; P=0.017) and adjusted odds ratio, aOR, 0.23 (95% CI 0.07-0.78; P=0.019) adjusted in model 2 (Table 2). In a sensitivity analysis, further adjustment of model 2 for use of aspirin did not significantly alter the association between high level of sCD40L and AA (aOR 0.25 (95% CI 0.07-0.87); P=0.029). In other sensitivity analyses, use of statins, weekly alcohol intake, and hypertension were not associated with AA and sCD40L.

Detectable level of D-dimer was associated with higher odds of AA, both univariably (OR 2.76 (95% CI 1.34-5.67; P=0.006)) and adjusted in model 1 (aOR 2.22 (95% CI 1.02-4.85; P=0.045)). Adjusting for traditional risk factors in model 2 did not change the parameter estimate, (aOR 2.24 (95% CI 0.99-5.04; P=0.052). There were aortic aneurysms among PLWH with undetectable D-dimer.

Syndecan-1 and thrombomodulin were not associated with AA (Table 2).

Including all four biomarkers into a single analysis did not significantly alter the associations compared to the individual associations between the biomarkers and AA.

In exploratory analyses with the biomarkers as continuous variables, a doubling in sCD40L concentration was associated with lower odds of AA; univariably with OR 0.81 (95% CI 0.66,0.98; P=0.028) and aOR 0.76 (95% CI 0.62-0.95; P=0.014) adjusted in model 2. A twofold increase in D-dimer concentration was associated with higher odds of AA; univariably with OR 1.51 (95% CI 1.22-1.86; P<0.001) and aOR 1.41 (95% CI 1.08-1.84; P=0.010) adjusted in model 2. Syndecan-1 was not significantly associated with AA. A twofold increase in thrombomodulin concentration was univariably associated with higher odds of AA (OR 1.82 (95% CI 1.02-3.26; P=0.043) but not in adjusted models (Table 3). Analyses of high versus low levels and with continuous variables are shown in Figure 1.

3.2 Aortic diameter and the association with markers of platelet activation, hemostasis, and endothelial disruption

The mean (SD) aortic diameters are presented in Figure 2 and Table 4. Diameter in ascending aorta 34.54 mm (4.30); in descending aorta 26.36 mm (2.70); in suprarenal aorta 24.97 mm (2.82); and in infrarenal aorta 21.65 mm (3.17). In sensitivity analyses, high level of sCD40L was associated with smaller suprarenal aortic diameter (0.46 mm (95% CI 0.01-0.91; P=0.045)) adjusted in model 2 but not univariably (0.40 mm (95% CI 0.14-0.93; P=0.144)). Detectable level of D-dimer was associated with larger infrarenal aortic diameter; univariably 1.11 mm (95% CI 0.38-1.84; P=0.003) and 0.97 mm (95% CI 0.30-1.63; P=0.004) adjusted in model 2. High level of syndecan-1 was univariably associated with larger diameter of the ascending aorta (1.04 mm (95% CI 0.23-1.85; P=0.012)) and with larger infrarenal aortic diameter (0.95 mm (95% CI 0.36-1.55; P=0.002)). High level of thrombomodulin was associated with larger infrarenal aortic diameter; univariably 1.41 mm (95% CI 0.82-2.00; P<0.0001) and 0.89 mm (95% CI 0.38-1.41; P<0.001) adjusted in model 2. Furthermore, high level of thrombomodulin was univariably associated with larger diameter of the ascending aorta (1.12 mm (95% CI 0.30-1.92; P=0.007)) and with larger suprarenal aortic diameter (0.68 mm (95% CI 0.14-1.21; P=0.024)) (Table 4 and Figure 2).

3.3 Aortic wall thickness and the association with markers of platelet activation, hemostasis, and endothelial disruption

The mean (SD) aortic wall thickness was: in ascending aorta 2.28 mm (0.59); in descending aorta 2.32 mm (0.60); in suprarenal aorta 2.22 mm (0.76); and in infrarenal aorta 2.09 mm (0.81). In sensitivity analyses, high level of sCD40L was not associated with aortic wall thickness. Detectable level of D-dimer was associated with greater infrarenal aortic wall thickness; univariably 0.46 mm (95% CI 0.36-0.56; P<0.001) and 0.41 mm (95% CI 0.30-0.51; P<0.001) adjusted in model 2. High level of syndecan-1 was univariably associated with greater infrarenal aortic wall thickness (0.16 mm

TABLE 2 Associations between AA and high levels of the biomarkers.

Variable	OR (95% CI), P-value	aOR[a] (95% CI), P-value	aOR[b] (95% CI), P-value
sCD40L (high vs. low)	0.23 (0.07,0.77), **P=0.017**	0.24 (0.07,0.81), **P=0.022**	0.23 (0.07,0.78), **P=0.019**
D-dimer (detectable vs. undetectable)	2.76 (1.34,5.67), **P=0.006**	2.22 (1.02,4.85), **P=0.045**	2.24 (0.99,5.04), P=0.052
Syndecan-1 (high vs. low)	1.55 (0.77,3.10), P=0.219	1.27 (0.61,2.64), P=0.515	0.89 (0.40,1.98), P=0.767
Thrombomodulin (high vs. low)	1.75 (0.88,3.47), P=0.109	1.31 (0.63,2.74), P=0.466	1.06 (0.48,2.36), P=0.877

[a]Model 1 and [b]model 2.
Bold values: significant associations.

TABLE 3 Associations between AA and the biomarkers as continuous variables.

Variable	OR (95% CI), P-value	aOR[a] (95% CI), P-value	aOR[b] (95% CI), P-value
sCD40L	0.81 (0.66,0.98), **P=0.028**	0.80 (0.65,0.98), **P=0.029**	0.76 (0.62,0.95), **P=0.014**
D-dimer	1.51 (1.22,1.86), **P<0.001**	1.44 (1.13,1.85), **P=0.004**	1.41 (1.08,1.84), **P=0.010**
Syndecan-1	1.66 (0.85,3.26), P=0.139	1.34 (0.66,2.72), P=0.417	0.90 (0.41,1.97), P=0.787
Thrombomodulin	1.82 (1.02,3.26), **P=0.043**	1.40 (0.77,2.56), P=0.273	1.15 (0.62,2.12), P=0.655

[a]Model 1 and [b]model 2. OR and aOR of AA per doubling in concentration.
Bold values: significant associations.
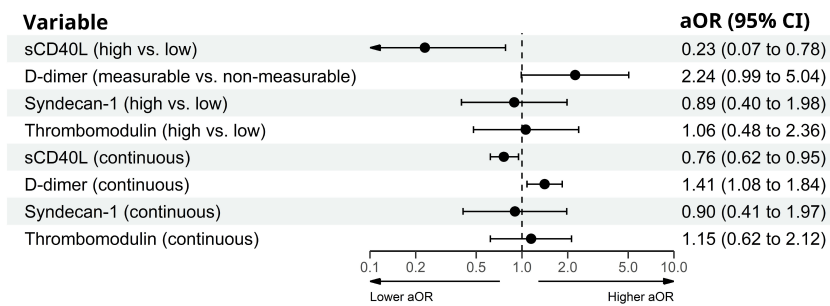
FIGURE 1
Primary (high vs. low variable) and exploratory (continuous variable, aOR associated with doubling of biomarker) analyses of the association between AA and the biomarkers in PLWH adjusted for age, sex, hypertension, smoking status, and BMI category.

FIGURE 2
Aortic diameter measured as maximum outer diameter. Mean diameter and larger or smaller diameter in mm (95% CI) associated with high level of the biomarker. Bold text: significant associations. *Adjusted for age, sex, hypertension, smoking status, and BMI category. (Made with biorender.com).

TABLE 4 Associations between aortic diameter and the biomarkers.

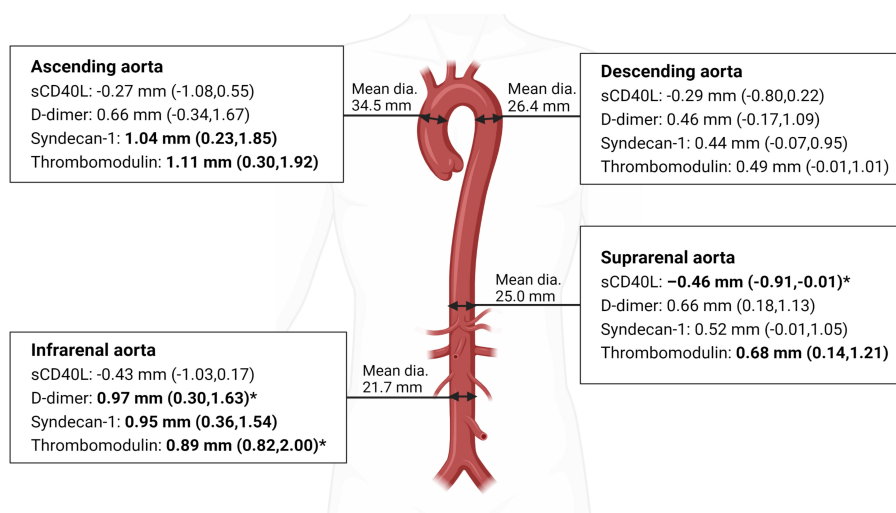| Variable	Larger or smaller diameter in mm if high level (95% CI), P-value	Larger or smaller diameter in mm if high level[a] (95% CI), P-value	Larger or smaller diameter in mm if high level[b] (95% CI), P-value
Ascending aorta			
sCD40L	-0.27 (-1.08,0.55), P=0.519	-0.18 (-0.92,0.57), P=0.643	-0.47 (-1.20,0.27), P=0.215
D-dimer	0.66 (-0.34,1.67), P=0.196	0.29 (-0.63,1-21), P=0.538	0.33 (-0.62,1.27), P=0.494
Syndecan-1	1.04 (0.23,1.85), **P=0.012**	0.51 (-0.24,1.26), P=0.182	0.54 (-0.22,1.29), P=0.167
Thrombomodulin	1.11 (0.30,1.92), **P=0.007**	0.68 (-0.06,1.43), P=0.073	0.59 (-0.16,1.34), P=0.121
Descending aorta			
sCD40L	-0.29 (-0.80,0.22), P=0.266	-0.17 (-0.59,0.24), P=0.408	-0.37 (-0.76,0.03), P=0.069
D-dimer	0.46 (-0.17,1.09), P=0.154	0.16 (-0.35,0.67), P=0.535	0.21 (-0.29,0.71), P=0.415
Syndecan-1	0.44 (-0.07,0.95), P=0.094	-0.08 (-0.50,0.34), P=0.723	-0.05 (-0.45,0.36), P=0.821
Thrombomodulin	0.49 (-0.01,1.01), P=0.057	0.12 (-0.29,0.54), P=0.573	0.10 (-0.30,0.50), P=0.636
Suprarenal aorta			
sCD40L	-0.40 (-0.93,0.14), P=0.144	-0.33 (-0.77,0.11), P=0.150	-0.46 (-0.91,-0.01), **P=0.045**
D-dimer	0.66 (0.18,1.13), P=0.056	0.35 (-0.23,0.92), P=0.235	0.38 (-0.21,0.97), P=0.213
Syndecan-1	0.52 (-0.01,1.05), P=0.057	0.04 (-0.41,0.49), P=0.860	0.01 (-0.46,0.47), P=0.980
Thrombomodulin	0.68 (0.14,1.21), **P=0.024**	0.29 (-0.15,0.74), P=0.203	0.26 (-0.19,0.72), P=0.1259
Infrarenal aorta			
sCD40L	-0.43 (-1.03,0.17), P=0.158	-0.32 (-0.85,0.21), P=0.239	-0.40 (-0.91,0.11), P=0.122
D-dimer	1.11 (0.38,1.84), **P=0.003**	0.88 (0.24,1.53), **P=0.008**	0.97 (0.30,1.63), **P=0.004**
Syndecan-1	0.95 (0.36,1.54), **P=0.002**	0.46 (-0.08,0.99), P=0.095	0.32 (-0.20,0.84), P=0.232
Thrombomodulin	1.41 (0.82,2.00), **P<0.0001**	1.06 (0.54,1.59), **P<0.0001**	0.89 (0.38,1.41), **P<0.001**

[a]Model 1 and [b]model 2. Larger or smaller diameter in mm associated with high levels of the biomarkers.
Bold values: significant associations.

(95% CI 0.01-0.31; P<0.040)). High level of thrombomodulin was associated with greater infrarenal aortic wall thickness; univariably 0.27 mm (95% CI 0.12-0.42; P<0.001) and 0.18 mm (95% CI 0.03-0.33; P=0.018) adjusted in model 2.

4 Discussion

In a large cohort of well-treated PLWH, we found high level of sCD40L to be associated with lower odds of AA and D-dimer above 290 ng/mL (FEU) to be associated with higher odds of AA. The same associations were found in analyses with biomarkers treated as continuous analyses.

4.1 sCD40L

The primary source of sCD40L is activated platelets (33). A study reported decreased platelet counts in HIV-negative AA patients compared to controls (34), and it is possible that lower platelet count in the PLWH with AA may contribute to the inverse association between AA and sCD40L, since platelets are critical for vascular endothelial integrity and health (35). Other studies have

reported aspirin to suppress (36) or block (37) the release of sCD40L from platelets. Furthermore, hypertension is associated with higher concentration of sCD40L (26), statins reduce sCD40L in CVD patients (38, 39), and alcohol is proposed to partially activate platelets (40) resulting in decreased sCD40L production. However, our analyses showed no such associations, so this cannot explain the contrast to previous reports of sCD40L being increased in plasma from HIV-negative patients with abdominal AA compared to matched controls (11) and the lacking finding of associations between sCD40L and AA (41).

In line with the emerging focus on the role of endothelial cells in AA pathogenesis (9, 10), we hypothesized that the biomarkers may be involved in the pathogenesis prior to the aortic diameter exceeding the aneurysmal limit. Therefore, we performed sensitivity analyses regarding associations between aortic diameter and the biomarkers. We found sCD40L to be negatively associated with suprarenal aortic diameter, while, at present, studies regarding associations between aortic diameter and sCD40L have not been published. Another measurement of interest is the aortic wall thickness, which studies have suggested the incorporation of into the risk analysis of AA (42, 43). Therefore, we investigated associations between wall thickness and the biomarkers but found no association with sCD40L.

4.2 D-dimer

D-dimer, an important marker of hemostasis, has been reported elevated in HIV-negative patients with abdominal AA compared to controls without abdominal AA (13–17). Furthermore, a study reported D-dimer to be 49% higher in PLWH on ART aged 45-76 years than in population controls (adjusted percent difference) (27). In line with these findings, our results highlight D-dimer as a possible biomarker, not only associated with abdominal AA, but all AA in PLWH. However, adjusting for known risk factors resulted in an only borderline significant parameter estimate, perhaps caused by an insufficient sample size, as plasma from 83 patients lacked measurement of D-dimer. The reported association between D-dimer and larger infrarenal aortic diameter is consistent with a previous study (44) and suggests a possible role of D-dimer as an indicator of larger infrarenal aortic diameter. Additionally, the reported associations between D-dimer and greater infrarenal aortic wall thickness may indicate increased fibrinolytic activity in the infrarenal aorta. However, as D-dimer is a general marker of fibrinolysis and not disease-specific, combination with other biomarkers may increase the specificity.

4.3 Syndecan-1

A previous study reported concentrations of syndecan-1, a key constituent of the endothelial glycocalyx and a regulator of inflammation, to be elevated in aortic tissue, though not in plasma, from patients with ascending AA compared to matched controls (18). Additionally, a study reported elevated syndecan-1 in PLWH compared to uninfected controls (23). Syndecan-1 is a key constituent of glycocalyx on most endothelial cells in the body, and its soluble form is generated by shedding from the endothelial glycocalyx indicative of damage to endothelial cells (45). As suggested by a previous study (18), it is possible that increased local shedding of syndecan-1 from the aneurysm is masked by the total pool of the biomarker, resulting in non-significant associations with AA in this study. Furthermore, since we only measured syndecan-1 in plasma and not aortic tissue, this may altogether explain why we do not reproduce the previously found association. Syndecan-1 being univariably associated with larger diameter in ascending and infrarenal aorta suggests an association between larger aortic diameter and endothelial disruption.

4.4 Thrombomodulin

Another biomarker shed from endothelial cells indicating endothelial cell injury (46), is soluble thrombomodulin, which has been reported elevated in serum from HIV-negative patients with abdominal AA compared to healthy controls (21, 22). Moreover, several studies have reported thrombomodulin to be elevated in PLWH compared to uninfected controls (24, 25). We found thrombomodulin to be univariably associated with AA when kept as a continuous variable, but not when used as a dichotomous variable. However, this finding was not significant when adjusted for known risk factors in our predefined models and we considered it

to be an incidental finding. A potential reason why we found no independent association between thrombomodulin and AA in PLWH is the above-mentioned possible masking of shedding from the aneurysm by the shedding from all endothelial cells. The reported association between thrombomodulin and larger infrarenal aortic diameter suggests thrombomodulin as a possible marker of aortic diameter, though at present, no studies regarding this association have been published. Moreover, as with syndecan-1, the association between thrombomodulin and greater infrarenal aortic diameter indicates an association between larger aortic diameter and endothelial disruption. However, further studies into these associations are required.

4.5 Diameter of aneurysms

The patients in the majority of the previous studies were undergoing surgery for AA, meaning that the diameters of the aneurysms were larger than those in our study, in which the vast majority of aneurysms had diameters below the surgical threshold. A study reported the size of abdominal AA to be associated with concentration of D-dimer (47), while another study suggested endothelin-1 (ET-1), which is released from endothelial cells in response to among other factors vascular injury, as a marker of aneurysm diameter (48). Though such associations have not presently been reported regarding the other biomarkers, it is possible that the smaller diameters of the aneurysms in our study precluded the use of these biomarker concentrations to identify AA, and may, thus, partly explain the negative inverse association between sCD40L and AA and the lack of associations between AA and high levels of syndecan-1 and thrombomodulin, respectively.

4.6 Strengths and limitations

A limitation of this study is its cross-sectional design, making it difficult to investigate causal relationships between the biomarkers and AA. Moreover, the cohort is predominantly male and in fact, all AA were found in men. Strengths include a large cohort of well-treated PLWH and the opportunity to adjust for known risk factors for AA. Lastly, the prevalence of AA is high, making it possible to investigate associations with AA.

5 Conclusions

In conclusion, in this large cohort of well-treated PLWH, we found high sCD40L to be associated with lower odds of AA, and we confirmed previous findings of the association between high D-dimer and higher odds of AA. Furthermore, thrombomodulin and D-dimer were associated with larger aortic diameter and sCD40L with smaller aortic diameter. These findings regarding sCD40L and AA call for further investigation into its utilization as a biomarker of AA in PLWH. D-dimer holds promise as a possible marker, though not specific of AA, why further examinations of specific biomarkers remain necessary to aid in the early diagnosis of AA in PLWH.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Regional Ethical Committee of Copenhagen. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Design of the COCOMO study was carried out by SN et.al (29). and data collection was performed by the COCOMO-team from the Viro-Immunology Research Group. SO contributed to the Luminex® analysis and interpretation of the analysed biomarkers. Statistical analysis plan and design of this sub study was written by SG under supervision by SN and JH. Statistical analyses, interpretation of data, and writing of the manuscript was performed by SKG under supervision by SN, JH and AK. Review of the manuscript was performed by MP, PS, AF, JK, LK, JG, TB, SO, and KK. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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