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The relationship between aortic aneurysm sac thrombus volume on coagulation, fibrinolysis and platelet activity

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ABSTRACT

Aim: Abdominal aortic aneurysm (AAA) is associated with chronic mural inflammation and a pro-thrombotic diathesis. It has been suggested that both may be related to biologically active intra-sac thrombus. The aim of this study was to examine the relationship between thrombin generation, fibrinolysis, platelet activity and AAA sac thrombus volume.

Methods: 30 patients (29 men) of median (IQR) age 75 (71–82) years with an infra-renal AAA > 5.5 cm in antero-posterior diameter were prospectively studied. AAA, lumen and thrombus volumes were calculated using a CT workstation (Vitrea). Plasma thrombin-antithrombin (TAT), plasminogen activator inhibitor (PAI)-1, and soluble (s) P-selectin were measured as biomarkers of coagulation, fibrinolysis and platelet activity, respectively

Results: Median (IQR) AAA total, lumen and thrombus volumes were 188 (147–247) cm^3, 80 (54.3-107) cm^3 and 97.6 (63–127) cm^3 respectively.

TAT levels were significantly higher (median, QR, 7.15 [4.7-31.3] μg/L, p = <0.001) and sP-selectin levels significantly lower (median, IQR, 80.5 [68–128] ng/ml, p = <0.0001) than the normal range. PAI-1 levels (median, IQR, 20.9 [8.4-50.7] ng/ml) were normal. There was no correlation between AAA thrombus volume and PAI-1 (r = −0.25, p = 0.47), sP-Selectin (r = 0.26, p = 0.43) or TAT plasma levels (r = −0.21, p = 0.54).

Conclusion: The present study confirms that patients with AAA demonstrate haemostatic derangement, but the extent of the haemostatic derangement does not correlate with AAA sac thrombus volume.

Introduction

AAA are characterised by chronic inflammation and the presence of mural thrombus [1]. In contrast to atherosclerotic arterial plaques, blood flow is maintained through the mural thrombus thereby acting as an exchange interface between the systemic circulation and thrombus mass [1]. The thrombus microstructure is highly complex consisting of a network of inter-connecting canaliculi capable of delivering macromolecules between the arterial lumen-thrombus and thrombus-arterial wall surfaces. The canaliculi often contain cellular infiltrates including neutrophils, macrophages and platelet in a state of degranulation. This may result in a sub-clinical disseminated intravascular coagulation caused by the consumption of platelets and coagulation factors. [2] Thus, the mural thrombus represents a biologically active entity with the ability to trap polymorphonuclear leukocytes, absorb circulating plasma components and aggregate platelets as well as being implicated as a source of proteolysis and fibrinolytic activity thought to be implicit in AAA progression [1,3,4].

Patients with asymptomatic AAA demonstrate a hypercoagulable and hypofibrinloytic state that is associated with cardiovascular morbidity following open and endovascular repair [5,6]. We have previously reported a positive correlation between serum levels of Interleukin-1α and maximum AAA size [7]. Few studies, often with contradictory results, have investigated the relationship between AAA thrombus volume and this prothrombotic diathesis [8–11]. The aim of this study was to examine the relationship between novel biomarkers of thrombin generation, fibrinolysis, platelet activity and AAA size/sac thrombus volume.

Methods

Patients presenting to the Vascular Surgery Units of the Heart of England NHS Foundation Trust and University Hospital Birmingham NHS Trust between January 2007 and January 2009 with an infra-renal abdominal aortic aneurysm measuring ≥ 5.5 cm in maximum diameter

Abbreviations: AAA, Abdominal Aortic Aneurysm; ADP, Adenosine diphosphate; APC-PCI, activated protein C-protein C inhibitor complex; cm^3, cubic centimetres; CTA, computed tomographic aortograms; IQR, Inter-quartile range; PAI-1, Plasminogen activator inhibitor-1; sP-Selectin, Soluble P-selectin; TAT, Thrombin-antithrombin III-complex.

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and due to undergo endovascular abdominal aortic aneurysm repair were assessed for study inclusion suitability. A patient was considered unsuitable for study inclusion if they had undergone any recent (vascular and non-vascular) surgical or an endovascular procedure, they suffered with an hereditary or acquired conditions that could effect their coagulation, fibrinolysis or platelet function including but not limited to: significant lower limb peripheral vascular disease (Fontaine Classification IIb-IV) [12], concomitant thoracic or suprarenal aortic aneurysm or dissection, peripheral arterial aneurysm, history of malignancy and/or chemotherapy within the last 5 years; or the patient was unable or unwilling to give fully informed consent.

All patients gave informed consent to participate in the study, which was approved by the Birmingham East, North and Solihull Research Ethics Committee and South Birmingham Research Ethics Committee (reference: 06/Q2703/44).

Blood Sample Collection

All patients had a resting venous blood sample drawn pre-procedure Blood was drawn into a standard syringe utilising a 21 g hypodermic needle and then immediately transferred to specific tubes; a 2.7 ml sample was collected into EDTA anticoagulant (1.6 mg/ml) and a 3 ml sample into sodium citrate anticoagulant (0.106 mol/l). Samples were placed immediately on ice and transferred to the laboratory within 30 minutes of collection. Plasma was separated by centrifugation at 3,000 revolutions per minute for 30 minutes at a temperature of 4°C (equivalent to 1400 g). Plasma and serum were separated utilising a standard 1 ml graduated Pasteur pipette and stored in cryogenic vials at - 80 C for batch analysis.

Markers of Thrombin generation, fibrinolysis and Platelet activity

Plasma thrombin antithrombin III-complex (TAT) (healthy range, <4.2 μg/L) (Assaypro®) was assayed as a marker of thrombin generation. Plasma plasminogen activator inhibitor 1 (PAI-1) (healthy range, 7-43 ng/ml) (American Diagnostica Inc.®) was assayed as a marker of fibrinolysis. Soluble P-selectin (sP-selectin) (healthy range, 92–212 ng/ml) (Immuno-Biological Laboratories, Inc ®) was assayed as a marker of platelet activity. All assays were analysed using a Triturus® (*Grifols*) fully automated enzyme immunoassay analyser. The manufacturer determined the healthy range for each haemostatic marker.

Assessment of Thrombus Load

All patients underwent computed tomographic aortograms (CTA) performed according to a standard acquisition protocol using an Aquilion system (Toshiba). Images were uploaded to a Vitrea Fx (Vital Images) workstation for post-processing thrombus load calculation. The Vitrea Fx workstation has a semi-automated thrombus load estimation function that estimates total aneurysm, lumen and thrombus volumes through a process of interpolation. The start and endpoints were standardised to the aorta immediately distal to the lowest renal artery and the aortic bifurcation, respectively. If the software incorrectly identified thrombus as lumen and vice versa, thrombus/lumen contours were manually corrected prior to thrombus calculation. Thrombus volume is measured in cubic centimetres (cm^3).

Statistics

Differences between patient characteristics were assessed using Fisher's exact test for categorical variables and Mann–Whitney test for continuous variables.

Correlation analyses were performed using the Spearman rank test. Where levels of assays were below the lower limit of assay detection, this level was assigned for statistical analysis. A probability (p-) value of less than .05 was regarded as statistically significant. Data are presented as median (inter-quartile range). All analyses were carried out using StatsDirect version 2.7.2 (StatsDirect Ltd, Cheshire, UK).

Results

General

30 patients (29 men and 1 women of median (IQR) age 75 (71–82) years) were prospectively studied. Ten patients had a previous history of cardiac ischaemic disease, four patients suffered with diabetes, four patients suffered with chronic renal failure (Kidney Disease Outcomes Quality Initiative (KDOQI) Stage <3), one patient was an active smoker and one patient suffered with peripheral vascular disease (Fontaine Classification <IIb). [12,13] Sixteen patients were being treated with an anti-platelet agent (aspirin = 14, clopidogrel = 1, aspirin & clopidogrel = 1); thirteen patients were being treated with a statin.

Haemostasis

The plasma levels of sP-selectin were significantly lower than the normal range: median (IQR) = 80.5 (68–128) ng/ml), p = <0.0001. The use of a statin (median (IQR); 72 (66–81) vs. 84 (80–117) ng/ml, p = 0.0165) resulted in lower plasma sP-selectin levels. The use of an anti-platelet (median (IQR); 73 (66–81) ng/ml vs. 84 (76–99) ng/ml, p = 0.076) tended to result in lower sP-selectin levels. The plasma levels of TAT were significantly higher than the normal reference range: median (IQR) = 7.15 (4.7-31.3) μg/L, P = <0.001. There was no correlation between anti-platelet (p = 0.13) or statin (p = 0.21) pharmacotherapy and TAT levels. The plasma levels of PAI-1 (median (IQR) = 20.9 (8.4-50.7) ng/ml) were within the normal values. (See Table 1)

Hameostasis and Thrombus Volume

Median (IQR) maximum aneurysm diameter was 66.5 (57.3-70.3) mms. Median (IQR) total aneurysm volume and aneurysm lumen volume were 188 (147–247) cm^3 and 80 (54.3-107) cm^3. Median (IQR) aneurysm sac thrombus volume was 97.6 (63–127) cc. There was no correlation between aneurysm thrombus volume and maximum aneurysm diameter: r = 0.45, p = 0.15. There was no correlation between aneurysm thrombus volume and PAI-1 (r = −0.25, p = 0.47), sP-Selectin (r = 0.26, p = 0.43) or TAT plasma levels (r = −0.21, p = 0.54).

Discussion

Abdominal aortic aneurysms (AAA) represent a chronic inflammatory pathology and is usually characterised by the presence of biologically active mural thrombus directly proportional to the maximum AAA diameter [14]. To date only six studies have investigated the effects of AAA thrombus volume/maximum thickness on coagulation and fibrinolysis using a variety of biomarkers [8–11,15,16]. (See Table 2) Both Yamazumi et al and Aho et al reported a correlation between AAA total thrombus volume/maximum thrombus thickness and changes in biomarkers of systemic fibrinolysis [11,15]. However, only Yamazumi et al reported a positive correlation between maximum thickness of intraluminal AAA thrombus and heightened thrombin generation [15]. In direct contradiction, Kolbel et al, using APC-PCI as a marker of

Table 1
Values of markers of haemostasis. The arrows indicate comparisons to normal reference ranges. (PAI-1 = Plasminogen activator inhibitor-1, sP-Selectin = Soluble P-selectin, TAT = Thrombin-antithrombin III-complex).

Assay	Normal Range	Pre-operative Values (median (IQR)
		Cohort 2
PAI-1	7-43 ng/ml	20.9 (8.4-50.7)
sP-Selectin	92-212 ng/ml	80.5 (68–128) ↓
TAT	<4.2 mg/L	7.15 (4.7-31.3) ↑