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SHORT REPORT

Relationship Between Intraluminal Thrombus Volume and Circulating ADAMTS-13 Activity in Abdominal Aortic Aneurysms

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Introduction: Abdominal aortic aneurysms (AAAs) with intraluminal thrombus (ILT) are suggested to be more prone to rupture than AAAs without. Prior studies indicate that the von Willebrand factor (vWf) plays a role in the formation of ILT since a positive correlation between ILT volume and vWf has been shown. vWf mediates the tethering of platelets at sites of endothelial injury, and the protease ADAMTS-13 cleaves larger forms of vWf, thus counteracting the thrombosis cascade and maintaining haemostatic balance. When investigating the largest quantifiable thrombus in the human body, it was hypothesised that circulating ADAMTS-13 activity may be associated with ILT size in patients with AAA and the aim was to explore this potential relationship using 3D contrast enhanced ultrasound (3D-CEUS) for ILT volume determination.

Report: In this retrospective, exploratory study, 60 patients with AAA were evaluated, and the association between ILT volume and thickness and ADAMTS-13 was estimated using 3D-CEUS. ADAMTS-13 activity was measured in plasma samples obtained the same day. No association between ILT volume ($r = -0.03$, $p = 0.84$) or ILT thickness ($r = 0.02$, $p = 0.87$) and ADAMTS-13 activity was found. Likewise, when subdividing the group into lowest and highest 50% of ADAMTS-13 activity, the half with the lowest ADAMTS-13 activity (mean ILT volume ±standard deviation [SD]: 32 ± 34 mL) did not differ from the half with the highest ADAMTS-13 activity (43 ± 24 mL) when comparing ILT volume ($p = 0.172$, $F = 2.95$) and thickness ($p = 0.070$).

Discussion: After evaluating the largest quantifiable intraluminal thrombus in the vasculature, it was concluded that, surprisingly, circulating ADAMTS-13 activity seems unrelated to ILT formation in AAA.

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INTRODUCTION

Abdominal aortic aneurysm (AAA) is a localised enlargement of the abdominal aorta, which leads to weakening of the arterial wall and may lead to rupture. AAA growth is a complex process influenced by several mechanisms: proteolysis, primarily through enzymes like matrix metalloproteinases degrading the extracellular matrix; oxidative stress damaging the cellular components, contributing to the dysfunction of vascular smooth muscle cells (VSMCs); an inflammatory immune response perpetuating a cycle of chronic inflammation, attracting immune cells, which in turn release cytokines, exacerbating tissue damage; and lastly, apoptosis of VSMCs diminishing the repair and structural support of the aorta, collectively leading to degraded structural integrity of the aortic wall, gradual expansion and ultimately rupture.[1]

AAAs with intraluminal thrombus (ILT) are suggested to expand faster and carry a higher risk of rupture than AAAs without.[2,3] ILT of varying sizes is present in 80% of all AAAs,[2,4] and the mechanisms behind ILT formation, and AAA growth and rupture are unclear. Disruption of the endothelial lining may be one event linking ILT and AAA growth.[5] However, ILT formation is most likely multifactorial, involving concerted interaction among haemodynamics, coagulation cascade, and inflammatory cell activation. Monitoring AAA biomarkers could have a significant clinical application, particularly in early detection, monitoring of disease progression, and predicting clinical outcomes. Blood biomarkers could offer a minimally

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invasive, cost effective means of identifying high risk patients, enabling timely intervention and personalised treatment strategies.

Von Willebrand factor (vWf) is essential in haemostasis and is released from the endothelium in response to inflammation or damage.[6] The elongation and formation of vWf multimers on the endothelial surface, as part of a haemostatic activation, is tightly regulated by the vWf cleaving protease, also called a disintegrin and metalloproteinase with thrombospondin type 1 motif, member 13 — or ADAMTS-13.[7] The primary function of ADAMTS-13 is to cleave vWf anchored on the endothelial surface and at the sites of vascular injury, thereby modulating thrombus formation and vWf mediated platelet recruitment.

The literature on the relationship between ADAMTS-13 activity and the risk of arterial thrombotic events is conflicting, and both negative and positive associations between ADAMTS-13 activity and the risk of myocardial infarction (MI) have been shown.[8,9] A previous study found a positive correlation between ILT volume and vWf activity in patients with AAA,[10] but the potential relationship between AAA, ILT, and ADAMTS-13 activity in the circulation remains unclear. When investigating the largest quantifiable thrombus in the human body, it was hypothesised that circulating ADAMTS-13 activity may be associated with ILT size in patients with AAA and the aim was to explore this potential relationship using 3D contrast enhanced ultrasound (3D CEUS) for ILT volume determination.

REPORT

Subjects

In this retrospective, single centre study, 60 patients with an asymptomatic infrarenal AAA were randomly chosen from the COpenhagen Aortic CoHort (COACH) using a MATLAB (The MathWorks, Inc., Natick, MA, USA) generated randomisation sequence. The randomisation sequence was set to ensure an even distribution of AAA diameters. The main group was subdivided into two groups, defined by the median AAA diameter. Age, sex, medication, and comorbidity were matched between the two groups. However, when performing the analysis the initial two groups were redefined. The median activity of ADAMTS-13 was used to subdivide the main group into upper and lower 50% of ADAMTS-13 activity (Table 1). The maximum diameter was determined from the leading edge of the adventitia anterior wall to the leading edge of the adventitia posterior wall using 3D-CEUS.

COACH is a single centre cohort of AAAs, consecutively and prospectively included from the outpatient clinic and enrolled in an advanced US follow up surveillance programme. The exclusion criteria were iliac or thoracic aortic involvement, since these segments are inaccessible with 3D-CEUS. Written informed consent was obtained from all subjects, and the local ethics committee approved the study (H-6-2014-056).

Ultrasound imaging and analysis

All subjects underwent 3D-CEUS at inclusion, using a previously validated protocol.[4] Before 3D-CEUS acquisition, a venous catheter (18 gauge) was placed into the right antecubital vein, allowing blood sampling and subsequent administration of US contrast (Sonovue, Bracco, Milan, Italy). A 1.5 mL bolus of reconstituted US contrast was administered and flushed with 10 mL of 0.9% saline solution. All 3D-CEUS acquisitions were performed with an X6-1 xMATRIX array transducer on a Philips EPIQ 7 US system (Philips Medical Systems, Bothell, WA, USA). The 3D-CEUS acquisitions were analysed offline with 3D interactive Philips software (AAA prototype v.2.0, Philips Research, Suresnes, France), quantifying partial AAA, ILT volume, and ILT thickness, as described and validated previously.[4] Quantification of the ILT size (volume) was accomplished in two steps. First, the proximal and distal extent of the aneurysm were manually defined, restricting the segmentation length

Table 1. Baseline patient ($n = 60$) demographics and characteristics.

Characteristics	Lower 50% ADAMTS-13 ($n = 30$)	Upper 50% ADAMTS-13 ($n = 30$)	p value
AAA characteristics			
Age — y	75 ± 7.3	72 ± 7.1	.67
Male sex	26 (87)	26 (87)	1.0
Hypertension	15 (50)	19 (63)	.29
Chronic obstructive lung disease	9 (30)	6 (20)	.56
Current or previous smoker	27 (90)	20 (67)	.73
Statin treatment	20 (67)	23 (77)	.39
Anticoagulant therapy	3 (10)	3 (10)	1.0
Antiplatelet therapy	25 (83)	22 (73)	.35
No antithrombotic therapy	2 (7)	5 (17)	.23
AAA absolute measurements			
AAA maximum diameter — mm	49 ± 9	49 ± 8	.99
AAA volume — mL	80 ± 36	87 ± 29	.39
ILT thickness — mm	9 (1—32)	14 (4—30)	.070
ILT volume — mL	32 ± 34	43 ± 24	.17
ADAMTS-13 — kIU/L	0.64 (0.2—0.76)	0.89 (0.76—1.27)	

Data are presented as n (%), mean ± standard deviation, or median (range). AAA = abdominal aortic aneurysm; ILT = intraluminal thrombus.

(60 mm). This was followed by an automatic outlining of the vessel wall to determine the AAA volume. The procedure was subsequently applied on the contrast filled lumen. The AAA volume was subtracted from the contrast volume to determine the ILT volume. For each pixel located on the vessel wall surface, and within the defined segmentation length, the minimum distance to the lumen surface was computed and the maximum ILT thickness was selected. Likewise, the ILT volume was calculated by adding the predefined volume of each pixel within the segmented area.

ADAMTS-13 activity

Peripheral venous blood was collected into citrate sodium blood collecting tubes (Vacuette, Greiner bio One International GmbH, Kremsmünster, Austria). Samples were centrifuged for 20 minutes at1 950*g* (4°C) to platelet poor plasma and stored at −80°C in cryotubes containing 1 700 µL of bolus, within 30 minutes of collection. The ADAMTS-13 activity was measured using the HemosIL automated method (Instrumentation Laboratory, Werfen Company, Bedford, MA, USA), and the chemiluminescent signal was quantitated as relative light units by the BioFlash optical system and transformed to a value of ADAMTS-13 activity using a calibrator curve.[11]

Data analysis and statistics

Due to the exploratory nature of the study, a simple correlation r ($r = .4$) of n observations was anticipated. Using a two sided test, 5% significance level test ($\alpha = .05$) with 80% power ($\beta = 0.2$), the required sample size was 47 ($n = 47$).

The Shapiro—Wilk test was used to test for normality. Normally distributed data are presented as mean with standard deviation, and median with range for skewed data. Median activity of ADAMTS-13 was used to subdivide the main group into two groups: upper and lower 50% of ADAMTS-13 activity. A box plot was used to illustrate the two groups against the corresponding ILT size. Pearson's correlation analysis was performed to compare baseline ILT volume, ILT thickness, and activities of ADAMTS-13. Means and medians were compared using the parametric independent t test and the non-parametric test, respectively. Multivariable analysis was not intended for the original subdivision (median AAA diameter) due to randomisation. No multivariable analysis was needed in the new grouping (Median ADAMTS-13), since the two groups were without statistically significant differences.

Statistical Analysis System Enterprise Guide 7.11 (SAS Institute, Cary, NC) was used for this study.

DISCUSSION

In this retrospective study, 60 patients were included (female, $n = 8$). All patients were recruited consecutively in a prospective manner. None of the 60 patients had undergone recent major surgery (<60 days) or were on anti-inflammatory or immunosuppressive medication. The overall mean AAA maximum diameter was 49 ± 9 mm, and the median ADAMTS-13 activity was 0.76 kIU/L (0.2—1.27 kIU/L). The ADAMTS-13 activity was representative of healthy subjects.[12]

No association between ILT volume ($r = -.03$, $p = .84$) or ILT thickness ($r = .02$, $p = .87$) and ADAMTS-13 activity was found (Fig. 1A and B).

Epidemiological studies have shown conflicting results on the relationship between ADAMTS-13 activity in circulation and the risk of MI and ischaemic stroke (IS). A recent meta-analysis demonstrated that low ADAMTS-13 activity increased the risk of MI, but without any mediation by vWf.[13] Another study of younger females by Andersson *et al*. examined the association between plasma vWf and ADAMTS-13 activity and the risk of MI and IS in blood samples taken in the chronic phase (median 95 months, range 23—146 months).[14] Andersson *et al*. found a concentration dependent association between vWf and ADAMTS-13 activity (inverse association) and the probability of MI and IS (negative association). The aforementioned negative association was later disproven in a large population based case control study, where plasma samples obtained more than six months after an MI showed a positive association with ADAMTS-13 activity.[9] Another single study, with a small number of patients, suggested the vWf/ADAMTS-13 ratio taken immediately after an MI as a potential predictor for new cardiovascular events.[8]

The time point of blood sampling seems important in these studies (sampling in the acute or chronic phase). Thus, the chronicity of ILT in patients with AAAs may influence ADAMTS-13 activity, and it could be hypothesised that ADAMTS-13 is only affected in times of ILT activity: expansion or degradation.

The patients with AAA in the current study were all enroled in a standardised AAA surveillance programme and were primarily referred after random AAA detection. Thus, it was not possible to determine the physiological age of the AAA and the chronicity of the thrombus. Likewise, it was not possible to evaluate whether the ILT was in an active or passive state, since ILT evolution and mechanism are still so poorly understood, making it difficult to establish such a state. However, considering ILT as the largest quantifiable thrombus in the vasculature, and due to the crucial role of ADAMTS-13 in thrombus formation and degradation, a detectable association between circulating ADAMTS-13 activity and ILT size was hypothesised. The half with the lowest ADAMTS-13 activity (mean ± SD ILT volume: 32 ± 34 mL) did not differ from the half with the highest ADAMTS-13 activity (43 ± 24 mL) when comparing ILT volume ($p = .17$, $F = 2.95$) and thickness ($p = .070$). However, the ILT thickness box plots (Fig. 1C) were further apart, with minor overlapping of the confidence intervals compared with ILT volume (Fig. 1D). Since only a marginal difference between the two ADAMTS-13 groups was found ($p = .070$) an error of omission could be suspected (type 2 error), suggestive of small sample size or high variability of ADAMTS-13 within the groups. One condition adding to the variability within the groups could be blood group information, which was lacking and is a known factor regarding levels of vWf. Prior studies have suggested ILT to facilitate vessel wall hypoxia due to the prolonged

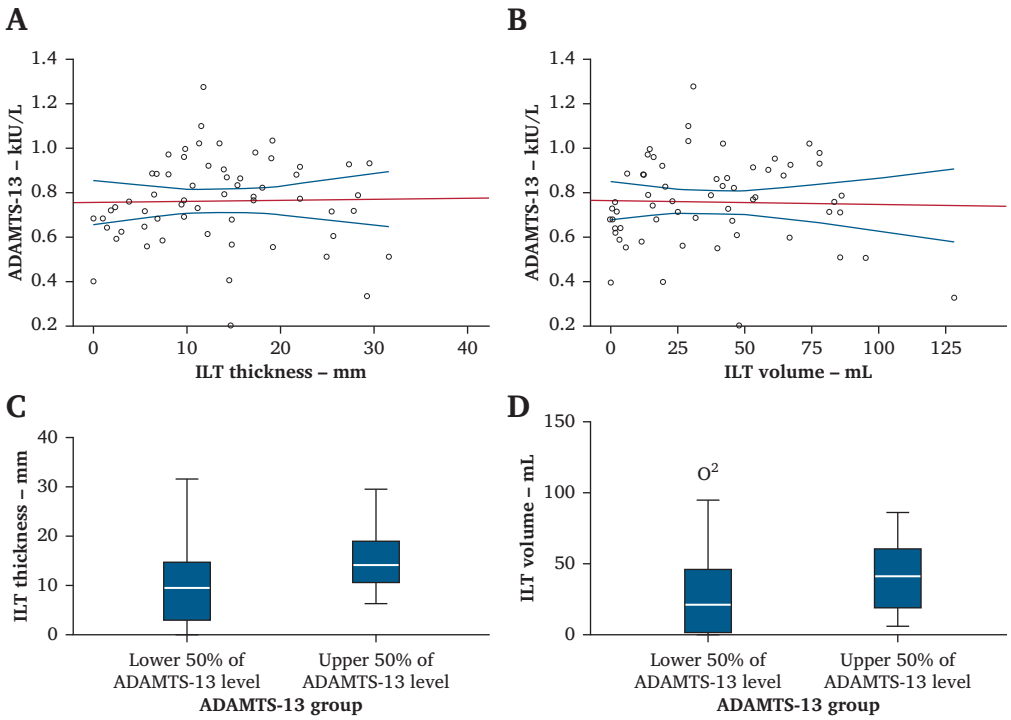

Figure 1. Correlation between ADAMTS-13 activity and (A) intraluminal thrombus (ILT) thickness and (B) ILT volume. Distribution of (C) ILT thickness and (D) ILT volume, when split according to the median activity of ADAMTS-13.

distance from the lumen to the vessel wall,[15,16] underlining ILT thickness, instead of volume, to be the driver of volatile vessel wall degeneration.

In addition to the small number of patients, the multifactorial nature of ILT evolution may have blurred ADAMTS-13 detectability. It can be speculated that sampling of blood far from the ILT, or source, may potentially affect the detectable ADAMTS-13 activity. Blood sampling from the aortic lumen, close to the ILT (e.g., during endovascular aneurysm repair) could shed some light on this dilemma. Lastly, the two groups were matched according to prescribed antithrombotic medication, but the duration of such a prescription was not registered, which inherently may have influenced ILT activity and size. However, antiplatelet therapy does not have any influence on ADAMTS-13 activity, being a hepatic enzyme. Thus, a future study should encompass peripheral and aortic lumen blood samples, larger sample size, and ILT classification, either by using an imaging modality (magnetic resonance) or histologically.

In conclusion, when investigating the largest quantifiable ILT in the vasculature, no association between ILT volume, ILT thickness, and ADAMTS-13 activity was evident.

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