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Atherosclerotic risk factors and atherosclerotic postoperative events are associated with low inflammation in abdominal aortic aneurysms

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ABSTRACT

Objective: Evidence is emerging that abdominal aortic aneurysm (AAA) formation cannot completely be explained by systemic atherosclerosis and is in part due to other pathophysiological mechanisms such as local immune reactions. The aim of the present study was to study variance in AAA wall inflammation, and relate that to clinical patient characteristics.
Methods: Ventral walls from 201 patients with intact AAAs undergoing open repair were prospectively collected and processed for histology and protein measurements. Patients were monitored for 3 years postoperatively.
Results: The amount of lymphocytic infiltrate was used to distinguish 96 lymphocyte-poor AAAs from 105 lymphocyte-rich AAAs. The walls of lymphocyte-rich AAAs had higher concentrations of various inflammatory markers, including interleukin (IL) 6, IL8, matrix metalloproteinase (MMP) 8; however, MMP9 levels were comparable. Patients with lymphocyte-poor AAAs had more atherosclerotic risk factors: type 2 diabetes (22% vs. 9%, $P = .008$), hypertension (81% vs 66%, $P = .019$), and serum cholesterol levels (mean[SD] 5.2[2.5] vs. 4.2[1.0] mmol/L, $P = .023$). Intimal lesions in the AAAs revealed more frequently an extracellular lipid pool in lymphocyte-poor AAAs (66% vs. 52%, $P = .026$). Lymphocyte poor AAAs were associated with a worse survival during 3 years of follow-up, although this association did not reach statistical significance when correcting for other cardiovascular predictors (24% vs. 14%; HR 1.9 −2.3).
Conclusion: Low amount of inflammation in AAAs is associated with more atherosclerotic risk factors, more advanced local atherosclerotic lesions and more postoperative atherosclerotic adverse events. This observation supports the view that AAA development is a multi-factorial process in which part of the patient population has a closer relation with systemic atherosclerotic disease, while in other patients local inflammatory reactions might play a larger role.

The pathophysiological mechanisms of abdominal aortic aneurysm initiation and progression are poorly understood. Risk factors for the development of AAA, such as male sex, advanced age, dyslipidemia, and smoking overlap with those for atherosclerosis [1–3]. Patients with AAAs frequently have atherosclerotic disease such as coronary heart disease and peripheral atherosclerotic occlusive disease. However, not all patients with advanced atherosclerotic disease develop an AAA and vice versa not all patients with AAA have atherosclerotic disease in other vascular territories. It is therefore unknown whether the association between AAA and atherosclerosis is causal or due to common risk factors. [4].

Abbreviations: AAA, abdominal aortic aneurysm; EvG, elastin von Gieson; H&E, hematoxylin and eosin; IFN, interferon; IL, Interleukin; MMP, Matrix metalloproteinase; PET/CT, positron emission tomography/computed tomography; SMC, smooth muscle cell; TNF, tumor necrosis factor; vWF, von Willebrand factor.

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More evidence is emerging that the development of AAA cannot completely be explained by systemic atherosclerosis and is at least partly caused by other pathophysiological mechanisms. Case control studies did not find more coronary, carotid or peripheral atherosclerosis in AAA patients [5–7]. In a recent study in 6446 patients no dose-response relationship was found between atherosclerosis and abdominal aortic diameter. From these results it was suggested that aneurysm formation and atherosclerosis, under influence of some common risk factors, develop in parallel but as partly independent processes. [8].

Evidence that AAA formation and atherosclerosis do not have identical pathophysiological mechanisms has also been provided by tissue studies. Already in the 1990s it was demonstrated that inflammation and proteolytic activity are far more pronounced in AAAs than in atherosclerotic occlusive disease [9,10]. Recent studies have provided evidence that local immune reactions are involved in the initiation and propagation of the inflammatory response in aortic tissue. [11–14].

Risk of rupture increases with diameter, but rupture also occurs in patients with a small-diameter AAA, suggesting other factors may substantially contribute to this risk [15–18]. Furthermore, growth rate analyses of small AAAs in a large screening study showed that initial AAA diameter followed a unimodal distribution that in 5 years evolved to a bimodal distribution: half of the small AAAs remained quiescent with only little growth, whereas the other half expanded substantially, leading to surgical repair or rupture [19]. These observations suggest that variation exists in the composition of the AAA wall between patients. The variation in AAA wall composition was confirmed in histopathological studies where it was demonstrated that especially the amount of inflammation in the AAA wall varies among patients [20].

Thus far histopathological studies that study AAA wall composition in relation to clinical patient's characteristics and clinical follow up are lacking. The aim of this cross-sectional observation study was to study variance in AAA wall inflammation, and relate that to clinical patient characteristics such as atherosclerotic risk factors and pre- and postoperative manifestations of systemic atherosclerotic disease.

1. Methods

1.1. Aneurysm-express biobank

Aneurysm-Express is a prospective cohort study that includes a biobank with vascular tissue from patients undergoing open AAA surgical repair in 2 Dutch hospitals (University Medical Center Utrecht and St Antonius Hospital, Nieuwegein). The primary study objective is to investigate the relation between tissue characteristics at baseline and clinical outcome during follow-up [21]. The study was approved by the Ethical Review boards of both hospitals, and written informed consent was obtained from all patients.

All consecutive patients scheduled for open repair of their intact AAA in the 2 participating hospitals were asked to participate in this study. The criteria to perform open surgical AAA repair were met in accordance with international guidelines and performed when endovascular treatment was not suitable [22]. Exclusion criteria for follow-up were presence of a malignancy and unwillingness or physical incapability to participate (e.g., severe dementia).

Baseline clinical parameters, including cardiovascular risk factors and medication use, were recorded. According to established guidelines, a symptomatic AAA was defined as back pain not attributable to any other cause than the AAA [22]. Patients completed an extensive questionnaire at baseline based on the Rose cardiovascular survey [23]. Inconsistencies were resolved by contacting the patient's referring hospital or general practitioner.

1.2. Aneurysm tissue collection and characterization

During elective open AAA repair, a full-thickness specimen of the ventral aneurysm wall next to the ventral aortotomy was collected at the site of the maximum AAA diameter (minimum dimensions tissue specimen 10 × 20 mm) shortly after the proximal clamp was placed. This tissue specimen was immediately transported to the laboratory, where it was cut into 5-mm segments. The middle segment was used for histological analyses, and one adjacent segment was used for protein isolation. Other segments were stored for future use.

The middle segment was fixed in formalin and embedded in paraffin. Immunhistochemical stains were performed on the Bond-Max staining robot (Leica Microsystems, Wetzlar, Germany). Consecutive sections were stained with hematoxylin and eosin (H&E), elastin van Gieson (EvG), and Sirius red, as well as α-actin, von Willebrand factor (vWF), CD68, CD45, CD3, CD20, and CD138 immunostains. Used antibodies are specified in Appendix Table 1. Histologic examination was performed by 2 independent observers (R.H., A.V.) blinded for clinical data. In case of discrepancies in judgment, sections were reanalyzed. Consensus was reached in all cases.

Structural wall components were semiquantitatively scored as (1) minor or (2) moderate to heavy staining in all 3 wall layers (intima, media, and adventitia) separately for collagen (Sirius red) and smooth muscle cells (SMCs)/myofibroblasts (α-actin).

Table 1
Clinical characteristics of the study population.

Characteristics[a]	Lymphocyte-rich AAAs ($n = 105$)	Lymphocyte-poor AAAs ($n = 96$)	P value
Age, mean (SD), *y*	68.9 (8.1)	70.6 (7.5)	0.116
Male sex	82 (78)	82 (85)	0.115
Current smoker	52 (50)	40 (42)	0.345
Diabetes mellitus type 2	9 (9)	21 (22)	0.008[b]
Hypertension	69 (66)	78 (81)	0.019[b]
Coronary artery disease	27 (26)	32 (33)	0.344
Peripheral artery disease	15 (14)	19 (20)	0.298
Chronic obstructive pulmonary disease	26 (25)	16 (17)	0.159
Body mass index, mean (SD), kg/m^2	26.0 (4.1)	26.0 (3.3)	0.541
Serum cholesterol, mean (SD), mmol/L	4.2 (1.0)	5.2 (2.5)	0.023[b]
Serum creatinine, median (IQR), μmol/L	89 (80–101)	92 (80–113)	0.390
Glomerular filtration rate, mean (SD), mL/min/1.73m^2	75.1 (26.1)	74.7 (24.4)	0.682
AAA diameter, mean (SD), mm	64.8 (12.1)	62.7 (12.8)	0.174
Symptoms attributable to AAA	14 (13)	10 (10)	0.524
History of any other aneurysm detected	12 (11)	11 (11)	0.986
Statin use	49 (47)	50 (52)	0.410
Aspirin use	71 (68)	64 (67)	0.944
Angiotensin-converting enzyme inhibitor use	33 (31)	33 (34)	0.461
Angiotensin II receptor blocker use	17 (16)	17 (18)	0.565
Oral antidiabetic drug use	7 (7)	14 (15)	0.065
Insulin use	2 (2)	6 (6)	0.110

[a] Data are presented as No. (%) unless otherwise indicated. AAA is abdominal aortic aneurysm. Body mass index was calculated as weight in kilograms divided by height in meters squared. Glomerular filtration rate (GFR) was calculated using the Cockroft-Gault formula.

[b] $P < .05$.