Discrepancies in abdominal aortic aneurysm expressions and repair

Rob Hurks

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Discrepancies in abdominal aortic aneurysm expressions and repair

Discrepanties in aneurysma aortae abdominalis expressies en herstel
(met een samenvatting in het Nederlands)

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General introduction

1

"At 3.30 AM the next morning, I was awoken by an excruciating abdominal pain. I can only describe its intensity as inhuman, evoking dreaded images of horror films in which the victim is perforated by an industrial drill."[1] In this excerpt, a physician described the symptoms he encountered when his abdominal aortic aneurysm (AAA) ruptured. He was lucky to be inside a hospital at the time with a vascular surgeon on call, which in the end allowed him to write this narrative afterwards.

Epidemiology and risk factors for AAA development

The existence of AAAs was first described by the sixteenth century Belgian anatomist Vesalius.[2] An aneurysm is defined as a focal dilatation of a blood vessel with respect to the original or adjacent artery. For the diagnosis of an AAA it is required to have an aortic diameter of at least one and one-half times the diameter measured at the level of the renal arteries. Clinically, a diameter exceeding 3 cm is used as a cut-off.[3] The incidence of AAA is increasing in Western Countries[4, 5], reaching 4.1% in Dutch men and 0.7% in Dutch women of 55 years and over.[6] During the first decade of this century, 10733 men and 4884 women were recorded to die from AAA in the Netherlands[7], both numbers are hampered by the low number of postmortem examinations and therefore represent an underestimation. In the US, AAAs are the 15th leading cause of death in patients 55 years of age and older, and in this age category 9800 patients die annually diagnosed with this disease of whom 6500 die of aneurysm rupture.[8, 9]

This number is likely to increase due to aging of the population and increased life expectancy, and is reported more and more in common press media thereby increasing public awareness.[10, 11] The number of diagnosed AAAs will furthermore increase because of the introduction of screening programs. For instance, the U.S. Preventive Services Task suggested in January 2005 that men between 65 and 75 who have ever smoked be screened for AAA trough a one-time ultrasound examination.[12] The basis for this was a meta-analysis of 4 screening trials, which showed a protective effect of inviting patients to attend screening for AAA (OR 0.57[0.45-0.74]) on AAA related mortality in this specific high-risk group of patients.[13]

Several risk factors for development of AAA exist. The most prominent being tobacco use, both current and a history of smoking. Interestingly, smoking is associated with AAA in men 2.5 times more frequently than it is with coronary artery disease.[14] Other important risk factors for AAA include male gender, advanced age, atherosclerosis, dyslipidemia and a first degree relative with an AAA.[14-16] Remarkably, in contrast with the strong association with arterial occlusive disease, diabetes mellitus appears to be protective for AAA development and progression.[15, 17]

Rupture risk and (preventive) treatment

AAA rupture carries a great risk for the patient, leading to a community mortality rate of 80%. For patient reaching the operating room the mortality rate is approximately 50%.[18-22] The main determinant of rupture is AAA size: AAAs with diameters ranging from 3-4 cm have an annual rupture risk of 0%; 4-5 cm 1%; 5-6 cm 1-11%; 6-7 cm 10-22% and >7 cm AAAs are at a 30-33% risk of rupture per year.[23-25] Other factors that are known to elevate rupture risk include: rapid expansion, eccentric aneurysm shape, female gender, smoking, hypertension, and COPD.[20, 26-33]

Albert Einstein was subjected to one of the first methods of AAA repair in 1948 when he was 69 years old. The surgeon (Dr. Rudolph Nissen) was unable to remove the AAA, and chose to wrap it with polyethene cellophane in an attempt to prevent growth and rupture. Cellophane is a tissue irritant, producing marked fibrosis which was hoped to strengthen the vessel wall and prevent expansion. Despite this attempt Einstein's AAA ruptured 7 years later, which caused his death.[34]

Later on in 1953 Charles Dubost used homografts to repair AAAs.[35] After some years, these homografts became aneurysmatic themselves because of the preservation methods. Blakemore and Voorhees were the first to describe the use of a nylon graft for aneurysm exclusion[36], which later on evolved to the use of Dacron and PTFE grafts. This method, in the current thesis, is referred to as open AAA repair and is still common practice. However, the proportion of open repair is rapidly declining in favor of endovascular aneurysm repair (EVAR), that was first performed by Parodi in 1990.[37] It was reported that the introduction of EVAR decreased the annual number of deaths from intact and ruptured AAA significantly in a national study covering 1993-2005.[38]

The described procedures for AAA repair can also be applied in the urgent setting of a ruptured AAA. Nevertheless, the vast majority is performed on intact AAA as a preventive procedure. Even in such a setting it is a procedure with high mortality and morbidity. The early method with the homograft had a mortality of >25%.[35] The current open AAA repair has a perioperative mortality of 4.8% and EVAR 1.2% in 45,000 matched patients of the US Medicare population.[39] Other prominent perioperative complications were higher after open repair: myocardial infarction (9.4% and 7.0%), pneumonia (17.4% and 9.3%), acute renal failure (10.9% and 5.9%) and need for dialysis (0.5% and 0.4%). In most analyses the strongest predictors of perioperative mortality are older age, renal disease, and heart failure.[39]

Aims of this thesis

Overall AAA disease is a rising healthcare problem, both because of the disease itself but also because of the (preventive) interventions. In this thesis we focused on strategies to decrease the burden after AAA repair.

1. Variance and variants in AAA

Much is still to be learned of AAA pathophysiology. There are shared factors with atherosclerotic disease, which might infer that one has a capacity to predict events of the other. This can become relevant for (post procedural) follow-up of these patients. We wanted to analyze whether all AAA are identical or that different variants of the same disease exist as different growth patterns were described, but no variation on tissue level was identified yet. Furthermore, virtually all current knowledge on pathophysiology is based on a specimen of the ventral AAA wall. We are curious whether that really represents the entire AAA, as it is a large structure which can exceed 17cm in diameter.

2. Improving outcome after repair

Besides patient- and AAA characteristics, many factors influence outcome after repair. There are multiple ways of gaining access to the common femoral artery, which is needed to perform an EVAR. We wanted to investigate if the least invasive method (percutaneous access) provides

a better outcome than the current standard in many centers (femoral cutdown access). Moreover, we wanted to analyze the effect of the treating physician on outcome. Physicians with different specialist backgrounds perform AAA interventions, and the relation with outcome and annual volume and experience for these different specialists remains unknown.

Outline of this thesis

We provide a historical overview of the advancing knowledge in cardiovascular disease in **Chapter 2**, starting with the presence of atherosclerotic lesions in pharaoh mummies of over 3500 years ago. Developments in knowledge are discussed leading to the present with a need for biobanks with vascular tissue. **Chapter 3** describes the rationale and design of the Aneurysm-express biobank, which provides the basis for the majority of the Chapters in this thesis.

Zooming in on AAA wall inflammation, **Chapter 4** investigates the predictive value of a protein called osteopontin on the occurrence of postoperative adverse events. **Chapter 5** focuses on osteoprotegerin and its role in AAA proteolysis and degradation. The effects of commonly prescribed statins on the vascular wall are investigated in **Chapter 6**. We compare different statins to patients who do not use statins to analyze differences in AAA inflammation.

Next, we analyze heterogeneity of aneurysm expressions. In **Chapter 7** we investigate variance among AAA to see if we could identify differences in clinical characteristics and outcomes based on the extent of in wall inflammation. Furthermore, because the AAA is such a large structure and rupture occurs at specific sites, we were curious to see if there is a variance inside the AAA, which is the focus of **Chapter 8**. Often regarded expressions of the same disease at a different location, in **Chapter 9** we compare AAA with popliteal aneurysms, which received recent media coverage when ex-US vice-president Dick Cheney was treated for this disease.[40]

Post procedural outcome is dependent on the choice of treatment, but also on the many choices on how to execute a specific procedure. We wanted to analyze the effects on outcome of using percutaneous access instead of femoral cutdown access for EVAR. **Chapter 10** addresses this question using the clinical database of the US National Surgery Quality Improvement Program, whereas **Chapter 11** focuses on the experience at a high-volume tertiary academic center.

Management of AAA evolves over time with advancing knowledge. Specialist type, level of experience and volume load likely influence the choice of treatment and outcome. We analyze the effect of the introduction of EVAR on these factors over time (2001-2009), using a US national database in **Chapter 12**. The management of small AAA is most challenging as the balance between risk of surgery and risk of AAA rupture is more delicate. Choosing treatment or a conservative approach can have a substantial impact on a patient's outcome. In **Chapter 13** we analyze what the determinants are for making that choice, what weights they have, and recommendations are subsequently made.

Chapter 14 concludes this thesis, providing a general discussion and summary of the findings of this thesis

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1

Historical overview of cardiovascular disease and current biobank concepts

2

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Summary

Multiple risk factors have been associated with progression of atherosclerosis. To identify the individual patient who is at risk for disruption of a vulnerable plaque, leading to a cardiovascular event remains a major challenge. Current screening methods, based on traditional risk factors, do not allow risk stratification on an individual level. The discovery of new biomarkers would aid in identifying specific patient groups at risk for adverse cardiovascular events due to atherosclerotic disease progression. The current definition of the vulnerable plaque, e.g. atheromatous inflammatory plaque with a thin fibrous cap, has been based on cross-sectional post mortem studies. The predictive value of these histological characteristics of the vulnerable plaque is likely to be low, because they are also frequently observed at multiple locations in symptomatic and asymptomatic patients.

The Athero-express study follows a new concept to search for the atherosclerotic patient who may suffer from adverse events. In this study, we investigate the predictive value of local plaque composition for adverse events in other vascular territories, regarding the plaque as a concentrated expression of this systemic disease. First results from this longitudinal biobank study show that the local plaque hides strong predictive value for cardiovascular events elsewhere in the vascular tree. Longitudinal biobank studies will facilitate the identification of novel local plaque markers. The search for the plaque protein signature that is predictive for adverse events might enable patient stratification that will allow individualized tailor made medicine and subsequently guide the choice for therapeutic interventions.

Introduction

Historical concepts of atherosclerosis

Morphological findings in Egyptian mummies at the beginning of the 20th century confirm the presence of atherosclerotic lesions in pharaoh mummies over 3500 years ago.[1, 2] Hippocrates (469-377 B.C.) linked symptoms found in living to findings in the dead and described sudden (cardiac) death.[3] In both cases conserved material was used for medical research and these studies can therefore be considered as the first examples of biobanking. Biobanks are well-organized resources of biological samples with associated clinical characteristics used for scientific investigation.[4] Over the years, collecting tissue has become more and more sophisticated, leading to rapidly increasing scientific results and insights in pathogenesis of disease since the middle of the 19th century. In the current review we will focus on biobanks that consist of atherosclerotic tissue specimens.

Biobanks of atherosclerotic plaques have brought, and subsequent detailed (immuno-) histochemical studies have resulted in, major steps in the understanding of the pathogenesis of atherosclerosis. Rudolph Virchow described cellular inflammatory changes in the atherosclerotic vessel walls.[5] It was the first in-depth study of the histological characteristics of the atherosclerotic lesion: the term 'endarteritis deformans' was introduced. Virchow represented the atheroma being a product of an inflammatory process within the intima and described that the fibrous thickening evolved as a consequence of a reactive fibrosis induced by proliferating connective tissue cells within the intima. Virchow maintained that mechanical

forces initiated the irritative stimulus and that the inflammation was part of a repair mechanism[5]. This concept of local intima injury as the initiating 'irritative' is currently still accepted and it has been extended to include other factors besides mechanical factors.

The importance of the cellular component in atherosclerotic disease was noticed via observations of large amounts of smooth muscle cells raising the possibility of a similar pathogenesis as observed with neoplasms.[6] As a result it was hypothesized in 1977 that human plaques are monoclonal of origin. To appreciate this hypothesis it is important to understand the inactive X-chromosome hypothesis. This concerns the view that both X-chromosomes are active in adult females, but only one is active in any cell. Furthermore each cell's offspring expresses the same active X-chromosome as its parent cell, except in early embryogenesis. This observation means that all females are mosaics composed of two phenotypically different X-chromosome linked cell types.[7, 8] An X-linked gene product, glucose-6-phosphate dehydrogenase, was measured in both female atherosclerotic plaques and female healthy arterial walls using electrophoresis. Females were selected on heterogeneity of the enzyme. Specimens were obtained approximately 20 hours after death. Results showed that 24 of 30 plaques were of 1 enzyme type, whereas only 2 of 59 healthy arterial wall specimens were of 1 enzyme type and accordingly 57 healthy specimens consisted of a mixture of 2 enzyme types.[9] This was interpreted as a confirmation of the proposed hypothesis that human plaques were monoclonal, and further research for specific mutagens (chemical, nutritional or mechanical) was expected to be necessary.

Russell Ross provided other explanations for these findings that were also translated from cancer research.[10] He pointed out that the observation of a single enzyme phenotype in a lesion does not necessarily imply a clonal origin; it could arise from several cells containing the same isozyme. Another possibility is that lesion development could be characterized by repeated cycles of cell death and growth, in which case repetitive sampling could lead to a single enzyme phenotype, despite multicellular origin. Furthermore, clonal selection with evolution toward single enzyme phenotypes conceivably occurs in some types of hyperplasia.[10]

Nevertheless, Ross underlined the importance of arterial smooth muscle cells in atherosclerosis.[11] He suggested a focal increase of smooth muscle cells as the earliest phase of lesion development, followed by the deposition of either intracellular or extracellular lipid. He hypothesized a sequence of events starting with increased cell proliferation, lipid deposition and synthesis of connective tissue matrix components ultimately leading to the formation of fibrous plaques, and that subsequent cell disintegration, calcification, and deposition of blood products eventually cause the formation of complicated lesions. He realized the difficulties of establishing any kind of sequence because of the impossibility of studying a single site in the arterial vasculature more than once.

Response to injury theorem

Ross was inspired by the response-to-injury hypothesis as proposed by Virchow. Evolution in research lead to the adjusted hypothesis of factors involved in atherosclerotic lesion formation such as hyperlipidemia, hormonal dysfunction, and the increased shear stress in hypertension that may 'injure' the endothelium and alter the nature of the endothelial barrier to the passage of blood constituents into the arterial wall.[10] Focal desquamation of the endothelium exposes the underlying subendothelial connective tissue to platelets and other elements in the

circulation. The massive infiltration of platelet factors, plasma lipoproteins and possibly other plasma constituents at these sites of injury leads to focal proliferation of arterial smooth muscle cells, to formation of large amounts of connective-tissue matrix by these cells and to deposition of lipids both within the cells and in their surrounding connective tissue matrix. Restoration of the endothelial barrier ultimately occurs and the lesions regress if both the injury and the tissue response to it are limited. However further proliferation of smooth muscle cells and accumulation of connective tissue and lipid occur if injury to the endothelium is continuous or repeated, suggesting a critical balance between re-endothelialization, cell proliferation and cell destruction and removal that determines size and evolution of the lesion. Ross suggested that risk factors might directly influence the endothelial balance. The concept of endothelial damage leading to smooth muscle cell proliferation was supported by pathological observations and he performed in vivo experiments earlier on. Arterial lesions identical in appearance to the atherosclerotic lesions seen in man were induced in nonhuman primates by removing the arterial endothelium with an intravascular balloon catheter.[11] This experiment was conducted in macaques and from 10 minutes to 6 months and after removal of the endothelium the arteries were examined by light and electron microscopy. During the first 24 hours platelet microthrombi were seen adherent to the exposed internal elastic lamina; 3 to 5 days after injury the endothelium had begun to regenerate, and within 1 week after injury medial smooth muscle cells were observed extending through fenestrae of the internal elastic lamina into the intima. By 14 days the endothelium had regenerated and the intima was thickened containing 5-10 layers of smooth muscle cells. Three months after injury the lesion contained as many as 15 layers of smooth muscle cells surrounded by collagen and immature elastic fibers. After 6 months the intima was much thinner and contained only one to two layers of smooth muscle cells.

Later on in the 1990s Russell Ross summarized the latest modification of the response-to-injury theorem.[12, 13] This hypothesis suggests that the numerous, different forms of insult to the endothelium and to the cells of the arterial wall begin with a chronic, inflammatory response, featuring peripheral blood monocytes and T-lymphocytes, which adhere to the endothelium and invade the artery wall. These are all features that could be observed in numerous pathological specimens. Once inside the arterial wall the monocytes become macrophages and express a series of genes, including genes for cytokines and growth regulatory molecules, and undergo replication. Some of the secreted factors may result in smooth muscle migration and proliferation in the intima.

Arterial remodeling

Another important concept based on systematic analyses of large numbers of atherosclerotic plaques originated from Seymour Glagov. His concept of compensatory enlargement of human atherosclerotic coronary arteries has been explored in 1986.[14] He studied 136 autopsy specimens of the left main coronary artery to examine the diameter of the lumen in relation to the plaque size. Results showed that before lumen stenoses are greater than 40 percent, the actual lumen area seemed to remain independent of the plaque area, reflecting the corresponding increase in arterial size. Only when more than 30 to 40 percent of the 'potential' lumen area, as defined by the internal elastic lumen area, is occupied by plaque a sharp decline in lumen area with an increasing percentage of stenosis became evident. During this period,

it appeared that arterial enlargement no longer kept pace with increases in plaque size. Arterial size also increased with increasing age and heart weight, but their contributions on arterial wall size turned out to be small in regression analysis. Although this remodeling and its preservation of the patency of the lumen seems beneficial, this process is associated with unstable phenomena as shown in 1998.[15] In our lab, we used 50 postmortem obtained femoral arteries to systemically analyze morphology and histology of atherosclerotic lesions. We demonstrated that immunohistologic markers for unstable plaques, being presence of inflammatory cells, lack of vascular smooth muscle cells and collagen, and a large percentage of atheroma in the plaque, are associated with a larger plaque area and a larger vessel area.[16] This suggests that arterial shrinkage (or failure of expansive remodeling) will accelerate chronic luminal narrowing, but reduces the risk of rupture and therefore acute luminal narrowing or occlusion. Interestingly, not small but rather large plaques, which may not produce significant luminal narrowing, appeared to be the vulnerable lesions that may develop rupture and subsequent thrombosis.

Atherothrombosis

Michael J Davies defined 3 stages of thrombus evolution due to plaque rupture; intraplaque thrombus, transitional/mural thrombus and occluding thrombus.[17, 18] He provided an overview of previous studies[19-22] that showed plaque vulnerability to be a function of increased numbers of macrophages, increased expression of tissue factor, reduced levels of smooth muscle cells, a lipid core that occupies a high proportion of overall plaque volume and a thin plaque cap. When all these factors coincide, the plaque is at high risk of disruption. Each of the parameters that determine vulnerability has a wide variation, and they are not directly linked.

The age of intracoronary thrombi was investigated by another group to further investigate the time interval between plaque disturbance and the onset of symptoms (of e.g. myocardial infarction).[23] This was conducted by aspirating the thrombi during angioplasty in patients with an acute myocardial infarction, immediately fixating the thrombus with formalin followed by histological assessments. Thrombi were classified into three categories: fresh (<1 day), lytic (1-5 days) and organized (>5 days). Based on analysis of 199 cases, the authors demonstrated 49% consisted of fresh thrombus, 35% of organized, 9% of lytic thrombus and 7% combined fresh and organized thrombus. Older thrombi are apparently present in 51% of patients with acute myocardial infarction, which might indicate that acute coronary occlusion is often the final stage in a series of successive thrombotic events that occurred in the preceding days or weeks.

Plaque erosion

Endothelial damaging has been shown by Davies, when using freshly treated explanted hearts from 6 heart transplant patients.[24] Dissected coronary arteries were viewed using electron microscopy, revealing normal endothelium in healthy segments and irregularly organized endothelium over atherosclerotic lesions with gaps and missing endothelial cells. At the site of these plaque erosions platelets were often present, suggesting a role in pathogenesis.[24]

Morphology of plaque-related vascular thrombosis has been described in 50 cases of sudden death due to coronary thrombosis by the group of Virmani.[25] Post-mortem angiography was used to select areas of >50% stenosis, which were fixated in formalin and cut for histological

analyses. They described two categories of coronary thrombosis: the first as a disruption of a fibrous cap over a lipid core with contact of the acute thrombus with the lipid pool, and a second showing direct contact between the intimal plaque and an acute thrombus via a superficial erosion without plaque rupture or contact with the lipid pool. They found that 22 out of 50 acutely thrombosed carotid artery plaques were superficial erosions. These plaque's luminal surfaces were irregular, eroded, and lacked endothelial cells. Besides, these eroded plaques were distinct from ruptures in that they were rich in SMC and proteoglycans, with relatively few inflammatory cells.[25] Relevance of this finding is the knowledge that fibrous atherosclerotic plaques (i.e. without any atheroma) and plaques with a thick fibrous cap overlying a necrotic core can still cause acute arterial thrombosis via plaque erosion.[25-27] To further evaluate the prevalence of erosions a large biobank containing the entire epicardial coronary tree and necropsy heart samples from 2304 patients with or without ischaemic heart disease was used.[26] After selecting patients that suffered from fatal myocardial infarction without using any coagulation influencing drugs, material from 298 patients remained for analysis. Results showed 25% of the acute thrombosis was due to a plaque erosion, whereas in 75% an underlying plaque rupture could be identified. Plaque erosion was more common in women compared to men (37.4% and 18.5%, respectively). An important limitation of these post-mortem studies is the sole inclusion of fatal myocardial infarction and sudden deaths. To study true prevalence, analysis should be extended to for instance non-fatal myocardial infarctions, but of course that would lead to practical difficulties. Another important factor might be the exclusion of patients using any coagulation influencing drugs, as these patients are expected to have an increased risk for developing cardiovascular disease.

The same group used a biobank of 113 formalin-fixated coronary arteries and frozen blood serum, of male patients after dying suddenly, to correlate coronary risk factors to plaque morphology.[28] Analysis of this material revealed that smoking was associated with acute thrombosis in multivariate analysis, regardless of the mechanism of plaque disruption (rupture or erosion) or the number of vulnerable plaques. Elevated serum cholesterol was associated with the rupture of vulnerable plaques. They also demonstrated a strong correlation between serum cholesterol and the number of vulnerable plaques, independent from other risk factors. They posit that cholesterol lowering may be particularly beneficial to patients with vulnerable plaques, and that anti-thrombotic therapy may be especially effective in cigarette smokers at risk for sudden death due to coronary events.[28]

Vulnerable plaque

Vulnerability of the plaque is a fascinating concept. Several mechanisms have been proposed to be part of the cascade leading to plaque rupture. Overviews of those findings have been published over the years together with histological classifications.[17, 29-31] The ruptured vulnerable plaque can cause local thrombosis and thereby unstable clinical syndromes like myocardial infarction or stroke in 60% of cases.[30, 32] Multiple definitions of the vulnerable plaque have been documented but three different atherosclerotic plaque phenotypes potentially leading to thrombotic arterial occlusion have been proposed. The typical, and most common, type of vulnerable plaque contains a plaque with a large lipid core, covered by a thin fibrous cap. This fibrous cap is prone to rupture, resulting in thrombus formation when the core is exposed to the blood.[33] A second type involves a plaque with a superficial endothelial erosion, resulting

in direct contact between blood and the thrombogenic sub-endothelial tissue, as described previously. A third type of vulnerable plaque encompasses a calcified nodule protruding into the lumen and is considered to induce thrombosis. Typical histological features of the vulnerable plaque are besides the large lipid core and thin fibrous cap, infiltration of inflammatory cells, particularly macrophages, reduced levels of smooth muscle cells, neovascularization with intra-plaque hemorrhage and outward remodeling.[20, 22, 32-36]

An original method to study the frequency of thin cap fibroatheroma and ruptured plaques was used by Cheruvu et al.[37] They harvested postmortem coronary arteries of 50 patients which were infused with green dye, enabling the dye to enter plaques with erosion or rupture. This process was followed by longitudinal sectioning and analyzed using histology and computer-aided morphometry to assess the amount of green plaques (erosions/ruptured plaques) and thin cap fibroatheroma. They found per heart a mean number of 0.38±0.70 ruptured plaques and 0.46±0.95 plaques with a thin cap fibroatheroma.

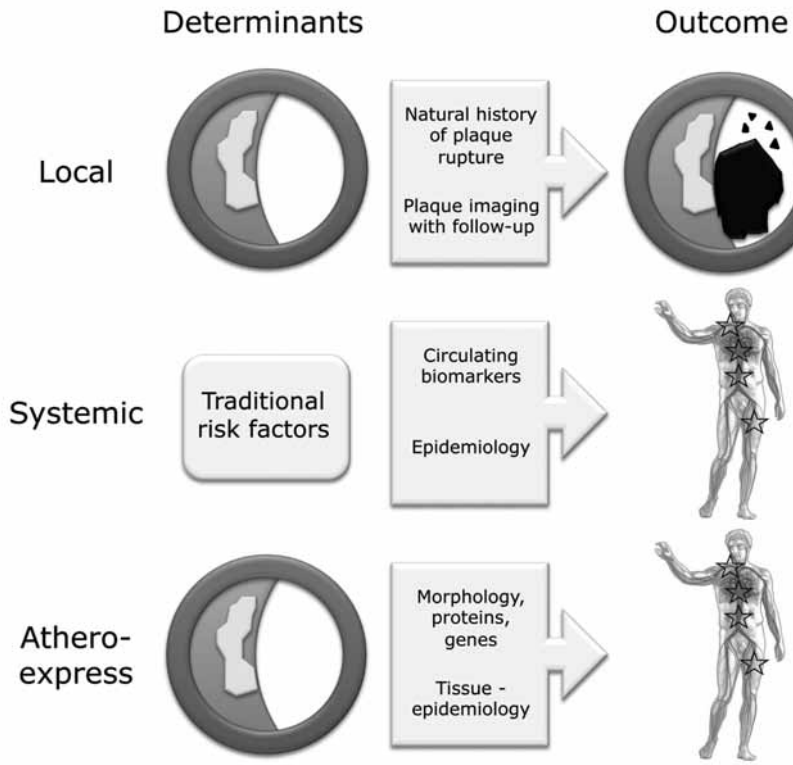

Figure 1 | Overview of study designs for identifying markers to predict future cardiovascular events
Traditionally, systemic risk factors are used to define the risk of cardiovascular events (systemic determinants to predict systemic outcome; epidemiology). An upcoming approach is the identification of vulnerable plaques with plaque imaging coupled to follow-up (local determinants for local outcome; natural history of plaque rupture). The Athero-express study follows a third and novel approach aiming at identification of local plaque characteristics (morphology, protein expression, genes) that are predictive of systemic outcome (local determinants for systemic outcome; tissue epidemiology)

Intraplaque thrombi

Another intriguing observation in pathology specimens has been reported recently, demonstrating that erythrocyte membranes could be a major constituent of lipid lakes in atherosclerotic plaques. Investigators used plaques from 39 patients with plexogenic pulmonary hypertension (Eisenmenger syndrome) to investigate the role of hypertension in plaque formation and from 28 patients with chronic thromboembolic pulmonary hypertension for investigating the corresponding role of thrombi.[38] Their results showed fibrous-rich plaques in plexogenic, and plaques with a glycophorin-rich core in thromboembolic pulmonary hypertension. Glycophorin A is an erythrocyte specific protein and thereby a role for erythrocytes in atheromatous lesion formation was suggested.[38, 39] As intraplaque hemorrhage is found to be common in advanced coronary atherosclerotic lesions, the role of the erythrocyte was subject of further assessment. In a study of the coronaries of 100 patients who died of a heart disease, the presence of erythrocyte membranes in plaques with and without recent hemorrhage was investigated.[40] Histological samples were stained using antibodies for hemosiderin and glycophorin A. Findings indicated previous hemorrhages in lesions with late cores and those prone to rupture. The degree of reactivity of glycophorin A and the level of iron accumulation corresponded to the size of the necrotic core, and the increase in these variables paralleled the increase in the density of macrophages, raising the possibility that the hemorrhage itself serves as an inflammatory stimulus. To test this hypothesis an experimental model of intramural hemorrhage was developed in rabbits. The injection of autologous erythrocytes into existing lesions produced plaques with crystalline cholesterol, lipid, iron, and foam cells, whereas control lesions had little free cholesterol and few macrophages. The finding that intramural hemorrhage in an experimental atherosclerotic lesion induces the formation of cholesterol crystals with the recruitment of macrophages supports the hypothesis that erythrocyte membranes in the necrotic core of human coronary lesions can cause an abrupt increase in the levels of free cholesterol, resulting in expansion of the necrotic core and the potential for the destabilization of plaque.

As neoangiogenesis is reported to be closely associated with plaque progression in the search for the origin of erythrocyte membrane accumulation[41], presence of extensive intraplaque angiogenesis itself inspired Sluimer et al[42] to investigate hypoxia in atherosclerosis. To do so they infused a hypoxia marker (pimonidazole) prior to carotid endarterectomy in 7 symptomatic patients. This hypoxia marker and protein expression of hypoxia-inducible transcription factors (HIF) appeared to be present in lesions, especially in macrophage rich centre areas. Hypoxia correlated with presence of a thrombus, angiogenesis and expression of CD68, HIF and VEGF.

Considering the amount of macrophages within atherosclerotic plaques, and their expected role in the different stages of plaque formation, Allard van der Wall described different subpopulations of macrophages within one plaque in 1992.[43] He used arterial material of the descending aorta and the coronary and carotid arteries, collected 2-9 hours after death. The 34 collected specimens were divided in 3 groups (intimal thickening, fatty streak and atheromatous plaques) and stained using immunohistochemistry. Variation was seen from one individual to another in the amount of macrophages, moreover also at different levels within the same vessel. The phenotypic expression of the macrophages, on the other hand, varied only in relation to the underlying lesion. He suggested that its phagocytic function is

accompanied by a shift in phenotypic expression. These changes occurred in parallel with an increase in both fat uptake and lysosomal activity of the macrophages. Such findings suggest a differentiation and maturation of the same cell lineage and could relate to inflammatory functions.

Limitations

Current evidence is largely based on cross-sectional and retrospective studies and prospective evidence is lacking. It is therefore unknown if the above mentioned (vulnerable) plaque characteristics hide positive predictive value to identify plaques that are prone to rupture. Autopsy studies provided insights in the prevalence of histological characteristics, such as increased macrophage infiltration, large lipid core and the presence of a thin fibrous cap, which have been associated with increased plaque vulnerability. In addition, these observational studies showed that the predictive value of the vulnerable plaque characteristics is probably minimal because lipid rich inflammatory plaques are also frequently observed in asymptomatic patients, and plaques lacking typical vulnerable histopathological characteristics are able to cause clinical events and plaque rupture itself is asymptomatic.[25, 44-48] These cross-sectional biobank studies are mainly descriptive and therefore do not allow inferences regarding causality.

Pathological studies have also been executed to examine the systemic distribution of vulnerable plaque characteristics. Postmortem studies revealed that the presence of inflammatory cells in the cap of the plaque does not seem associated with the presence of cap inflammation at another location in the arterial system. This observation indicates that plaque inflammation is not homogeneously distributed throughout the circulation, independent of its prevalence. On the other hand, postmortem studies demonstrate that the presence of a large lipid core in one artery is associated with its presence in a contralateral artery. In summary, the phenotype of the vulnerable plaque remains to be elucidated since prospective studies have not been performed.[49]

Current and novel concepts of atherosclerosis

The vulnerable patient

To identify individual subjects at risk for developing future adverse cardiovascular events, many other factors, besides local plaque characteristics, can be considered. For instance, diabetes mellitus, hypertension, smoking and hypercholesterolemia, have been established risk factors for a long time. Naghavi et al. [50] introduced the term "the vulnerable patient" who could be determined by different factors like: the vulnerable plaque, thrombogenic blood (vulnerable blood) and electrical instability of myocardium (vulnerable myocardium). According to Naghavi et al., plaques with similar characteristics may reveal different clinical presentations because of the properties of the vulnerable blood and/ or the vulnerable myocardium. Atherosclerosis is a multi-system chronic disease, therefore it is essential for accurate risk assessment to appreciate the total patient vulnerability and not just the characteristics of a single vulnerable plaque. The traditional risk assessment strategies have already shown to predict for long-term outcome of atherosclerotic disease. An integrated approach of defining vulnerable plaque

characteristics, traditional risk factors, systemic biomarkers and genetic profiling may facilitate personalized medicine and predict risk for the individual patient. Ideally, screening for the vulnerable patient should be inexpensive, non invasive, reproducible, applicable to an asymptomatic patient population and it should add predictive value to the measurements of the established risk factors.[50, 51]

Biomarkers

Current screening for patients who are at risk for adverse events due to progression of atherosclerotic disease is based on traditional risk factors. However, this approach does not allow specific risk stratification on an individual level to determine who will or will not suffer from a clinical event such as myocardial infarction or stroke.

One of the most considered approaches for risk assessment is executing tests on peripheral blood samples for the presence of a specific atherosclerosis marker. Because atherosclerosis is a systemic inflammatory disease, known inflammatory markers and acute phase reactants have been studied for this purpose. These reactants are mainly produced by hepatocytes, and the increased expression is driven by cytokines, which are produced by activated macrophages and other cells.[60] According to the United States National Institutes of Health a biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, or pharmacological responses to therapeutic intervention.[61] One of the most extensively studied serum biomarkers for cardiovascular disease is C-reactive protein (CRP), which is highly elevated in patients suffering from most forms of inflammation, infection or trauma. In the absence of these events it has a value as a biomarker for atherosclerosis, since the concentration was directly correlated to the presence and severity of coronary, cerebral and peripheral artery atherosclerosis.[62] CRP is a robust marker because of its analytical stability, reproducible results in clinical studies, and high sensitivity assays with good precision are commercially available.[63] It was shown that an increased production of CRP was common in patients with angina and turned out to be associated with an increased risk of myocardial infarction and sudden death.[64] CRP has been associated with traditional risk factors, such as smoking, obesity and serum triglycerides, but it demonstrated an independent predictive value for the occurrence of cardiovascular events. Treatment with statins, which are known to reduce plaque inflammation, leads to decreased levels of CRP.[65] Despite this, the predictive value of CRP as a biomarker for atherosclerosis is only moderate and less compared to traditional risk factors and clinical application is therefore not widely accepted.[66] In addition, many other inflammatory markers have been identified in relation to atherosclerosis. For instance circulating interleukin-6 levels predicts future coronary events in patients with acute coronary syndromes.[67, 68]

A good biomarker needs to be specific for disease development or progression, to have a high predictive value and should reflect successful treatment. Atherosclerosis is a multi-factorial disease and therefore one single biomarker will not be sufficient to reach these objectives. However, with the currently available circulating biomarkers, even the use of multiple biomarkers only adds moderate predictive value to the traditional cardiovascular risk factors. In the Framingham heart study, a cohort of 3209 patients was analyzed to evaluate if this multimarker approach could enhance risk stratification with currently available and previously reported individual biomarkers.[69] A combination of 10 biomarkers was assessed, including:

CRP, B-type natriuretic peptide, N-terminal pro-atrial natriuretic peptide, aldosterone, rennin, fibrinogen, D-dimers, plasminogen-activator inhibitor type 1, homocysteine, and the urinary albumin-to-creatinin ratio. After a follow-up of 7.4 years the hazard ratio for cardiovascular events was only 1.84 for the people in the highest quintile scores, compared to the two lowest quintiles. The C statistic, used to measure the ability to classify risk, increased only slightly not reaching significance (0.76 to 0.77). This paper has been criticized for the limited number of major events and for its definition of these major events, when including heart failure and coronary insufficiency.[70]

Another approach was proposed by investigators of a community-based cohort of 1135 elderly Swedish men [71], it was stated that current risk factors do not directly reflect myocardial cell damage, left ventricular dysfunction, renal failure and inflammation, all being clinical conditions associated with an increased risk on cardiovascular disease and death. They tested the added value to patients risk stratification of a combination of biomarkers involved in named pathophysiological processes, including troponin I, N-terminal pro-brain natriuretic peptide, cystatin C and high sensitivity CRP. The C statistic increased significantly (0.664 to 0.766) when these 4 biomarkers were added in a model containing established risk factors, thereby suggesting an improved risk assessment in the cohort of elderly Swedish men. The absence of validation of these findings in other (independent) patient groups makes it hard to generalize found results to other age groups, women and ethnic groups. Some concerns were raised concerning possible confounding illnesses in the enrolled elderly men, such as chronic liver disease, potentially raising the levels of the tested biomarkers.[72]

Given these results there is a pressing need for more specific and prognostic biomarkers to be added to the established risk factors to optimize risk prediction. Due to the lack of longitudinal studies, the local atherosclerotic plaque has not been considered as a source for biomarkers to predict future adverse cardiovascular events. Longitudinal studies (including imaging) will be necessary for the discovery of novel local plaque markers and circulating (cell) markers: the assembly of multiple plaque biomarkers may lead to the discovery of protein plaque signatures that will facilitate the identification of different patient groups. Until recently, atherosclerotic pathological biobanks have not been considered as a source for biomarkers in a longitudinal study design. The Athero-express study is the first atherosclerotic pathological biobank that will elucidate the positive and negative predictive power of local histological plaque characteristics and potential biomarkers, and determine whether plaque markers hide prognostic value for future events.

Athero-express

In two Dutch hospitals the Athero-express study started in 2002. This study is an ongoing prospective cohort study collecting both blood and endarterectomy specimens of the carotid and femoral artery. All cohort members will be followed for carotid restenosis for 2 years and the occurrence of adverse cardiovascular events for a minimum of 3 years. In January 2008 over one thousand plaques were included from patients who underwent a carotid endarterectomy. Main objective is to determine the predictive value of local morphological plaque characteristics and biomarkers as determinants for local restenosis or future cardiovascular events elsewhere in the body, such as myocardial infarction, stroke or peripheral intervention.[73] The concept is based on the fact that atherosclerosis is a systemic disease and

it is hypothesized that not just one single plaque but that all plaques in the vascular system share information about the stability of the atherosclerotic lesions irrespective of the vascular territory. In the Athero-express study dissected carotid plaques are stored and after a three-year follow up, cases and control patients are defined. Subsequently plaques from cases and controls are compared on a histological and protein level. Characteristics that demonstrate differential expression between cases and controls are validated in another cohort of patients that encompasses about 300 patients who underwent femoral endarterecomy with a 3 year follow up.

The first observations based upon the Athero-express biobank were cross-sectional studies and revealed differences in plaque phenotype from symptomatic patients compared to asymptomatic patients.[74] Symptomatic lesions demonstrated predominantly an atheromatous plaque phenotype in comparison with asymptomatic lesions which showed a more fibrous phenotype. Subsequent studies demonstrated that atherosclerotic lesions from women are associated with a more stable plaque phenotype, which might suggest that women may benefit less from a carotid endarterectomy.[75] In the Athero-express biobank also restenotic lesions have been harvested that had developed after previous endarterectomy procedures. Restenotic lesions that became hemodynamically significant after 5 years had an inflammatory plaque phenotype, characterized by macrophage infiltration and large lipid core size, comparable to the plaques of primary symptomatic lesions.[76]

Predicting local atherosclerotic disease progression

One of the objectives of the Athero-express was to examine whether local dissected plaques hide characteristics that could predict restenosis. If so, then these plaque features could serve as prognostic markers and serve as surrogate markers for future restenosis. Hellings et al. [77] analyzed the progression of restenosis after carotid endarterectomy as a function of immuno-histochemical plaque composition. Carotid plaques of patients undergoing primary carotid endarterectomy were collected and subjected to histological examination. Patients underwent duplex follow-up to assess patency of the target vessel at 1 year and clinical follow-up at 1 to 3 years after surgery. Results of 500 patients showed that vessels with previous stable fibrous plaques (low macrophage and lipid content) are more prone to develop restenosis after endarterectomy. In contrast, lipid and macrophage content were associated with lower rates of restenosis. This finding was somewhat unexpected, since it is generally assumed that progression of restenotic lesions is driven by inflammation.[13, 78] Early restenosis is mainly caused by smooth muscle cells and collagen deposits, which is different from late restenosis which resembles primary atherosclerosis.76 Inflammatory arteries may be protected from early restenosis due to increased expansive geometrical remodeling of the vessel.[77] This was the first time that it was demonstrated that locally dissected plaques still hide prognostic value for ongoing vascular occlusive disease.

Predicting systemic atherosclerotic disease progression

A concept that has gained much attention is the identification of the vulnerable patient as described above.[50, 51, 79] Each year, many patients suffer from their first cardiovascular event, such as a stroke or myocardial infarction. If it were possible to identify these patients before they become symptomatic, this might dramatically decrease the burden of cardiovascular

disease. For patients with manifest atherosclerotic disease, it is important to predict the chance of a second vascular event. Since secondary cardiovascular events can occur anywhere in the vascular tree, the research focus shifts from natural history and determinants of the local vulnerable atherosclerotic plaque to markers of systemic cardiovascular vulnerability.

In the search for new biomarkers that predict major adverse events, one of the approaches is to develop a serological test for molecules that are known to be present in unstable plaques or suggested to be involved in the mechanisms of plaque destabilization. High serum levels of these "plaque-markers" could provide a fingerprint of the vulnerable patient. This concept was supported by studies which discovered that IL-6 was increased in blood samples distally from the coronary plaque after percutaneous intervention of the coronary artery, suggesting secretion of IL-6 from the plaque.[80] However, the choice for these biomarkers should be considered with great care since they mostly originate from observational pathological studies. If a protein is co-expressed in ruptured lipid rich plaques, then it is unknown whether the protein is related with the cause or consequence (repair!) of plaque destabilization or an innocent aspecific bystander.

Still, major biomarker studies have been initiated and are ongoing based on expression profiles that are associated with the current concepts of the vulnerable plaque. The search for the set of biomarkers with a strong risk prediction is still ongoing.

Instead of searching for histological characteristics that are associated with plaque rupture or systemic biomarkers that are predictive for adverse systemic events, there is increasing evidence that local plaque characteristics could also be predictive for cardiovascular events elsewhere in the vascular tree. Several studies showed that the instability of the vascular wall is a systemic process rather than only local inflammation and that the molecular structure of the atherosclerotic vascular wall at one side could hold information about the stability of the whole system.[81-83] Thus, local atherosclerotic lesions, harvested with surgical endarterectomy, may hide predictive biomarkers for adverse systemic cardiovascular events in any vascular territory.

Identification of the vulnerable patient

Since sero- epidemiological research (e.g. systemic markers predictive for systemic outcome) has not yielded biomarkers that are strong enough for individual risk stratification, other approaches have been attempted. As described, there is large interest in prospective imaging trials to predict local plaque instability (local determinants for local outcome). In the Athero-express study, we follow a third and novel approach. We investigate the predictive value of the local plaque composition for systemic cardiovascular outcome, regarding the plaque composition as a concentrated expression of a systemic disease. We hypothesize that local plaques contain molecular information that is predictive for atherothrombotic events in other vascular territories.[55] In this case the local atherosclerotic plaque may act as a source to identify prognostic biomarkers for all adverse cardiovascular events. A nested case control study design has been applied in the first discovery phase. Proteomics studies have been executed to compare plaque proteins between 100 patients that had developed (multiple) adverse cardiovascular events during their follow-up and 100 controls without events during follow-up. Preliminary observations clearly show that local plaque proteins are a source for biomarkers with strong predictive value for future cardiovascular events in all vascular territories.[84] Lists

of potential markers have been discovered using this proteomics approach and these are now being validated in the whole cohort. In this protein biomarker discovery, the traditional concept of the vulnerable plaque is not taken into account. These proteins with biomarker properties are likely expressed in both stable and unstable plaques and can be considered as sensors for stability of the atherosclerotic process throughout the vascular system. This is not surprising as individual plaques may undergo stabilization and destabilization over time. The ideal biomarker is expressed in plaques that are active (inflammatory, unstable) and can become activated (stable plaques that can become destabilized). Further research should be focused at identifying the best independent protein markers in the plaque. Markers that are validated positively will undergo external validation in femoral or other plaques. The next step will then be to combine multiple markers into a plaque protein signature, in order to improve risk prediction and identify the vulnerable patient. The first results have stimulated the project group to set an ambitious threshold for risk prediction. We hypothesize that within 1 year we will be able to identify which 25% of the patients who undergo an endarterectomy have a 70% risk of suffering from a secondary adverse event within three years. On the other hand we will then also be able to identify which patients will suffer from a less then 5% risk for a secondary event. This will be a great leap forwards to identify the vulnerable patient. In addition, genomic analyses are being executed and bioinformatics will assist us to identify subgroups of patients who are at risk. The above mentioned genomic and proteomic approaches in atherosclerotic plaques, could introduce the discovery of a whole new set of prognostic biomarkers that will be specific for certain types of adverse cardiovascular events.

The predictive value of local plaque characteristics is currently limited to patients who undergo vascular surgery. To make the predictive value of the plaque characteristics available to more patients and make the step towards primary prevention, the targets identified in our study should be translated to markers, which can be measured in patients who do not undergo surgery. The first possibility is to measure the circulating levels of the protein signature identified in the plaque. Proteins from atherosclerotic carotid plaques can be secreted into the systemic circulation and measured in a peripheral blood sample.[80] Circulating levels might also be predictive for future cardiovascular events, but secretion of the specific protein by other tissues adds noise to the signal of plaque derived proteins. Nevertheless, when a strongly predictive signature can be obtained in the plaque, the circulating levels of these proteins may still possess good predictive value for future vascular events. Another option that will be considered is the identification of the plaque protein markers in circulating cells.

Future perspective

There are also other possibilities to determine the Athero-express biomarkers in patients for (primary) prevention. In theory, plaque proteins with predictive value could be imaged with contrast agents coupled to specific antibodies. Although not yet clinically applicable, developments in the nanochemistry of MRI contrast agents have brought molecular imaging closer,[85] In the context of the non-invasive detection of biomarkers, present possibilities include imaging agents that may be directed to atherosclerotic sites using specific targeting of cell surface receptors that are either expressed at the vasculature or inside plaques with

neovascularization.[86, 87] This is realized by conjugating an imaging probe with one or several targeting ligands. These ligands can potentially be chosen to target specific biomarkers. Current limitations of these techniques restrict its use to biomarkers that are being expressed in the cell membrane. Besides this, toxicity and clearance of the used products is an important issue for this field in research. For this, SPECT analysis might be a quicker route to the clinic as toxicity and clearance is less of a problem and quantification is easier. It is evident that a plaque biomarker that is detected in one or a few plaques and that hides strong predictive value for events in all vascular territories has an enormous potential since this would bring non invasive imaging (of the carotid artery) to predict risk of a coronary event much closer.
Other clinical applications for discovered predictive biomarkers are to serve as surrogate endpoints in clinical trials. The intima-media thickness is used as a surrogate endpoint in clinical trials investigating the effects of lipid lowering drugs. If plaque molecular imaging or plaque biopsies would be optional, then the local plaque biomarkers could serve as a strong surrogate marker for drug efficacy. Plaque biopsies could also be executed in non-stenotic segments, e.g. femoral artery when a coronary catheterization is executed. In United States about 4 million vascular invasive interventions are executed on an annual basis in which plaque biopsy could be considered.

Due to the massive amount of data gathering from pathological atherosclerotic biobanks (genomics, proteomics and forthcoming biomarkers) there is an increasing need for extending biobank analyses to multiple disciplines.[88] Systems biology coupling clinical, genetic and protein data is likely to be the next phase in the search for the vulnerable plaque and patient. The use of bio-informatics will lead us to a systems biology approach and will provide new possibilities to obtain an optimal predictive protein profile. This will require integration of skills in pathology, bioinformatics, molecular biology and genetics.

Conclusions

It is clinically important to identify the individual patient who is at risk for both local and systemic cardiovascular events. For this purpose identifying biomarkers and plaque characteristics that are predictive for events is crucial. Combining biomarkers from plaques in a longitudinal study might lead to the discovery of plaque signatures for different patient groups, and will be useful for clinical decision-making and therapeutic interventions. Prospective longitudinal biobank studies may fulfill a pivotal role in this first step towards personalized medicine.

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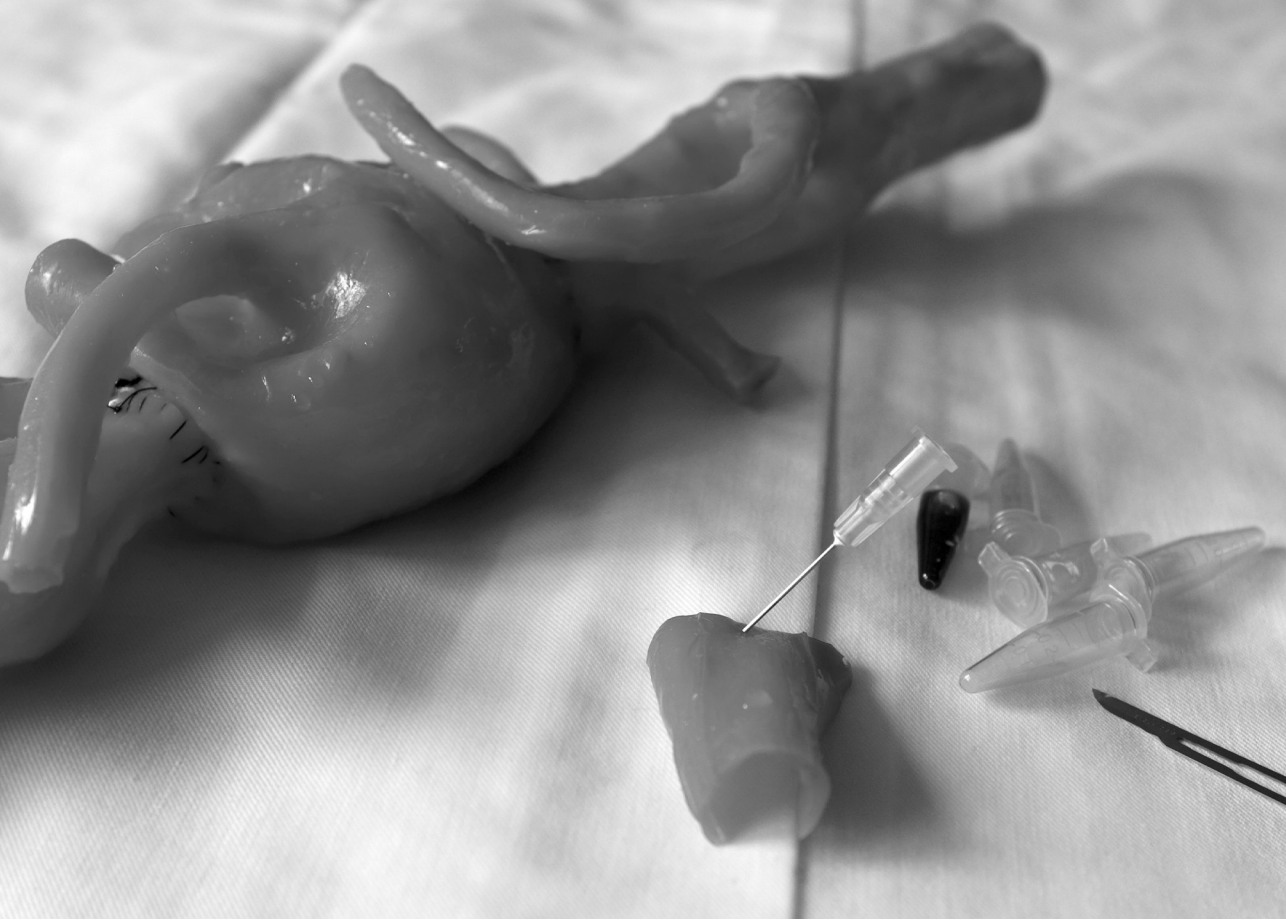

Aneurysm-express: human abdominal aortic aneurysm wall expression in relation to heterogeneity and vascular events. Rationale and design

3

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Abstract

Objective

Elective repair of Abdominal Aortic Aneurysms (AAA) is associated with significant morbidity and mortality. Large amounts of AAA tissue are necessary to assess heterogeneity among AAA, and to correct for potential confounders like known risk factors. The Aneurysm-express study aims to identify different types of AAA using inflammatory markers in the aneurysm wall that predict postoperative cardiovascular adverse events and mortality, therefore allowing individual risk assessment.

Methods

The Aneurysm-express is an ongoing prospective cohort study including AAA patients undergoing open repair. At baseline, blood is drawn, relevant clinical data are collected and the standard diagnostic modalities are performed. During surgery a specimen of the ventral AAA wall is collected and processed to study protein expressions and histology.

Interim results

The study commenced in 2003 in 2 medical centers and currently holds information and material of over 300 AAA patients, making it the largest reported aneurysm biobank. Patients are followed for 3 years after surgery for occurring cardiovascular events. The current mean follow-up is 2.1 ± 1.3 years with an event rate of 27%.

Conclusion

The large amounts of structurally stored tissue and blood combined with clinical characteristics and follow-up provide an excellent soil for in-depth pathophysiological analyses, with assessment of AAA heterogeneity in combination with postoperative clinical outcome.

Introduction

Abdominal Aortic Aneurysms (AAA) have gained increasing interest in the past decade (PUBMED search on December 15, 2009, using MeSH term "aortic aneurysm, abdominal" resulted in a total of 9,948 hits of which 6,621 in the last 10 years). The incidence of AAA is increasing in Western Countries with an aging population, reaching 5% of the older men who have smoked.[1, 2] In the USA ruptured AAA causes 4,500 deaths each year, with an additional 1,400 deaths resulting from the 45,000 annual preventive repair procedures.[3] This has caused vivid discussions about the need and benefit of AAA screening programs.[4, 5] Morbidity and mortality are significant after both endovascular repair and open repair of AAA, with 30-day mortality being 1.8% and 4.0%, respectively.[6, 7] Most complications are cardiac (4.2% and 7.2%, for endovascular and open repair, respectively) and respiratory (3.8% and 12.8%).[6] To predict adverse events following AAA repair, patients are screened for existing risk factors preoperatively. A variety of risk models has been tested in large patient cohorts, including (cardiovascular) preoperative morbidity and peri-operative variables. However, identification of high-risk patients yet remains unreliable using current scoring systems (e.g. Glasgow Aneurysm Score).[8] More accurate prediction is therefore essential to improve the postoperative outcome. Current studies mainly focus on peripheral blood markers, based on their role in AAA pathogenesis. These biomarkers, such as interleukin-6 (IL-6) and C-reactive protein (CRP), are used to seek associations with AAA presence.[9] The Aneurysm-express study aims to identify different types of AAA via markers in the aneurysm wall, predicting (postoperative) cardiovascular adverse events and mortality during follow-up, therefore allowing individual risk assessment.

Methods

Study Design

The Aneurysm-express study is a prospective cohort study, which is being executed in 2 medical centers in the Netherlands: the University Medical Center Utrecht in Utrecht and the St Antonius Hospital in Nieuwegein. Enrolment started in April 2003 and is ongoing. All patients in the cohort will be followed for adverse cardiovascular events and death for a minimum of 3 years.

All consecutive patients that are being referred to the vascular surgery department of both participating centers for open AAA repair are enrolled. Other open treated aneurysms are also included, such as aneurysms of the ascending aortic arch, descending aorta, iliac arteries, femoral arteries, popliteal arteries and carotid arteries. Both patients with asymptomatic or symptomatic aneurysms are eligible for inclusion, when scheduled for open repair. The indications for intervention were according to international established standards.[10] In short, AAA diameter exceeding 5.5 cm or expanding rapidly, AAA with symptoms attributable to the aneurysm, and AAA rupture are being treated. When endovascular treatment is not suitable, open surgery is performed and patients are enrolled. Patients with terminal malignancies are excluded from surgical repair and this study. This study has been approved by the medical ethics committees of the participating centers. When surgery is being scheduled, patients

receive oral and written information about the objectives of the study. All patients provide written informed consent and fill in an extensive questionnaire, based on the Rose cardiovascular questionnaire.[11]

Baseline

AAA diameter and morphology are assessed via computed tomography angiogram or magnetic resonance angiography. Blood is collected and the following fractions are stored: serum, ethylenediaminetetraacetic acid plasma, citrate plasma and erythrocytes. Preoperative laboratory tests include hemoglobin, hematocrit, creatinine, total cholesterol, high-density lipoproteins and triglycerides. Patients fill in a questionnaire before being admitted to the hospital, containing questions concerning medical history, including history of cardiovascular disease (aneurysmal disease and coronary-, cerebral- and peripheral artery disease), risk factors (smoking, family history of cardiovascular disease, hypertension, hyperlipidemia, diabetes mellitus, chronic obstructive pulmonary disease and alcohol consumption) and medication use (including dosage and start date). Height, weight, body mass index and blood pressure are determined in all patients.

Aneurysm tissue collection and processing

AAA open repair implies excluding the aneurysm from the circulation using a prosthetic graft, which is sutured in via the inlay technique. Adjacent to the aortic incision a part of the ventral AAA wall is excised at the site of the maximal diameter, which is immediately processed by dissecting the specimen in pieces of 0.5 cm, with the middle segment labeled 0 and the adjacent segments -1 and +1. Segments -1, +1 and all subsequent segments (-2, +2 and up) are snap frozen in liquid nitrogen and stored at -80°C in metal cups. Segment 0 is fixated in 4% formaldehyde and embedded in paraffin. Of this paraffin segment 4-µm-thick sections are cut on a microtome for histological analyses. In case of ruptured AAA a second aortic wall specimen is collected at the border of the rupture. The following stainings are routinely performed for AAA wall analysis: Hematoxilin & Eosin (overview), Elastic van Giesson (elastin), Picro Sirius red (collagen), α-smooth-muscle actin (smooth muscle cells), CD68 (macrophages), CD3 (T lymphocytes), CD20 (B lymphocytes), CD138 (plasma cells) and Von Willebrand factor (vasa vasorum). In addition, 10 blank cross-sections are cut and stored for future analyses. All stained sections are examined via light microscopy. All stainings are scored semi-quantitatively on a scale from 0 to 3 (0 being no staining, 1 minor, 2 moderate and 3 heavy staining). Structural wall components (elastin, collagen, vasa vasorum) and cells (smooth-muscle cells, macrophages, T lymphocytes, B lymphocytes and plasma cells) are scored on a 0 to 3 scale separately for intima, media and adventitia.

Of all -1 or +1 segments protein is isolated. The arterial segments are crushed in liquid nitrogen and in part isolated using Tripure Isolation Reagent (Boehringer Mannheim, Mannheim, Germany); another part is isolated via Tris (Roche, Basel, Switzerland). The samples are further filtered via a Spin-X filter column (0.22 µm Nylon pores), and the protein concentration is assessed utilizing the BCA Protein Assay Kit (Pierce Biotechnology, Rockford, USA). Both isolation methods, the filter step and the protein concentration measurements are performed according to the manufacturers' protocol. Total amounts of matrix metalloproteinase (MMP) 8 and 9 are determined using the Bio-Plex system employing Luminex multianalyte profiling

technology, as described and performed previously.[12, 13]
In this study, we assume that protein expression in segments -1 and +1 strongly correlates with expression in segment 0, and the obtained histological slides. This has been shown before in our laboratory.[14]

Follow-up
The patients routinely visit the hospital postoperatively, and cardiovascular follow-up data are collected via clinical records for occurring events and interventions per- and postoperative. In addition, a questionnaire is sent yearly. When the patient does not respond, the general practitioner is contacted for follow-up data. In the Netherlands, general practitioners have a central role in health care being appointed as coordinators of patient care and they keep detailed patient records.[15] Reported events are judged by an outcome event committee of 3 physicians, who are blinded to laboratory results. All occurring events are independently assessed by 2 members of the committee; in case of disagreement the third member is consulted.
Primary endpoints include all adverse cardiovascular events during follow-up: vascular death (death resulting from myocardial infarction, cerebrovascular accidents or sudden death), non-fatal myocardial infarction, non-fatal cerebrovascular and vascular intervention. In case of cardiovascular events or death, the referring hospital or general practitioner is contacted for detailed information and appropriate medical files.

Control group
For comparison purposes, 27 normal aortic specimens were collected by a pathologist during autopsy. Abdominal aortas exhibiting aneurysms or rupture were excluded. During autopsy, specimens of the infrarenal aortic wall were collected and processed identically to the description above.

Statistical analysis
Clinical characteristics with discrete variables were summarized as frequencies and percentages and normally distributed continuous variables as means and standard deviations, whereas non-normally distributed continuous variables were presented as medians and interquartile ranges. To compare continuous variables Mann-Whitney tests were performed. Probability values of $<.05$ were considered statistically significant. All analyses were performed using statistical package of the social sciences version 15 (SPSS Inc., Chicago, IL, USA).

Interim results

The first patient in this study was included in April 2003. By December 2009, 300 AAA were included and this number is expected to increase by 65 each year. The clinical characteristics of the first 300 patients are summarized in table 1. The distribution of AAA diameters is shown in figure 1. From the first 250 patients with an AAA and 27 normal aortic specimens, MMP8 and MMP9 levels (in arbitrary units) were available for analysis. The MMP8 levels were 18.9 (8.2 - 45.4) and 6.4 (3.9 - 15.1) ($P = .00048$), and the MMP9 levels were 93.2 (36.1 - 231.8) and

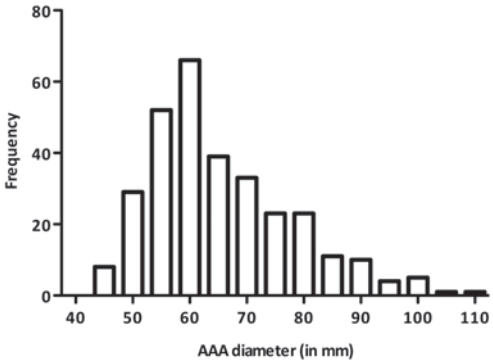

Figure 1 | Distribution of AAA diameters in the first 300 patients

Table 1 | Baseline characteristics of the first 300 AAA patients

Characteristic	
AAA diameter, mm	64 ± 14
Age, years	69.7 ± 7.5
Male gender	249 (83)
Current smoker	137 (46)
Hypercholesterolemia	189 (63)
Hypertension	216 (72)
Diabetes mellitus type 2	35 (12)
Body mass index, kg/m^2	26.1 ± 4.0
COPD	48 (16)
CAD	93 (31)
PAD	51 (17)
Previous reported aneurysm	36 (12)
Family history of aneurysm	30 (10)
Statin use	192 (64)
Postoperative follow-up, years	2.1 ± 1.3
Postoperative vascular events	81 (27)

Values are numbers of cases with percentages in parentheses or means ± standard deviations.
Abbreviations: COPD, chronic obstructive pulmonary disease; CAD, coronary artery disease; PAD, peripheral artery disease.

13.7 (5.5 - 51.5) (P =.00002), for AAA and normal aortas, respectively. Furthermore, 70 ascending aortic arch aneurysms, 30 popliteal aneurysms, 23 femoral aneurysms, 22 iliac aneurysms and 10 carotid aneurysms have been collected so far.

Discussion

Predicting outcome

Atherosclerotic biobank studies have already proven their value in predicting (secondary) local and systemic outcome.[16-19] Atherosclerosis is a systemic inflammatory disease and shares many factors with aneurysmal disease. Current screening for patients at risk for adverse events due to advancing atherosclerotic or aneurysmal disease is based on traditional risk factors. This approach does not allow assessment of risk on an individual basis to determine which patient will suffer from a cardiovascular event such as myocardial infarction. Studying peripheral blood samples for a specific marker has been most considered for risk prediction in patients suffering from atherosclerotic disease. The United States National Institute of Health defines a biomarker as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, or pharmacological responses to therapeutic intervention.[20] Known inflammatory markers and acute-phase reactants have been studied extensively for serving as biomarkers, but add only moderately as biomarkers for atherosclerosis and less compared to traditional risk factors.[18, 21, 22] The available circulating biomarkers in AAA have recently been reviewed9. Many studies focused on associating blood biomarkers with AAA presence, with choice of markers resulting from knowledge of inflammation in AAA pathogenesis. Circulating MMP9 has been studied most frequently; with initial research finding higher levels in patients with AAA as compared with healthy controls or atherosclerotic patients, whereas later studies could not confirm this finding.[23, 24] Reports regarding an association between serum levels of CRP and AAA presence are also conflicting.[25, 26] The most consistent marker associated with AAA is IL-6; showing increased levels in AAA tissue compared to normal aortic wall, increased plasma levels of IL-6 distal from the AAA suggesting IL-6 secretion from the aneurysm and a correlation of plasma IL-6 with aortic diameter in patients without aortic dilatation.[27-29] Furthermore, several small studies have shown increased IL-6 levels in the plasma of AAA patients as compared to atherosclerotic or healthy patients.[27, 30-32] Most biomarker studies in AAA contain only small sample numbers and show conflicting results. In addition, these studies focus on markers for AAA presence where reproducible determinants for AAA progression also remain to be elucidated.[9] Considering these results there is a pressing need for more specific and prognostic markers to be added to the established traditional risk factors. Atherosclerotic pathological biobanks are being used to determine positive and negative predictive power of local plaque characteristics and potential biomarkers, and determine predictive values of those plaque markers for future events. In this concept the plaque is regarded as a concentrated expression of a systemic disease, where a local plaque hides predictive information regarding cardiovascular events elsewhere in the vascular tree.[18] Several studies showed the instability of the vascular wall being a systemic process instead of only local inflammation and that a part of the atherosclerotic vascular wall at 1 site might hold information on the stability of the whole system.[33, 34] Local plaque markers have been identified to predict future cardiovascular events

elsewhere in the vasculature.[35] When an atherosclerotic plaque, only being composed of intima and media, hides predictive information it is likely that a sample of AAA wall, including all 3 layers of the artery, has even more predictive capacity especially since both vascular diseases have many shared factors.[36] To assess the predictive value of the AAA wall, large prospective tissue biobank studies are essential and currently lacking.

Differences among AAA

To improve the outcome of AAA treatment risk stratification is vital. Current risk stratification mainly focuses on determining risk factors for AAA development or general risk factors in AAA patients for rupture. Besides traditional risk factors, patients with increased white blood cell count and CRP have been identified for increased risk of AAA development; however, smoking, advanced age and male gender remain the most important determinants.[37, 38] Among AAA patients the diameter size and diameter increase are gold standards for the assessment of rupture risk, and therefore treatment cut-off.[10] While these screening parameters are widely accepted, markers for the identification of patients at risk for rupture or other cardiovascular events among AAA patients are lacking. Differences among AAA are likely to exist, as many large AAA remain stable for many years, whereas others tend to rupture at relatively small diameters.[39, 40] To investigate variations among AAA, aneurysm wall studies are required. Current research is hampered by low patient numbers. Studies are needed collecting aneurysm tissue from many patients as several risk factors (such as Diabetes) are likely to influence inflammatory processes on tissue level and therefore need to be corrected for. In addition, studies with low patient numbers lack discriminative power to distinguish different types of AAA. Unfortunately, collecting tissue is becoming increasingly difficult with the prominent place for endovascular treatment in AAA repair. The Aneurysm-express biobank study overcomes this limitation by already having included wall specimens of 300 AAA.

Aneurysm-express

The biobank currently holds information and material of more than 300 AAA patients, making it the largest reported aneurysm biobank. Histological assessment showed that the AAA wall composition varied substantially. Expression of factors, known to be involved in aneurysm development from animal studies, is currently being validated in a large number of AAA samples. When comparing total concentrations of MMP8 and 9 in the first 250 AAA to concentrations in 27 normal aortic specimens, both MMP8 and MMP9 were higher in AAA, consistent with previous reports.[41, 42] Due to the large sample size, the Aneurysm-express study provides the unique opportunity to correlate protein expressions to AAA phenotype and correct for potential confounders, such as clinical risk factors or medication use.

Validation studies in which newly discovered proteins have been examined in relation to AAA wall are mostly performed with low sample sizes, because of low availability of human AAA tissue, and therefore lack correction for confounders. AAA is a complex disease and both discovery and validation of new proteins should ideally be performed in a larger patient cohort, including all risk factors and different types of aneurysms. This study does not only allow cross-sectional research regarding histology and protein expression, but also facilitates studying wall composition in relation to cardiovascular outcome during long-term follow-up. Due to the

well-organized logistic structure in both participating centers the proportion of patients lost to follow-up will be minimal.
The Aneurysm-express study aims at identifying patient groups that have different postoperative cardiovascular outcomes, which will allow an individualized risk assessment (figure 2). Using this biobank study, we also aim at proving heterogeneity among patients with electively treated AAA, since current research does not distinguish between AAA variants.

Future perspective

The predictive value of local AAA wall characteristics is currently limited to patients undergoing open vascular surgery. To make the step towards primary prevention and thereby increasing the amount of patients eligible for this kind of risk stratification, the found biomarkers need to

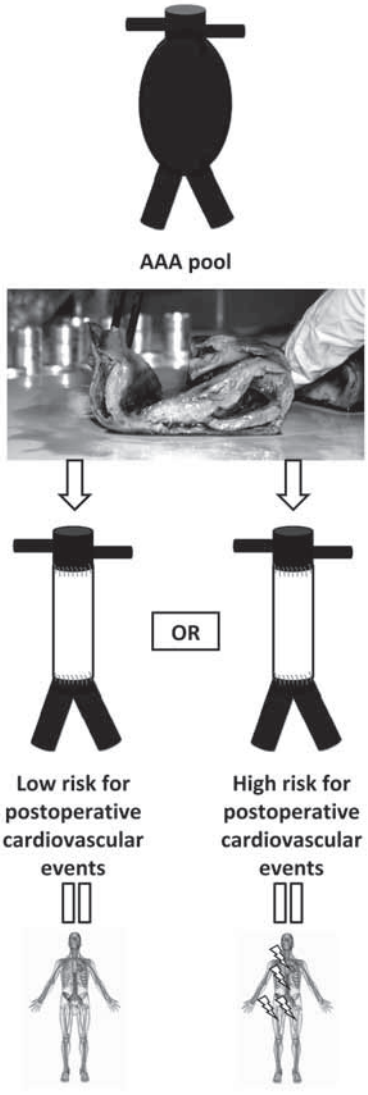

Figure 2 I Concept of the Aneurysm-express biobank; among the electively treated AAA, different types of AAA are expected to be identified. Instead of using traditional risk factors, this study is designed to distinguish between patients with high and patients with low risk for postoperative cardiovascular outcome.

be translated to markers available for measuring in patients who will not undergo vascular surgery, such as patients with small-diameter AAA. For instance, markers measurable in the peripheral circulation or detectable using imaging techniques would be appropriate candidates. Biomarkers in AAA form potential targets for medical treatment, can be used for detecting (small) aneurysms and, most importantly, can be a measure for AAA progression and outcome and may therefore have implications for future treatment.

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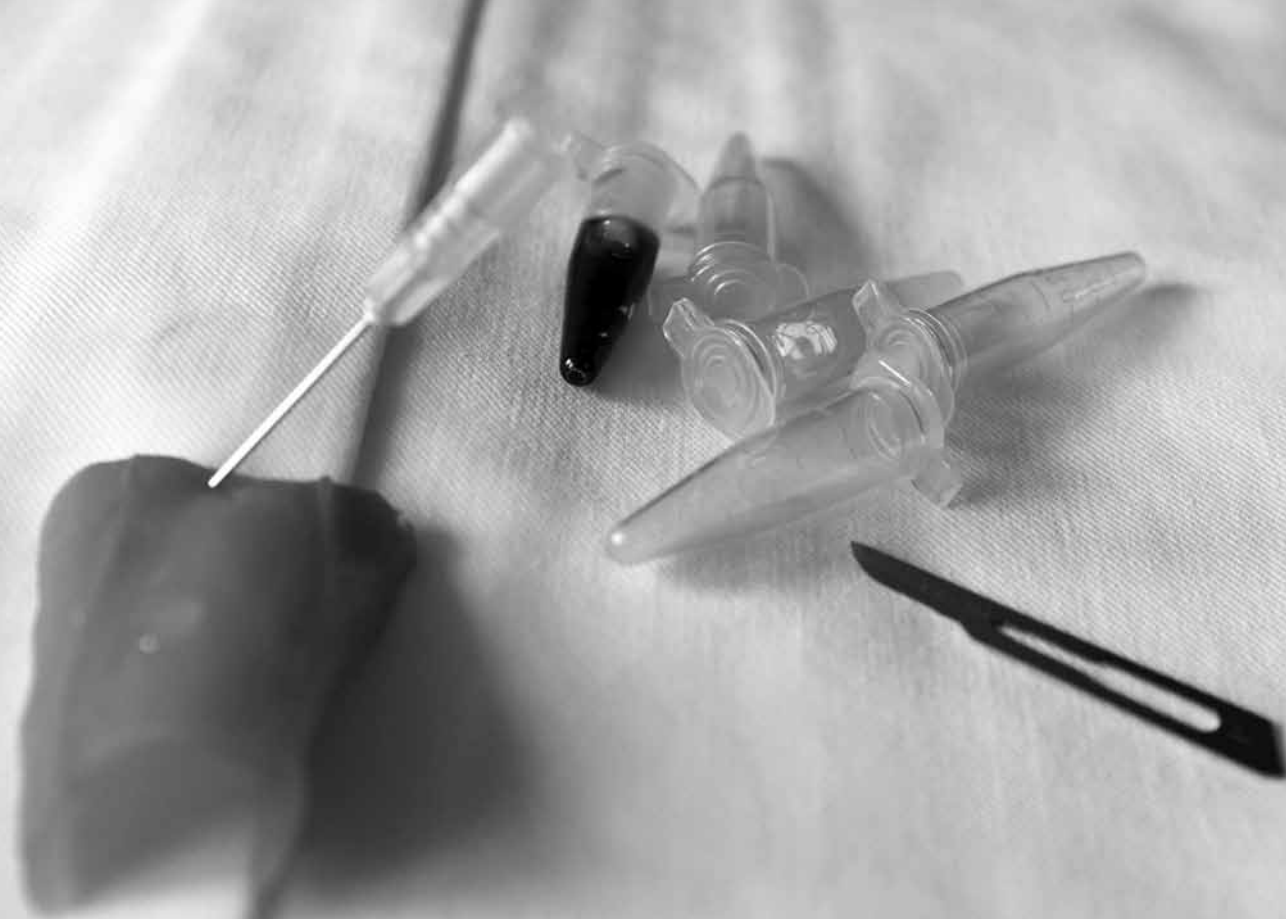

Osteopontin in the abdominal aortic aneurysm wall predicts adverse cardiovascular events

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Submitted

Abstract

Objective

Patients diagnosed with abdominal aortic aneurysm (AAA) are at increased risk of cardiovascular (CV) events. Circulating osteopontin (OPN) concentrations were associated with AAA presence and expansion. In addition, OPN in carotid plaques is predictive for secondary CV events. We hypothesized that OPN is present in AAA vessel walls, and predictive for postoperative CV events.

Methods and results

AAA biopsies were harvested from 219 patients. Patients underwent an annual follow up to 3 years (average 2.2 years). Primary endpoint was a composite of CV events and CV interventions. OPN was quantified in the AAA vessel wall and histological sections were stained for localization. Vessel wall OPN protein levels did not correlate with AAA diameter. High protein levels of OPN were predictive for CV events (HR 2.71 [1.30-5.62]), which was Independent of traditional risk factors. Subgroup analyses revealed that high OPN was also associated with major CV events (3.89 [1.53-9.91]) that mainly occurred shortly postoperatively as well as with minor CV events (2.70 [1.09-6.69]). Immunostaining showed clustering of OPN in calcifications and intimal macrophages.

Conclusions

OPN in the AAA wall is predictive for postoperative CV events. This observation supports the concept that local vascular markers hide predictive value for CV events in other vascular territories.

Introduction

Abdominal aortic aneurysm (AAA) disease often coexists with advanced atherosclerotic cardiovascular disease,[1] and systemic atherosclerosis is a defined risk factor for AAA formation.[2] Longitudinal studies observed an increased risk of cardiovascular (CV) events in AAA patients.[3-6] Also after AAA surgical repair an increased incidence of adverse CV events is observed, it was reported that 8.2% of patients suffered from an acute myocardial infarction (MI) after AAA repair in the US Medicare population.[3] Currently available risk scores underestimate the predicted event rate in vascular surgery patients. No risk indicators are known that may help to predict the higher risk that is evident for AAA patients.[7] Identification of patients at risk for CV events is needed to improve post-operative risk stratification.

Osteopontin (OPN) is an adhesive glycoprotein that is regulated through phosphorylation and glycosylation.[8-10] OPN was detected in many tissues, including vascular tissue, and regulates a variety of biological processes including inflammation, cell adhesion, angiogenesis and calcification. Cells described to express OPN included macrophages, T lymphocytes, smooth muscle cells and endothelial cells.[11] Animal studies showed that OPN had a role in altering arterial physiology, and promoting atherosclerosis.[12-14] In humans, extent of atherosclerosis in the abdominal aorta correlated with plasma OPN concentrations.[15] Circulating concentrations of OPN were found to be predictive for CV events.[16, 17] More recently a new concept was launched following the observation that atherosclerotic markers in local carotid plaques hide predictive value for adverse CV events in all vascular territories.[18-21] These observations support the view that characteristics of the local vascular wall reflect the stability of lesions throughout the circulation. Previously, OPN concentration from carotid and femoral plaques was found to be predictive for CV events during follow-up after carotid or femoral endarterectomy.[21] Thus far the search for local vascular components predictive for adverse events was limited to dissected atherosclerotic plaques. It is unknown whether the markers are also present in other vascular tissues and retain their predictive value. The aim of this study was to assess presence of OPN in AAA vessel walls and to determine whether these local OPN levels were predictive for CV events after AAA repair.

Methods

Aneurysm-Express biobank

The Aneurysm-Express study is a longitudinal biobank study that includes aneurysm tissue from patients undergoing open surgical AAA repair. The study design has been published previously.[22] The medical ethics committees of two Dutch hospitals approved the study and all participants provided written informed consent. For the current study we used ventral wall biopsies of intact AAA from consecutive elective patients who were included between April 2003 and January 2010. The indications to perform open AAA repair were based on current guidelines.[23] Open AAA repair was performed when endovascular aneurysm repair was not possible. Patients with terminal malignancies or severe dementia were excluded from this study. Clinical characteristics were prospectively collected via an extensive questionnaire based on the Rose cardiovascular survey,[24] and via patient clinical records.

Tissue processing

Biopsies from the ventral AAA wall were collected at the site of maximum diameter during open surgical repair. The tissue specimen was dissected into 5 mm segments. The middle segment was fixed in 4% formaldehyde, decalcified in ethylenediaminetetraacetic acid (EDTA), embedded in paraffin, and subjected to histological staining and analyses. To study the location of OPN in the vessel wall, a subset of 20 aneurysms was stained for OPN as follows. Sections were pre-treated in citrate buffer. A mouse anti-human OPN (IBL, Gunma, Japan; dilution 1 : 250) monoclonal antibody was used as decribed previously.[21] PowerVision Poly-AP-anti mouse IgG (Immunologic, Duiven, the Netherlands) and New-Fuchsin solution were used to visualize the signal. Sections were counterstained with hematoxylin. An isotype antibody was used as negative control. Adjacent segments were snap-frozen in liquid nitrogen, ground and dissolved in Tripure (Roche, Basel, Switzerland). Total protein concentration of every sample was quantified via a BCA protein measurement method (Pierce Biotechnology, Rockford, USA). OPN concentrations within AAA biopsies were quantified via an enzyme-linked immunosorbent assay (DOST00; R&D Systems, Minneapolis, USA).

Outcome

The primary outcome was a composite of CV events and interventions, which included vascular death (sudden death [unexpected death occurring within 1 hour after onset of symptoms, or within 24 hours given convincing circumstantial evidence] or death from stroke, myocardial infarction or congestive failure), non-fatal myocardial infarction (at least two of the following: Chest pain for at least 20 min, not disappearing after administration of nitrates; ST-elevation >1 mm in two following leads or a left bundle branch block on the ECG; CK elevation of at least two times the normal value of CK and a MB fraction >5% of total CK), non-fatal stroke (relevant clinical features, which have caused an increase in handicap of at least one grade on the modified Ranking scale, accompanied by a fresh infarction or hemorrhage on a repeat CT scan) and vascular interventions (percutaneous coronary intervention, coronary artery bypass grafting, peripheral artery interventions). In order to distinguish major from minor events, two secondary outcome measures were defined. Major CV events included (non-) fatal myocardial infarction, (non-) fatal stroke, and sudden cardiac death. Minor CV events were percutaneous coronary intervention, coronary artery bypass grafting and peripheral artery interventions.
Follow-up information that reflects the occurrence of postoperative CV events was collected via routine clinical visits and annual postal questionnaires. The referring hospital or the general practitioner was contacted when an event occurred for the collection of clinical data. Clinical records, electrocardiograms, laboratory tests and reports of scans or interventions were used for validation. Two members of an outcome committee (FM, JPdV, DK, and RH) validated reported CV events. In case of disagreement in judgment, a third member was consulted.

Statistical analysis

Discrete variables were reported as numbers and percentages, and continuous variables as median and interquartile range [IQR]. Comparisons were performed by χ^2 tests and Mann–Whitney *U* tests, where appropriate. Kaplan-Meier survival analysis was used to construct the event rate curves for an adverse outcome. Single and multivariable Cox regression analyses were executed to identify predictors of CV events. Traditional risk factor for atherosclerosis

(age, gender, hypertension, smoking and diabetes) were included in the multivariable analyses as well as variables with a univariable P value <.100. If a patient had multiple CV events during follow-up, the first CV event was included in the analysis. A cut-off for OPN was chosen based on the receiver-operating characteristic (ROC) curve and was used for all analyses. All tests were 2-sided, with P <.050 considered statistically significant. Statistical analyses were performed with PASW 18.0 software (SPSS Inc., Chicago IL, USA).

Results

A total of 219 AAA biopsies from patients were collected during surgery. Most patients were males (84%). The majority of patients were using statins (69%) or aspirin (68%). Additional baseline characteristics are listed in Table 1. OPN levels were skewed to the left, and therefore not normally distributed. OPN levels had a weak correlation with age (R =0.145 P =.032). OPN was higher in patients with PAD (6.17 [2.94-12.88] vs. 2.45 [0.91-9.51] ng/ml, P =.013). None of the other baseline characteristics had a relation with OPN levels. OPN in AAA biopsies did not correlate with AAA diameter (R =0.017, P =.808).

OPN in AAA biopsies in relation to events during follow up

The median duration of follow-up was 2.2 years (range, 1.1-3.0 years), with 5.9% lost to follow-up after hospitalization. We observed 46 primary outcome events. Occurring major CV events included 13 (non-) fatal MI, 8 non-fatal stroke, 5 vascular deaths, and 1 limp amputation.

Table 1 | Baseline characteristics.

Patient characteristics	AAA (n=219)	No events (n=174)	Events (n=45)	P-value
Age, y	70 [65-75]	70 [64-75]	71 [68-74]	.169
Male sex	187 (85)	150 (84)	37 (82)	.781
Current smoker	103 (47)	84 (47)	21 (47)	.894
Diabetes type 2	30 (14)	21 (12)	8 (18)	.374
Hypertension	168 (77)	129 (72)	39 (87)	.036
Coronary artery disease	91 (42)	66 (37)	23 (51)	.112
COPD	35 (16)	29 (16)	7 (16)	.936
Body mass index, kg/m2	25.6 [23.6-28.5]	25.3 [23.4-27.8]	27.7 [25.2-29.4]	.011
Serum creatinine, µmol/l	93 [82-111]	90 [82-107]	108 [85-122]	.009
Aneurysm diameter, mm	60 [55-68]	59 [54-66]	62 [56-70]	.097
Statin use	155 (71)	122 (68)	32 (71)	.665
Aspirin use	153 (70)	124 (69)	30 (67)	.834
ACEI use	74 (34)	55 (31)	21 (47)	.063
ARB use	45 (21)	32 (18)	11 (24)	.378

Data are presented as No. (%) or as median [interquartile range].
Abbreviations: AAA, abdominal aortic aneurysm; COPD, Chronic obstructive pulmonary disease; ACEI, Angiotensin-converting enzyme inhibitor; ARB, Angiotensin II receptor blocker.

Minor CV events were 21 peripheral vascular interventions, 4 coronary artery bypass grafting, and 2 percutaneous coronary interventions. For the analysis of the predictive value of OPN, a ROC-curve was used to determine the cut-off of 4.82 ng/ml, which resulted in the distinction of groups of AAA patients with high and low OPN (N=96 and N=123, respectively). High OPN in AAA biopsies were strongly related to primary outcome (Figure 1A), also after adjustment for traditional risk factors and potential confounders (HR 2.71 [1.30-5.62]; Table 2). High OPN was furthermore related to secondary outcome. Figure 1B illustrates the event rate curve for adverse major CV events, where high OPN was predictive after multivariable correction (HR 3.89 [1.53-9.91]; Table 3). OPN protein levels in AAA tissues were strongly related with the occurrence of major CV events within 30 days: 12 (12.5%) patients had a major CV event in the high OPN group, only 3 (2.4%) in the low OPN group (P =.003). MI was the most frequently registered event 30 days postoperatively (n=9 in high OPN group). OPN was also predictive for minor CV events (HR 2.70 [1.09-6.69]; Table 4). Figure 1C shows the time to event curve for minor CV events.

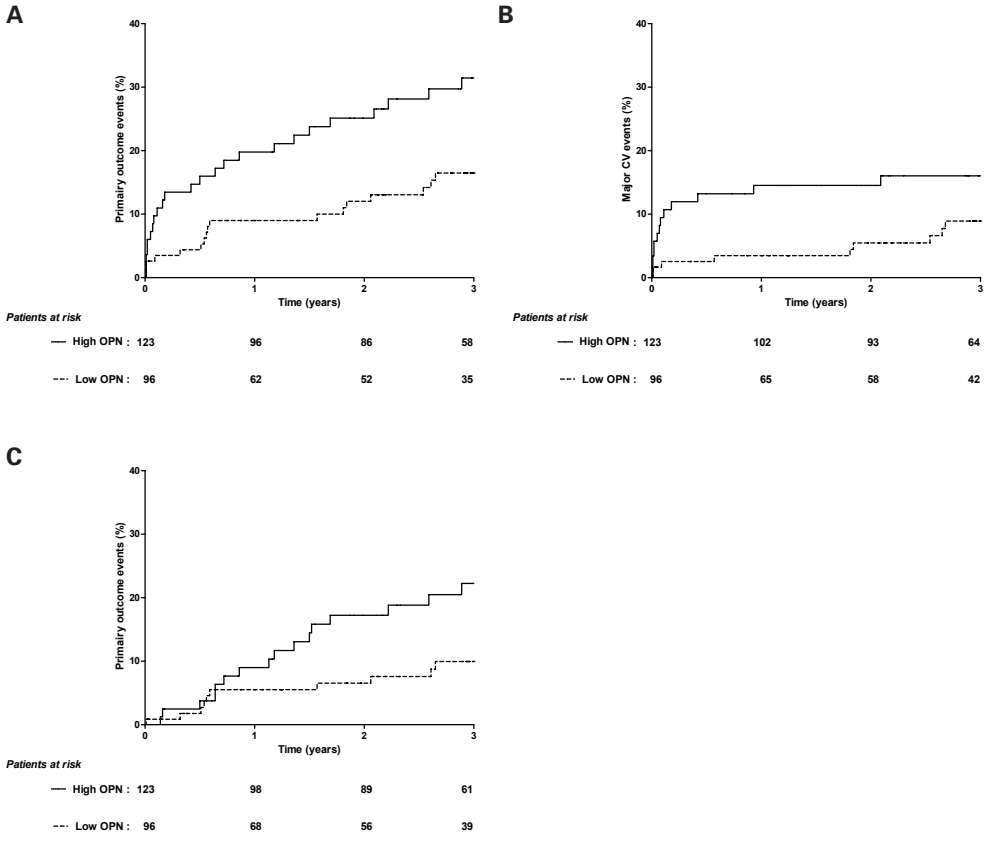

Figure 1 | Event rate curves for postoperative follow-up. A, primary outcome measure. B, major cardiovascular outcome. C, minor cardiovascular outcome.

Table 2 | Relation between characteristics and risk for primary outcome within 3 years.

Variables	Univariable HR [95% CI]	P-value	Multivariable HR [95% CI]	P-value
Age ≥ 70	1.37 [0.77-2.45]	.287	1.17 [0.57-2.44]	.669
Male sex	0.76 [0.36-1.63]	.482	0.40 [0.16-1.03]	.058
Current smoker	1.07 [0.59-1.93]	.830	1.06 [0.50-2.27]	.869
Diabetes type 2	1.80 [0.84-3.86]	.131	2.56 [0.96-6.82]	.060
Hypertension	2.36 [1.00-5.58]	.049	2.26 [0.73-6.98]	.156
Coronary artery disease	1.55 [0.87-2.77]	.136		
COPD	1.03 [0.46-2.30]	.945		
BMI ≥ 25	2.85 [1.24-6.54]	.014	2.38 [0.98-5.77]	.056
Serum creatinine*	2.19 [1.18-4.06]	.013	2.29 [1.01-5.16]	.046
Aneurysm diameter*	1.94 [1.03-3.65]	.039	1.57 [0.71-3.47]	.264
Statin use	1.19 [0.63-2.26]	.594		
Aspirin use	1.01 [0.55-1.88]	.969		
ACEI use	1.91 [1.07-3.42]	.028	1.39 [0.65-2.97]	.397
ARB use	1.38 [0.70-2.72]	.352		
OPN	2.34 [1.29-4.23]	.005	2.71 [1.30-5.62]	.008

*≥ median vs. < median
Abbreviations: AAA, abdominal aortic aneurysm; COPD, Chronic obstructive pulmonary disease; BMI, body mass index; ACEI, Angiotensin-converting enzyme inhibitor; ARB, Angiotensin II receptor blocker; OPN, osteopontin.

Table 3 | Relation between characteristics and risk for major CV events within 3 years.

Variables	Univariable HR [95% CI]	P-value	Multivariable HR [95% CI]	P-value
Age ≥ 70	1.96 [0.90-4.29]	.091	1.58 [0.65-3.84]	.315
Male sex	1.01 [0.35-2.91]	.991	0.61 [0.19-1.93]	.401
Current smoker	1.88 [0.85-4.20]	.121	1.73 [0.74-4.04]	.205
Diabetes type 2	2.71 [1.14-6.41]	.024	2.55 [0.92-7.08]	.073
Hypertension	4.27 [1.01-18.01]	.048	5.71 [0.74-44.06]	.095
Coronary artery disease	1.41 [0.66-3.00]	.373		
COPD	1.63 [0.66-4.04]	.293		
BMI ≥ 25	1.94 [0.70-5.39]	.204		
Serum creatinine*	4.65 [1.75-12.33]	.002	4.61 [1.55-13.74]	.006
Aneurysm diameter*	2.38 [1.00-5.67]	.050	1.77 [0.70-4.47]	.227
Statin use	0.77 [0.35-1.69]	.518		
Aspirin use	1.07 [0.48-2.38]	.873		
ACEI use	2.35 [1.11-5.02]	.026	1.70 [0.71-4.09]	.234
ARB use	1.24 [0.50-3.07]	.644		
OPN	2.48 [1.14-5.43]	.023	3.89 [1.53-9.91]	.004

*≥ median vs. < median
Abbreviations: AAA, abdominal aortic aneurysm; COPD, Chronic obstructive pulmonary disease; BMI, body mass index; ACEI, Angiotensin-converting enzyme inhibitor; ARB, Angiotensin II receptor blocker; OPN, osteopontin.

Table 4 I Relation between characteristics and risk for minor CV events within 3 years.

Variables	**Univariable** HR [95% CI]	**P-value**	**Multivariable** HR [95% CI]	**P-value**
Age ≥ 70	1.06 [0.50-2.26]	.874	0.81 [0.33-1.98]	.646
Male sex	0.58 [0.23-1.43]	.234	0.65 [0.22-1.94]	.440
Current smoker	0.45 [0.19-1.06]	.068	0.44 [0.14-1.37]	.157
Diabetes type 2	1.39 [0.48-4.03]	.542	1.22 [0.33-4.57]	.769
Hypertension	1.93 [0.67-5.57]	.226	1.17 [0.31-4.38]	.822
Coronary artery disease	1.66 [0.78-3.53]	.190		
COPD	0.75 [0.27-2.50]	.642		
BMI ≥ 25	3.05 [1.03-9.06]	.045	2.66 [0.85-8.34]	.094
Serum creatinine*	1.64 [0.74-3.62]	.220		
Aneurysm diameter*	1.25 [0.58-2.69]	.569		
Statin use	3.77 [1.14-12.53]	.030	2.31 [0.65-8,26]	.195
Aspirin use	0.93 [0.41-2.12]	.854		
ACEI use	2.07 [0.97-4.40]	.060	2.18 [0.86-5.53]	.100
ARB use	1.28 [0.52-3.17]	.594		
OPN*	2.47 [1.13-5.39]	.023	2.70 [1.09-6.69]	.032

*≥ median vs. < median
Abbreviations: AAA, abdominal aortic aneurysm; COPD, Chronic obstructive pulmonary disease; BMI, body mass index; ACEI, Angiotensin-converting enzyme inhibitor; ARB, Angiotensin II receptor blocker; OPN, osteopontin.

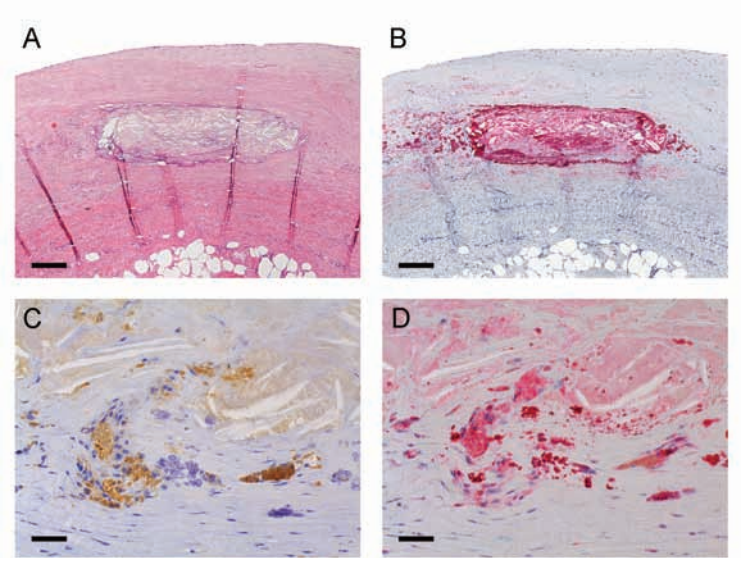

Figure 2 I Osteopontin (OPN) localization in abdominal aortic aneurysm (AAA). A, Hematoxylin and eosin staining showing atheroma in an atherosclerotic plaque. At the bottom of the picture adipocytes from the peri-adventitial fat are present. Bar = 200 µm. B, OPN immunostaining in red of consecutive section showing OPN at the boundary of the calcificated area. Bar = 200 µm. C, CD68 staining showing macrophages in brown at the border of an atheroma in an atherosclerotic plaque of an AAA. Bar = 20 µm. D, OPN immunostaining in red of consecutive section in which the macrophages and macrophage foam cells show immunoreactivity. Bar = 20 µm.

Immunohistochemistry
OPN was predominantly detected in the atherosclerotic plaque, whereas the media and adventitia did not reveal OPN immunostaining. Extra cellular staining of OPN was observed at the boundary of calcificated areas. In addition, immunostaining of OPN was detected in a subset of macrophages and macrophage foam cells (figure 2).

Discussion

The current study indicates that high OPN concentrations from AAA biopsies independently reveal predictive value for the occurrence of CV events in other vascular territories. The predictive value of OPN applies to both major and minor CV events. Previously, our group showed that high concentrations of OPN from carotid and femoral plaques were predictive for new CV events.[21] The present study demonstrates that this concept can be extended to the AAA wall.

Survival analyses revealed an independent relation of OPN with primary outcome. To further distinguish the prediction in types of events we also analyzed major and minor events. OPN was an independent prognostic factor for major CV events, which mainly occurred shortly postoperatively and MI was the most prominent event in this group. This is relevant for post-operative risk assessment and can therefore impact clinical decision-making. Even long after AAA repair, these patients remain at increased risk of CV events.[5] Interestingly, OPN predicted minor CV events that occurred late in the follow-up. Mainly peripheral interventions took place in the group with high OPN levels. The responsible mechanism for these findings needs to be clarified, and the results themselves need to be reproduced in a different cohort of patients.
OPN is involved in a variety of biological processes, including inflammation, cell migration, angiogenesis and calcification.[25, 26] Immunohistochemical staining showed staining of OPN in a subset of macrophages, smooth muscle cells, endothelial cells from neovessels, and clustering of OPN around calcification deposits. Mouse studies demonstrated evidence for a causal role of OPN in the development of atherosclerotic plaques.[12, 13, 27, 28] Circulating OPN was described to be associated with the presence of atherosclerosis (both coronary and aortic) and CV events.[15-17, 21] The present study demonstrated higher concentrations of OPN in AAA patients that experienced a CV event during follow-up.
OPN levels in blood have been associated with future CV events in a cohort of patients with chronic stable angina.[16] Our lab previously reported that OPN in blood had only half of the predictive capacity compared with OPN in plaques. In addition, OPN plaque levels had a weak correlation with blood levels.[21] Both indicative of a more prominent role of the vessel wall in the mechanism in which OPN is related to CV outcome.
We previously reported circumferential heterogeneity in the AAA wall.[29] We collected specimens of the AAA wall covering the entire circumference of the aneurysm at the site of maximum diameter. Already in 25 patients we found pronounced differences between ventral, dorsal and the lateral sides of the AAA. Inflammation, protease activity and microvessels were more pronounced laterally. OPN was also measured and had higher lateral expressions, when compared to the ventral wall, which was consistent with other measured inflammatory

markers.[29] This indicates that the predictive value might be different in the lateral AAA wall, however, current study shows in a larger cohort that the easily accessible ventral wall can already be predictive of CV events. It also underlines that large patient numbers are necessary for AAA tissue research to compensate for regional variance.
At this point, with the exact role of OPN in AAA pathogenesis still remains to be elucidated, it is unknown whether OPN is an innocent bystander or an active promotor of the disease. We have shown that it is present in the AAA wall. Given the pro-inflammatory properties of OPN, and the importance of inflammation in AAA development and progression[30, 31], it could infer that OPN has detrimental effects on the AAA. When this is further unrevealed, OPN might be an interesting therapeutic target. Administration of atorvastatin or olmesartan with or without pravastatin was reported to decrease plasma OPN concentrations.[32, 33]
The present study suffers from some limitations. As with other AAA tissue studies, the aneurysm wall can only be collected at one point in time and at a late stage of the disease when AAA size warrants repair. Because of this, temporal changes cannot be taken into account. Targeted imaging for OPN could potentially overcome this.

Prognostic biomarkers could improve risk stratification by identifying AAA patients who are at risk for CV events. High OPN in atherosclerotic plaques was previously reported to predict future CV events elsewhere in the vascular system. In the current study, we show that OPN levels in AAA walls also predict postoperative atherosclerotic events, including major CV events in the period shortly post AAA repair, and can therefore become a valuable addition in the identification of the patients at risk. These patients likely benefit from increased surveillance and more aggressive treatment of their concurrent atherosclerotic disease. Moreover, it might be beneficial for these patients to postpone AAA repair, as the balance between risk of surgery and the risk for rupture will be different.

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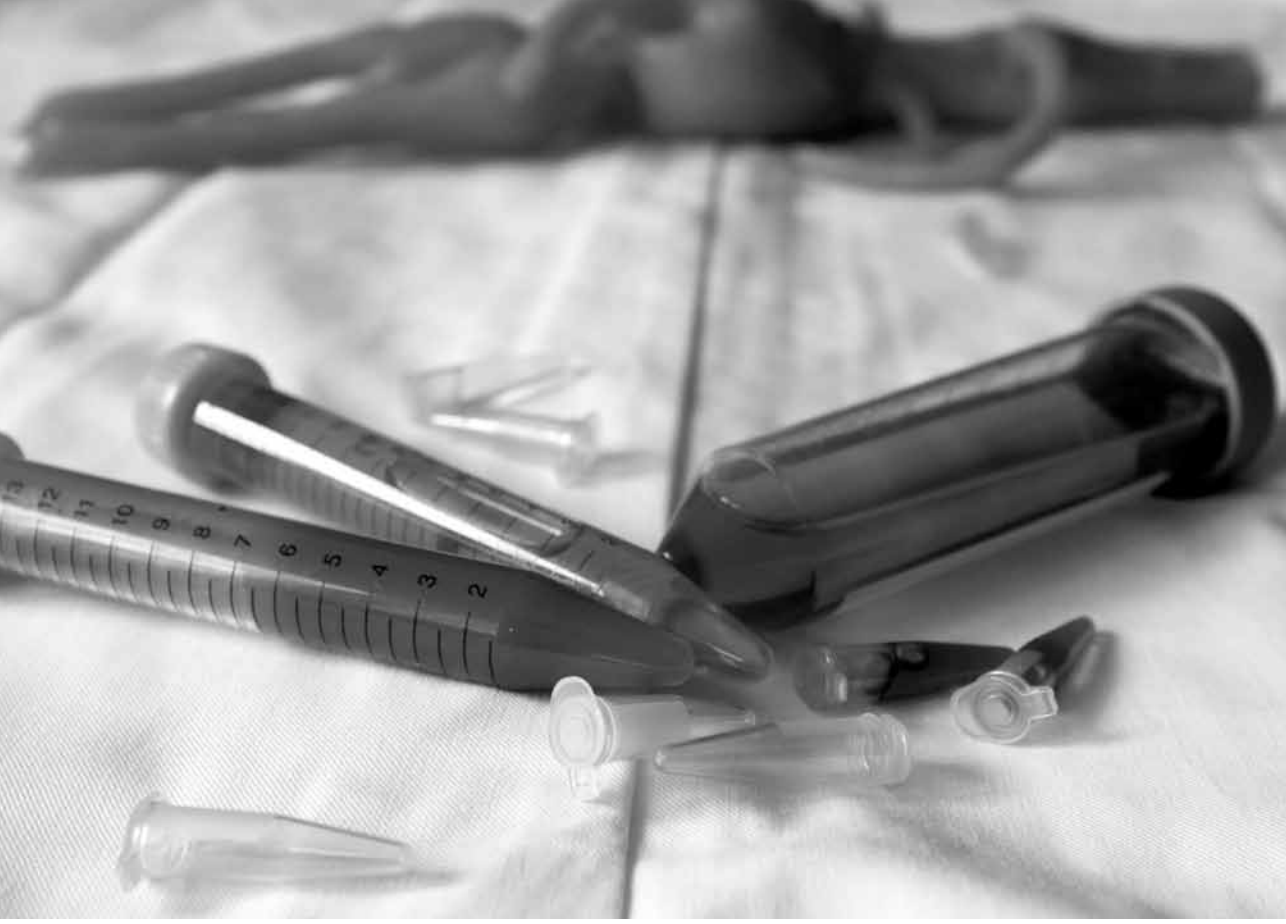

Osteoprotegerin is associated with aneurysm diameter and proteolysis in abdominal aortic aneurysm disease

5

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Submitted

Abstract

Objective

Serum osteoprotegerin (OPG) concentrations have previously been associated with growth of abdominal aortic aneurysms (AAAs). In vitro experiments showed that OPG promotes matrix metalloprotease (MMP) release from monocytes and vascular smooth muscle cells. We hypothesized that OPG expression is increased in human AAAs and is associated with proteolysis.

Methods and results

AAA biopsies were collected from 312 patients. We assessed the concentrations of OPG, cathepsins A, B and S as well as the activity of MMP2 and MMP9 . The AAA wall infiltration by macrophages, lymphocytes, and plasma cells was estimated by immunohistochemistry. The concentration of OPG correlated positively with aortic diameter (<55mm: 3452 (1324-6114) pg/ml; 55-70mm: 4100 (1913-7180) pg/ml; >70mm: 4496 (2687-9994) pg/ml, P =.024), cathepsin A (R =0.221, P =.013), B (R =0.384, P <.001) and S (R =0.467, P <.001), MMP-2 (R =0.180, P <.001), MMP-9 (R =0.178, P <.001) ; and the numbers of lymphocytes (P <.001) and plasma cells (P =.001). OPG immunostaining was predominantly demonstrated in lymphocyte and plasma cell cytoplasm.

Conclusions

The concentration of aortic wall OPG is positively associated with established markers of AAA severity and pathogenesis. OPG appeared to be associated with lymphocytes and plasma cells. These human data support previous experimental data suggesting a role for OPG in AAA pathogenesis.

Introduction

Abdominal aortic aneurysm (AAA) is an abnormal focal dilation of the aortic wall that affects approximately 5% of men aged over 60 years. The risk of aortic rupture increases with AAA size and AAA rupture is associated with a 80-90% mortality rate.[1] Currently the only treatment option for AAA is open or endovascular surgery, however there is significant interest in developing medical therapies to limit AAA growth and rupture.[2-4]

Osteoprotegerin (OPG) is a secreted glycoprotein member of the tumor necrosis factor receptor superfamily.[5] It acts as a decoy receptor for receptor activator of nuclear factor κB ligand (RANKL) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL).[6] Cells described to secrete OPG include osteoblasts, endothelial cells, human aortic vascular smooth muscle cells (VSMCs), dendritic cells, lymphocytes, and plasma cells.[7-9] OPG is a key regulator of bone remodeling[5, 10] but has also been implicated in tumourigenesis, immunological pathways and vascular diseases.[6, 7, 11-15] The precise role of OPG in vascular disease is currently controversial. Evidence from animal studies suggest that OPG prevents arterial calcification, and stabilizes plaque formation.[16, 17] However, clinical studies have associated serum OPG concentrations with the presence and progression of cardiovascular disease.[18-21] Circulating OPG concentrations have also been reported to be higher in patients with AAA and positively associated with AAA progression.[4,22] In vitro experiments have suggested that OPG stimulates matrix metalloprotease (MMP) secretion from human monocytes and VSMCs; and that OPG secretion is downregulated by irbesartan.[4] This data suggests a potential role of OPG in AAA, however, currently we are aware of no studies which have reported the concentration of OPG in human AAA biopsies.

The aim of this study was to assess the concentration of OPG in a large number of AAA biopsies and assess whether OPG expression was related to markers of AAA severity. We hypothesized that OPG is locally produced in vessel walls of patients with AAA disease, and is associated with markers of aortic proteolysis.

Methods

Aneurysm-Express Biobank

Aneurysm-Express is an ongoing longitudinal vascular biobank study.[23] All patients undergoing open AAA repair in two Dutch hospitals are asked to participate in the study. The Medical Ethics Committees of both hospitals approved the study and participants provided written informed consent. For the current research investigation we studied the ventral AAA wall biopsies from consecutive patients who were included between April 2003 and January 2011.

Patient inclusion

In this study, consecutive patients undergoing open repair of intact or ruptured AAA were included. The indications for intervention were based on current guidelines and included: AAA diameter exceeding 55 mm for males, AAA diameters between 50 – 55 mm for females, rapidly expanding aortic diameters (≥ 5 mm in 6 months with a minimum diameter of 40 mm), saccular aneurysms, symptoms attributable to AAA and AAA rupture.[1, 24] Patients with AAA

diameters between 50 and 55 mm were selected for surgery based on the clinical judgment of the surgeon and in consultation with the patient. Open repair was performed in patients in whom AAAs were not anatomically suited to endovascular repair. Patients with terminal malignancies, or severe dementia were excluded from this study.

Baseline characteristics

Risk factors and demographic data were obtained from clinical records and questionnaires at the time of recruitment. These questionnaires included history of vascular disease, cardiovascular risk factors (age, diabetes, gender, hypertension, smoking) and medication use. Aortic diameter and morphology were assessed via computed tomography angiography (CTA) or magnetic resonance angiography (MRA). Hypertension was defined as systolic blood pressure >130 mm Hg or usage of blood pressure–lowering drugs; diabetes was defined as use of insulin or oral hypoglycemic agents and smoking was defined on the basis of whether patients had smoked during the last weeks before AAA surgery. Chronic obstructive pulmonary disease (COPD) was recorded based on previous physician diagnosis.[25]

AAA tissue sampling and protein analysis

Biopsies of the ventral wall of the AAA at the site of maximal diameter were collected during open repair and dissected into 5 mm segments. The middle segment was fixed in 4% formaldehyde, decalcified for 1 week in ethylenediaminetetraacetic acid (EDTA) and embedded in paraffin. Adjacent segments were snap-frozen in liquid nitrogen, ground and dissolved in 1.5 ml 40 mmol TrisHCl. Total protein concentration of every sample was quantified via a BCA protein measurement method (Pierce Biotechnology, Rockford, USA). Concentrations of interleukin 6 (IL6), IL8, soluble ICAM-1 (sICAM-1), OPG, sRANKL, MMP8, cathepsins A, B and S were quantified employing Luminex multianalyte profiling technology [26, 27], using a Bio-Plex system (Bio-Rad, Hercules, CA). Activity of MMP2 and MMP9 were measured using Biotrak assays (Amersham Biosciences, GE Healthcare UK).[23] All measured concentrations were related to the protein concentrate of every sample. Inter-assay coefficient of variation ranged from 6 – 19% for the quantified markers.

Immunohistochemistry

The paraffin embedded segment of the AAA biopsy was cut into 4µm sections. Sections were stained for elastin using the elastin von Giesson method, for collagen using Picro-sirius red, and for VSMCs (α-smooth muscle actin), macrophages (CD68), T-lymphocytes (CD3), B-lymphocytes (CD20) and plasma cells (CD138) using immunohistochemistry, as previously described. [23] Immunostaining of sections with OPG was performed using the following primary antibody: a mouse monoclonal antibody to human OPG (clone 98A1071, Abcam; 1/500). To make the OPG epitopes accessible for the antibody, sections were boiled in citrate buffer (pH 6.0). PowerVision poly-AP-anti mouse IgG (Immunologic, Duiven, the Netherlands) and New-Fuchsin solution was used to visualize the staining. All sections were counterstained with hematoxylin. An antibody of the same isotype was used as negative control. Some histological specimens proved unsuitable for examination and were excluded. In total, we analyzed 214 AAA specimens. Staining was semi-quantified as no, minor, moderate or heavy staining as previously described.[23] Histological examination was performed by 2 independent observers

(R.H., A.V.) unaware of the clinical data. In case of discrepancies in judgment, sections were reanalyzed. Consensus was reached in all cases.

Cell culture

Human VSMCs were obtained from abdominal aorta of healthy individuals without atherosclerosis. VSMCs were isolated by combined collagenase and elastase digestion[28] and maintained in DMEM containing 10% FBS at 37°C, 5% CO_2. The human monocytic cell line THP-1 was maintained in RPMI 1640 (JRH) containing 10% FCS. Activation of THP-1 cells was induced with 10 µg/mL lipopolysaccharide (*S typhimurium*, Sigma). Human VSMCS and THP-1 cells were growth arrested for 18 hours in serum-free DMEM before incubation in experimental medium containing FCS (1%) in the presence or absence of rhOPG (ImmunoKontact; 0 to 20 ng per $1x10^5$ cells per 1 mL for 24 hours). Protein concentration of cell lysate and supernatant of each sample was determined by the Bradford micro-assay. The effect of rhOPG was assessed by measuring cathepsin S concentrations in pg/ml by ELISA (Human Total Cathepsin S DuoSet, R&D Systems, DY1183).

Statistical Analysis

Continuous values were reported as median with interquartile range (IQR). Discrete variables were reported as number and percentages. Spearman's correlation analysis and Kruskal-Wallis tests were conducted where appropriate. Multiple linear regression analysis was used to assess independent associations. To normalize the distributions and improve the linearity of the associations, the natural log transform was used during multiple linear regression analysis for non-normally distributed data. All tests were 2-sided, with P <.05 considered statistically significant. Statistical analyses were performed with SPSS 15.0 (SPSS Inc., Chicago IL, USA).

Results

Baseline characteristics

AAA biopsies were collected from 312 patients at open surgery. The study cohort consisted of 245 asymptomatic, 32 symptomatic and 35 ruptured AAAs. The baseline characteristics of the patients at the time of recruitment are shown in Table 1. There were 71 AAAs below 55 mm, 153 AAAs between 55 and 70 mm, and 88 AAAs above 70 mm. Patients with an aneurysm below 55 mm were younger in age (P <.001). No differences in co-morbidities were observed.

OPG and AAA diameter

The concentration of OPG in AAA biopsies was correlated with AAA diameter, R =0.186, p =.001. The median (IQR) OPG concentrations were 3452 (1324-6114), 4100 (1913-7180) and 4496 (2687-9994) pg/ml in biopsies removed from patients with <55mm, 55-70mm and >70mm AAAs, respectively, P =.024 (Figure 1). After adjustment for cardiovascular risk factors (age, gender, hypertension, diabetes, smoking, COPD and a history of MI) OPG concentrations were independently associated with AAA diameter (coefficient, 0.206; SE 0.011; P =.001).

OPG and protease expression in AAA

MMP2 (R =0.180, P <.001) and MMP9 (R =0.178, P <.001) activity were positively correlated with OPG concentration in the 312 AAA biopsies. There was also a significant correlation between the concentration of OPG in the 312 AAA biopsies and cysteine protease levels (Figure 2). Furthermore, OPG concentration was positively correlated with sICAM1 (R =0.498, P <.001), IL6 (R =0.343, P =.002), and IL8 (R =0.260, P =.007). We could not detect sRANKL in any biopsies examined.

Effect of OPG on secretion of cathepsin S from VSMCs and monocytic cells

Healthy human abdominal aortic VSMCs showed a trend for increased secretion of cathepsin S when incubated with increasing doses of OPG. Median (IQR) amounts of cathepsin S in conditioned media were 0.002 (0.000-0.005), 0.024 (0.018-0.029), 0.031 (0.021-0.031), and 0.028 (0.019-0.038) pg /µg protein when VSMCs were incubated in 0, 5, 10 and 20 rhOPG per 1x105 cells per 1 mL for 24 hours respectively, n=3, P =.074. Median (IQR) amounts of cathepsin S in VSMC lysates were 0.019 (0.016-0.019), 0.080 (0.069-0.094), 0.092 (0.081-0.100), and 0.096 (0.044-0.224) pg/µg protein when VSMCs were incubated in 0, 5, 10 and 20 rhOPG per $1x10^5$ cells per 1 mL for 24 hours respectively, n=3, P =.076. Increasing doses of rhOPG stimulated cathepsin S upregulation in activated THP-1 cells. Median (IQR) amounts of cathepsin S in THP-1 lysates were 0.042 (0.027-0.045), 0.101 (0.089-0.104), 0.111 (0.084-0.114), and 0.110 (0.108-0.121) pg/µg protein when cells were incubated in 0, 5, 10 and 20 rhOPG per $1x10^5$ cells per 1 mL for 24 hours respectively, P =.049. A similar but not significant effect of rhOPG on secretion of cathepsin S from activated THP-1 was demonstrated.

Table 1 | Baseline characteristics versus AAA diameter.

Variables	< 55 mm n=71	55 - 70 mm n=153	> 70 mm n=88	P-value
Age, y, median (IOR)	66 (61 - 73)	71 (66 - 76)	72 (66 - 77)	<.001
Male gender	58 (81.7)	125 (81.7)	80 (90.9)	.107
Smoking	30 (42.2)	69 (45.1)	35 (39.7)	.781
Diabetes	12 (16.9)	20 (13.1)	16 (18.8)	.781
Hypertension	47 (66.2)	112 (73.2)	54 (61.4)	.129
COPD	8 (11.3)	30 (19.6)	23 (26.1)	.164
History of MI	20 (28.2)	38 (24.8)	30 (34.1)	.590
Symptomatic or ruptured AAA	10 (10.4)	16 (10.5)	41 (46.6)	<.001
Saccular morphology	7 (9.9)	4 (2.6)	3 (3.4)	.064
AAA with dissection	7 (9.9)	1 (0.7)	0 (0.0)	.042
Creatinin, µmol/L, median (IOR)	91 (82 - 105)	95 (80 - 117)	99 (85 - 122)	.205
Statin use	52 (73.2)	88 (57.5)	51 (58.0)	.122
ACE-inhibitor use	22 (31.0)	47 (30.7)	28 (31.8)	.958

Characteristics are in n (%), unless otherwise indicated. Abbreviations: MI, myocardial infarction; COPD, chronic obstructive pulmonary disease; ACE, angiotensin converting enzyme.

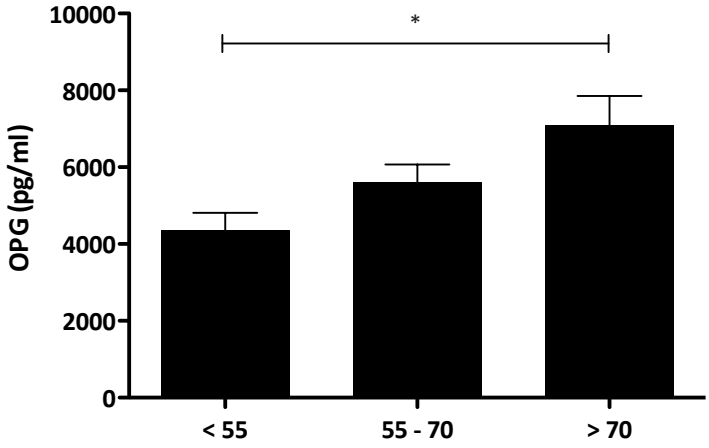

Figure 1 | Comparison of OPG concentrations in biopsies removed from AAAs with different diameters. OPG concentrations were higher in larger AAAs (*P =.024). Data are expressed as mean±SEM.

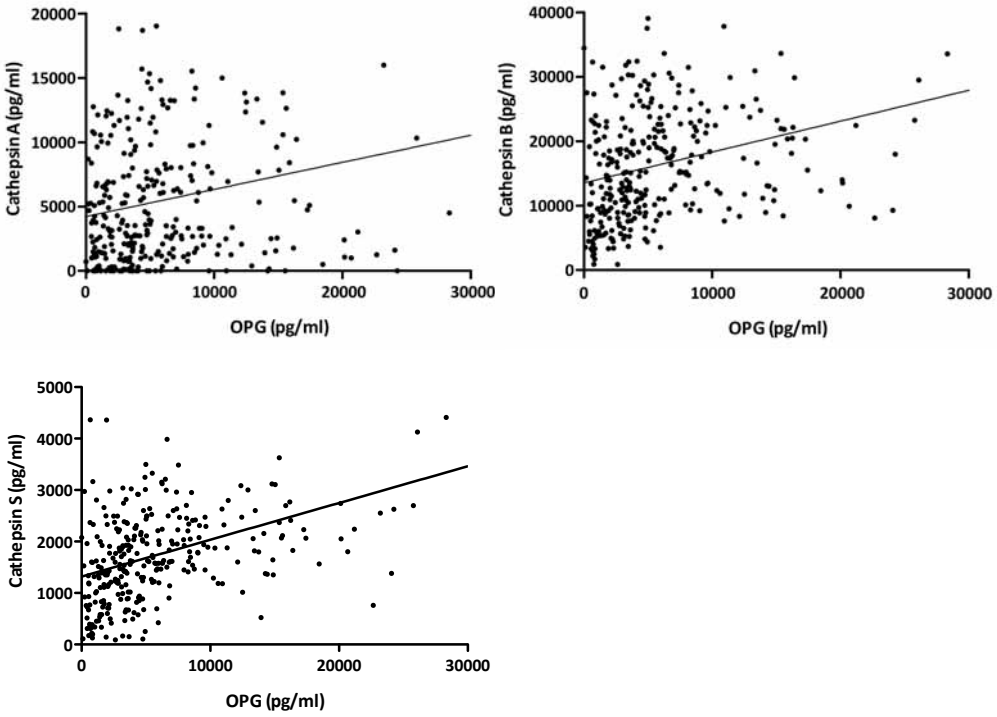

Figure 2 | Correlation between the concentrations of OPG and cathepsin A (R =0.221; P =.013), B, cathepsin B (R =0.384; P <.001) and, C, cathepsin S (R =0.467; P <.001) in AAA biopsies (n=312).

Median (IQR) amount of cathepsin S in conditioned media were 0.032 (0.032-0.036), 0.054 (0.042-0.059), 0.055 (0.039-0.077), and 0.055 (0.043-0.064) pg/µg protein when THP-1 cells were incubated in 0, 5, 10 and 20 rhOPG per 1x105 cells per 1 mL for 24 hours respectively, P =.092.

OPG and histology

We examined the relationships between OPG concentrations and the histology grading of AAA biopsies (Table 2). The AAA biopsy OPG concentrations were positively correlated with staining for B and T lymphocytes and staining for plasma cells (Figure 3). OPG concentrations were not associated with other histological characteristics of AAA biopsies (Table 2). Immunostaining for OPG was demonstrated to predominantly co-localize in the cytoplasm of lymphocytes and plasma cells (Figure 4).

OPG and advanced AAA calcification

There was no association between AAA biopsy OPG concentrations and histological grade of aortic calcification. Median (IQR) OPG concentrations were 4880 (2490-9500), 5662 (2960-8909), 4818 (2771-7280), 4311 (2471-5893) pg/ml in AAA biopsies with no, minor, moderate and heavy calcification, respectively; P =.759.

OPG concentrations in asymptomatic fusiform aneuryms

We performed a sub-analysis of OPG concentrations in asymptomatic fusiform AAAs. We excluded 67 symptomatic or ruptured AAAs, and 14 saccular AAAs. In total there were 231 asymptomatic AAAs with a fusiform morphology. The results of the sub-analysis are shown in Table 3. OPG concentration was correlated with AAA diameter and markers of proteolysis and inflammation.

Table 2 | Relation between AAA vessel wall histology and OPG expression levels .

Variables	n	No staining	n	Minor staining	n	Moderate staining	n	Heavy staining	P-value*
Calcifications	106	4880 (2940-9500)	39	5662 (2960-8909)	39	4818 (2771-7280)	30	4311 (2471-5893)	.759
VSMCs	43	4894 (3150-7641)	97	4495 (2664-7987)	53	5774 (3018-14415)	21	4496 (3262-9480)	.248
Elastin content	42	4648 (2934-7915)	106	5379 (3046-9181)	61	3968 (2673-8494)	5	6049 (5696-17349)	.442
Collagen content	0	-	60	5379 (2955-8487)	90	5708 (3291-9372)	64	3592 (2431-7483)	.291
Macrophages	126	4551 (2537-8273)	54	5050 (3296-9341)	18	6559 (3374-12278)	16	4894 (2940-9844)	.345
Neutrophils	165	5050 (2949-8871)	28	3687 (1880-7712)	16	4612 (2226-7952)	5	6642 (3216-10272)	.173
T-lymphocytes	55	3780 (1783-7296)	77	4379 (2956-7572)	50	5871 (2939-11074)	32	7466 (3648-14695)	.038
B-lymphocytes	31	3383 (1537-5731)	65	4140 (2420-7757)	72	5585 (3249-8493)	46	6321 (3408-14221)	<.001
Plasma cells	80	3612 (1663-6136)	59	4800 (2960-9127)	48	6025 (3860-9307)	27	8503 (3683-16153)	.001

* corrected for aneurysm diameter, age, gender, hypertension, smoking, diabetes, history of MI and COPD.

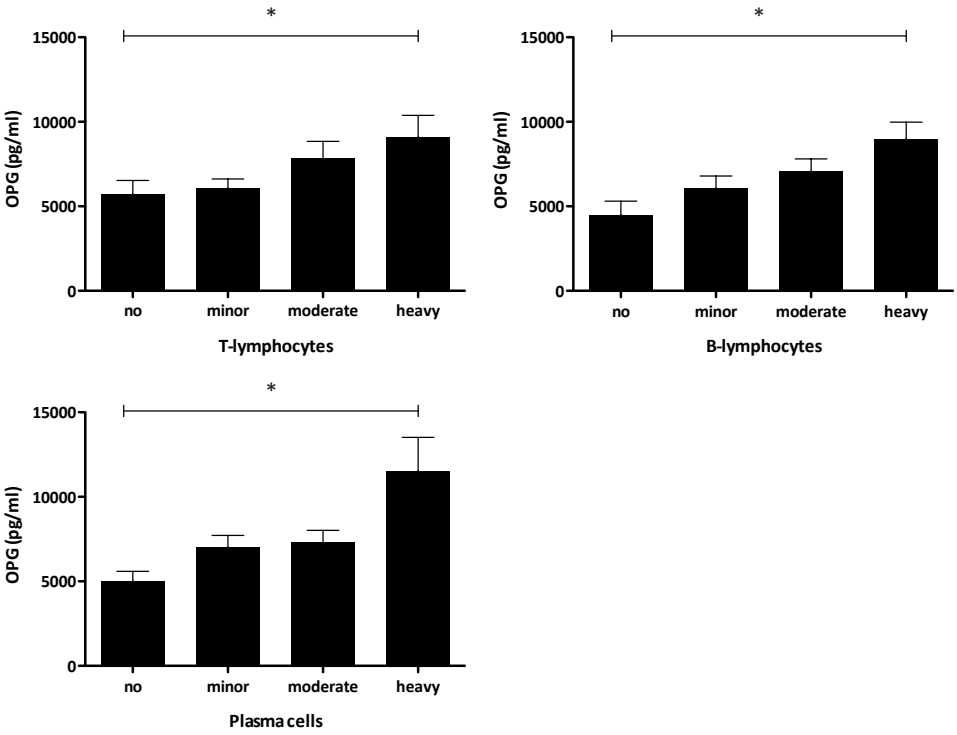

Figure 3 | OPG concentrations in AAA biopsies from patients with different severities of T-lymphocytes (*P =.013), B, B-lymphocytes (*P <.001), C, and plasma cell (*P <.001) infiltration. OPG concentrations were significantly higher in AAA biopsies with increased numbers of lymphocytes or plasma cells (n=214). Shown are mean±SEM.

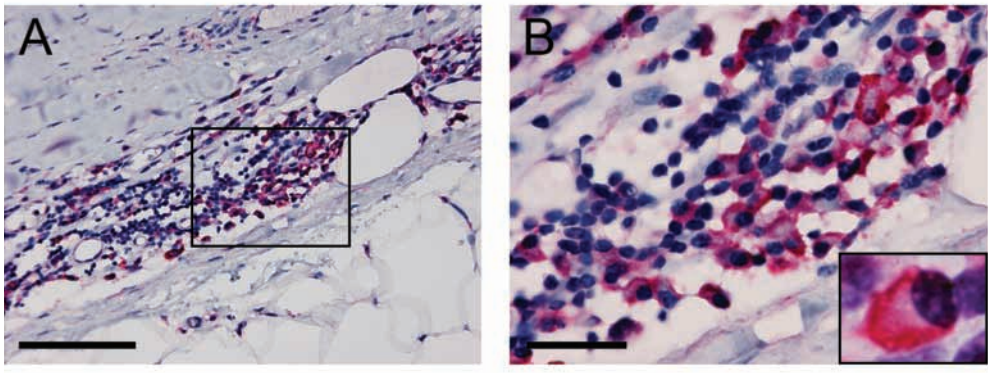

Figure 4 | Localization of OPG within an AAA biopsy. A. Immunohistochemical staining of OPG (shown in red) within the adventitia of the aortic wall. Bar = 100 µm. B. higher magnification of the indicated area in A. A high magnification of a plasma cell with OPG expression highlighted in the box. Bar = 25 µm

Table 3 I Association with OPG levels in asymptomatic AAAs.

Variables	Correlation coefficent	*P-value
AAA diameter	0.195	.002
MMP2	0.227	.001
MMP9	0.232	.004
MMP8	0.219	.200
Cathepsin A	0.228	.035
Cathepsin B	0.407	<.001
Cathepsin S	0.499	<.001
IL6	0.370	.003
IL8	0.290	.046
sICAM1	0.510	<.001
T-lymphocytes	-	.082
B-lymphocytes	-	.001
Plasma cells	-	.006

* corrected for aneurysm diameter, age, gender, hypertension, smoking, diabetes, history of MI and COPD.

Discussion

The main finding from the current study was that aortic concentration of OPG was associated with a range of markers of AAA severity. OPG concentration was positively associated with AAA diameter, markers of proteolysis and inflammation in a large collection of human AAA samples. In an in vitro study we found that OPG appeared to stimulate the production of cathepsin S by VSMCs and monocytic cells, suggesting a possible reason for the link between OPG and cathepsin concentrations.

The correlation between AAA OPG concentrations and aneurysm diameter remained significant after adjustment for traditional risk factors. These observations in human aortic biopsies are in line with previous observations in human blood samples. Moran et al. previously found a correlation between serum OPG concentrations and AAA progression.[4] Moran and colleagues also demonstrated that OPG stimulated MMP release from monocytes and VSMCs *in vitro*.[4, 29] In the current study, the aortic wall OPG concentrations and the activity of MMP2 and MMP9 were correlated in keeping with the previous findings of Moran et al. In the current study we also found a strong correlation between the aortic concentrations of OPG and cysteine proteases, especially cathepsin S. To investigate this association further we examined the effect of rhOPG on the expression of cathepsin S by VSMCs and monocytic cells. These studies suggested that OPG promoted cathepsin S production, although findings were of borderline significance. To our knowledge, the intracellular pathway via which OPG induces up-regulation of proteolytic enzymes in these cells is unknown. Nevertheless, it seems likely that OPG not only upregulates MMPs but also increases cathepsin S expression. These results further support the view that OPG promotes proteolysis in human AAA and could be acting as an amplifier of different proteolytic pathways.

Zauli et al. demonstrated increased adhesion and rolling properties of leukocytes in vitro and in vivo due to OPG administration.[30] In the current study we found a significant correlation between concentrations of OPG and sICAM1 in AAA biopsies. This finding is in line with a previous study from our group, which demonstrated that OPG promoted expression of adhesion molecules by pre-stimulated endothelial cells.[31] OPG was also associated with other markers of inflammation. Whether these correlations between OPG and pathological markers of AAA severity represent a causal link is not currently clear.

Immunodetection of OPG in AAA specimens localized the protein predominantly in the adventitia. OPG co-localized with plasma cells and lymphocytes. AAA biopsy OPG concentrations were correlated with staining for B and T lymphocytes and plasma cells. There was no correlation of OPG with grading of VSMCs numbers, elastin and collagen content. Most of the aortic media is degraded in advanced AAA disease and therefore any link between elastin loss and OPG may be difficult to detect in the samples we analyzed. In keeping with our current findings Li et al. have previously reported that lymphocytes and plasma cells were the major sources of OPG in the bone microenvironment.[8] Previous studies have implicated OPG in regulating the humoral immune response, which has been suggested to play a role in AAA.[11, 32-34] OPG might promote an immune response within the aneurysm wall, via dysregulated cathepsin S/MHC II activation, and thereby influence B cell activation, maturation and isotype switching.[35-37]

OPG is a decoy receptor for sRANKL and therefore binds sRANKL in the extracellular matrix. OPG – sRANKL complexes cannot bind to cell receptors. Because sRANKL has effects on the immune and vascular system and can form a complex with OPG [6, 14], we attempted to measure sRANKL expression in human AAA samples. sRANKL proved to be undetectable by Luminex technology. This could indicate low levels of sRANKL due to high levels of OPG or no production of sRANKL by the cells in the AAA vessel wall.

OPG has previously been associated with aortic calcification.[29, 38-40] In the current study there was no significant correlation between aortic concentrations of OPG and calcification. A reason for this observation could be the stage of the AAA disease. Most of the media was degraded in the biopsies examined and calcification was primarily located in the intimal layer. To our knowledge, OPG has been associated predominantly with medial calcifications in mice.[7, 29]

In conclusion, OPG is present in AAA biopsies and is predominantly expressed in adventitial lymphocytes and plasma cells. AAA wall OPG concentrations are associated with the concentrations of proteases and AAA diameter. The findings support previous work suggesting that OPG may be involved in AAA pathogenesis.

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Different effects of commonly prescribed statins on abdominal aortic aneurysm wall biology

6

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Abstract

Background

Pharmaceutical stabilization of the abdominal aortic aneurysm (AAA) wall can delay surgery and improve outcome. Observational studies indicate statins can be used to reduce AAA growth, but mechanistic data are scarce. In this study, our aim was to determine the pleiotropic effects of different statins on AAA wall composition.

Methods

We included 216 patients undergoing open AAA repair, of which 60 used simvastatin, 52 atorvastatin and 23 pravastatin. The AAA wall histology and protein expression (IL 1ß, 2, 4, 5, 6, 8, 10, 12, IFNγ, TNFα, ß, matrix metalloproteinase (MMP) 2 and 9 activities, total MMP 8, 9 and cathepsin A and B levels) between statin users and non-users were compared as also among the different statins.

Results

As far as histological inflammation goes, the AAA walls of statin users did not differ from those not using them. After multivariate adjustment for risk factors, pravastatin use was associated with tendencies of increased MMP8 (P =.022), active MMP9 (P =.040) and higher cathepsin B (P =.056) levels. AAA walls of simvastatin and atorvastatin users showed no differences in proteases or cytokines in multivariate analyses.

Conclusions

The use of statins was not associated with a decrease in protease levels or inflammation. The trends of elevated protease levels associated with pravastatin use suggest pleiotropic differences among the various statins, supporting the need for further research to target pharmaceutical AAA treatment.

Introduction

Abdominal Aortic Aneurysm (AAA) formation is a degenerative disease with a prevalence of 5.32 - 8.02%.[1] Progressive expansion of AAA eventually leads to rupture, it caused 6800 deaths in the UK in 2000.[1-3] Preventive exclusion of the AAA from the circulation is associated with a mortality of 9.6%.[4] As a consequence pharmaceutical stabilization of the AAA wall is a promising target to postpone intervention and thereby improve outcome.[5] Various medications have been suggested to inhibit AAA expansion, of which some, including roxithromycin[6] and doxycycline[7], have been tested in small randomized controlled trials. Observational studies indicate AAA growth reduction by hydroxymethylglutaryl-coenzyme A reductase inhibitors (statins)[8, 9], but mechanistic data are scarce. In rodents, atorvastatin and simvastatin are described to suppress AAA formation when compared to vehicle, independent of their lipid lowering effects.[10-12] Moreover, statins are described to have pleiotropic effects on the arterial wall, mainly described in atherosclerotic plaques.[13]

In AAA pathophysiology matrix metalloproteinases (MMP's) are key molecules for aortic wall degradation; especially MMP9 has been studied extensively.[14-16] Previous relatively small studies suggested that statin use attenuates MMP activity, leading to a decreased AAA growth rate.[17, 18] We aimed to confirm these findings and add both cytokine data and histology, while correcting for confounders to obtain more information on possible mechanisms. Besides this, other studies suggest dissimilar effects different types of statins have on serum lipid levels and atherosclerotic plaque composition.[19, 20] Therefore, our aim was also to address possible differences between commonly prescribed statins on AAA wall composition, including proteases, cytokines and histology in a large patient cohort.

Methods

Patients and materials

A total of 216 consecutive patients from 2 centers scheduled for operative open AAA repair were included in the Aneurysm-express cohort study. The indication for surgery was based on international standards.[21] The ethical review boards of the participating hospitals approved the study and all patients signed a written informed consent. Baseline characteristics of patients that participated in the Aneurysm-express study included medical history and medication use. These data were extracted from clinical patient records and a questionnaire based on the Rose cardiovascular survey was filled in by the patients.[22] In cases of doubt or inconsistencies, the patients' general practitioner or pharmacist was contacted for further details. The AAA diameter and morphology were assessed via computed tomographic angiography or magnetic resonance angiography. For all patients, protein was isolated from the AAA wall and used for measuring cytokine- and protein levels. Blood samples were available in 100 patients and histology for 129. Total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL) and high sensitivity C-reactive protein (hsCRP) were determined before surgery.

Tissue processing

During surgery a part of the ventral AAA wall was harvested at the site of maximum diameter, segmented and immediately processed. One segment was fixed using 4% formaldehyde, decalcified using ethylenediaminetetraacetic acid (EDTA) and paraffin-embedded for histological stainings. Adjacent segments were snap-frozen using liquid nitrogen and stored at -80 °C. The following stainings were performed for AAA wall analysis: haematoxilin and eosin (overview), elastica-Van Giesson (elastin), picrosirius red (collagen), alpha smooth muscle actin (smooth muscle cells (SMCs)), CD68 (macrophages), CD3 (T-lymphocytes), CD20 (B-lymphocytes) and CD138 (plasma cells). All stained sections were scored semi-quantitatively on a two-value scale (no to minor vs. moderate to heavy staining). Extracellular matrix components (elastin, collagen) and cells (SMCs, macrophages, T-lymphocytes, B-lymphocytes and plasma cells) were scored for the total vessel wall.

Adjacent segments were used for protein isolation. Samples were crushed in liquid nitrogen and afterwards partly dissolved in 1.5 ml 40 mmol TrisHCl (Roche) and centrifuged at max rpm and stored at -80°C. Another segment was isolated using Tripure Isolation Reagent (Boehringer Mannheim) according to manufacturer's protocol. Protein concentrations were measured via the BCA Protein Assay Kit (Pierce Biotechnology, Rockford, USA). Total amounts of MMP8, 9, cathepsin A and B were determined using the Bio-Plex system employing Luminex multianalyte profiling technology, as described and performed previously.[23, 24] MMP activities were measured using the Amersham matrix metalloproteinase 2 and 9 biotrak activity assay system (GE healthcare UK limited).

Levels of interleukin (IL) 1ß , 2, 4, 5, 6, 8, 10, 12p70, interferon (IFN) γ and tumor necrosis factor (TNF) α and ß were quantified in the aneurysmal wall by fluorescent bead immunoassay (Bendermed Systems).

Statistical analyses

Demographic and clinical characteristics with discrete variables were summarized as frequencies and percentages and normal distributed continuous variables as means and their standard deviation; non-normally distributed continuous variables were presented as median and interquartile range. Chi-square and Mann-Whitney tests were used for comparing discrete and continuous variables respectively between groups. Multiple groups were compared using the Kruskal-Wallis test. The association between statin groups and AAA-wall characteristics was adjusted for cardiovascular risk factors and baseline parameters showing an association ($P < .200$) with statin use in the multivariate logistic regression model. For this purpose, continuous variables were dichotomized at the median. Dependent variable was the measured protease, cytokine or histological parameter. For each variable 3 models were run; one with statin use included in the independent variables, a second with the different statins and a third with statins categorized according to their pharmacokinetic profile. The reported multivariate P represents the P-value of the independent variable in the model. To correct for the five simultaneous comparisons in the three models, we applied Bonferroni's method resulting in probability values of $<.010$ to be considered statistically significant. All analyses were performed using statistical package of the social sciences version 15 (SPSS Inc.).

Results

The AAA diameter and morphology did not differ between patients using statins and patients not using statins. Patients receiving statin therapy exhibited a higher prevalence of cardiovascular co-morbidities and risk factors as expected (Table 1). Nearly all patients used their statins at least one month prior to surgery. Blood was available from 100 patients included at the later period of the Aneurysm-express. Serum levels of total cholesterol (p=.001), LDL (P =.002) and HDL (P =.038) were significantly lower in statin treated patients, while triglycerides and C-reactive protein (CRP) were similar in groups (Table 1). No differences were found between groups using of ACE-inhibitors or angiotensin II receptor blockers.

Table 1 | Baseline characteristics.

	No statin	All statins	P	Simvastatin	Atorvastatin	Pravastatin	P
N	**81**	**135**		**60**	**52**	**23**	
Age, y	71.7±6.6	68.5±7.2	.001	68.4±6.8	68.0±7.4	69.8±7.7	.625
Male	59(73)	113(84)	.143	51(85)	41(79)	21(91)	.373
Diameter, mm	62.3±10.6	64.0±13.9	.682	64.6±14.0	61.7±13.3	67.2±15.0	.225
Fusiform morphology	68(84)	113(84)	.801	49(82)	45(87)	19(83)	.777
Current smoker	34(42)	63(47)	.722	30(50)	22(42)	11(48)	.495
Treatment for hypertension	49(60)	106(79)	.013	51(85)	41(79)	14(61)	.058
Diabetes mellitus	5(6)	23(17)	.025	10(17)	8(15)	5(22)	.794
COPD	17(21)	20(15)	.227	8(13)	7(13)	5(22)	.593
BMI, kg/m^2	24.9[23.2-27.8]	26.1[23.9-28.7]	.214	25.5[24.1-28.0]	26.1[23.1-29.0]	27.0[23.2-30.3]	.456
GFR, ml/min/1.73m^2	70.2[61.1-78.7]	69.6[54.4-92.7]	.981	67.9[54.9-86.8]	76.2[54.9-95.6]	66.5[51.8-93.2]	.388
History of myocardial infarction	9(11)	56(41)	<.001	21(35)	23(44)	12(52)	.357
ACE-inhibitor use	22(27)	43(32)	.502	19(32)	19(37)	5(22)	.550
Angiotensin II receptor blockers	13(16)	33(24)	.157	15(25)	13(25)	5(22)	.547
Peripheral artery disease	12(15)	26(19)	.429	16(27)	7(13)	3(13)	.151
Other aneurysm reported	9(11)	17(13)	.795	4(7)	9(17)	4(17)	.181
N	**33**	**67**		**24**	**32**	**11**	
Total cholesterol, mmol/l	5.41[4.31-6.47]	4.26[3.48-4.86]	.001	4.40[4.04-5.12]	3.81[2.86-4.68]	4.57[4.13-5.07]	.062
Triglycerides, mmol/l	1.38[1.00-2.13]	1.36[0.96-2.12]	.648	1.61[1.24-2.21]	1.28[0.79-2.07]	1.20[0.96-2.04]	.229
HDL, mmol/l	1.04[0.85-1.40]	0.90[0.74-1.09]	.039	0.91[0.77-1.08]	0.89[0.69-1.13]	0.94[0.78-1.23]	.749
LDL, mmol/l	3.46[2.49-4.35]	2.58[2.00-3.04]	.002	2.70[2.27-3.18]	2.20[1.50-3.01]	2.63[2.43-3.12]	.160
hsCRP, mg/l	4.4[2.4-10.2]	4.1[1.7-13.3]	.839	4.0[2.2-9.9]	4.0[1.2-16.6]	4.2[2.8-15.9]	.920

Data are shown as N (%), mean ± sd or median [interquartile range]. Abbreviations: COPD, chronic obstructive pulmonary disease; BMI, bodymass index; GFR, glomerular filtration rate; ACE, angiotensin converting enzyme; HDL, high density lipoprotein; LDL, low density lipoprotein; hsCRP, high sensitivity C-reactive protein. Values are mean ± standard deviation, median with [interquartile range] or in frequencies with (percentages). P values represent all statins combined versus no statin and differences among statin groups.

The AAA walls of statin-treated patients had trends for higher concentrations of active MMP2 (P =.042) and lower amounts of cathepsin A (P =.041) compared with patients not using statins; these tendencies did not sustain after multivariate adjustment for cardiovascular risk factors (Table 2). The analyses of effects of the different statins compared with no statin use are

Table 2 I Protease- and cytokine levels.

N	No statin 81	All statins 135	P [A]	Simvastatin 60	P [A]	Atorvastatin 52	P [A]	Pravastatin 23	P [A]
Active MMP 2	0.19 [0.03-0.33]	0.24 [0.11-0.47]	.536	0.23 [0.12-0.45]	.526	0.23 [0.08-0.40]	.838	0.36 [0.11-0.66]	.452
Total MMP 8	1919 [650-3826]	1819 [615-4324]	.452	1658 [604-4138]	.506	1406 [408-3839]	.794	3515 [1593-9822]	.022
Active MMP 9	0.16 [0-0.52]	0.31 [0.04-1.00]	.404	0.25 [0.05-0.93]	.298	0.24 [0.04-0.98]	.403	0.40 [0.07-1.74]	.040
Total MMP 9	8218 [2999-18680]	10668 [3703-24783]	.931	10340 [5228-22080]	.974	8522 [3145-29881]	.686	14121 [7040-60525]	.308
Total Cathepsin A	2950 [1270-5837]	1725 [149-4820]	.478	1192 [164-3766]	.357	1951 [93-5312]	.502	4546 [210-7050]	.848
Total Cathepsin B	13329 [9246-21219]	13359 [8352-20001]	.558	11815 [8352-18372]	.583	13359 [6525-19948]	.568	16611 [12462-25783]	.056
IL1ß	0.01 [0-0.58]	0.03 [0-0.41]	.630	0.11 [0-0.47]	.438	0 [0-0.30]	.703	0 [0-0.76]	.747
IL2	1.87 [0.81-5.43]	2.48 [0.97-4.06]	.126	2.51 [1.22-3.59]	.117	2.52 [1.02-4.79]	.100	1.37 [0.07-2.74]	.789
IL4	0.04 [0-1.13]	0 [0-0.69]	.884	0 [0-0.86]	.370	0 [0-0.77]	.792	0 [0-0.17]	.603
IL5	0.86 [0.16-2.41]	0.64 [0-1.98]	.600	0.55 [0-1.87]	.721	0.89 [0.11-3.03]	.350	0.42 [0-1.93]	.611
IL6	1.91 [0.13-8.35]	0.82 [0.15-2.96]	.800	0.67 [0.13-2.12]	.982	1.34 [0.21-5.40]	.749	1.20 [0.14-12.99]	.704
IL8	26.32 [10.82-59.12]	26.15 [11.52-54.61]	.664	24.30 [11.01-50.26]	.657	28.96 [7.76-65.27]	.934	23.79 [13.92-54.14]	.288
IL10	0.27 [0-1.36]	0.46 0-1.05]	.393	0.47 [0-1.03]	.453	0.58 [0-1.13]	.148	0.09 [0-1.13]	.305
IL12	0.46 [0.06-1.66]	0.29 [0-1.13]	.629	0.31 [0-1.02]	.984	0.32 [0-1.32]	.719	0.12 [0-0.92]	.244
TNFα	0.43 [0-1.17]	0.40 [0-0.79]	.463	0.41 [0-0.78]	.597	0.40 [0-0.91]	.690	0.26 [0.01-0.95]	.284
TNFß	0.35 [0-3.70]	0.33 [0-2.32]	.737	0.78 [0-2.08]	.560	0.28 [0-3.10]	.768	0.15 [0-2.86]	.723
IFNγ	0.73 [0.28-2.11]	0.71 [0.14-1.73]	.873	0.69 [0.34-1.76]	.655	0.92 [0.16-2.13]	.555	0.25 [0.02-1.36]	.163

MMP is matrix metalloproteinase, IL is interleukin, TNF is tumor necrosis factor and IFN is interferon. Total proteases are in arbitrary units, protease activities are in ng/ml/12 ug protein and cytokines are in ng/ml. Values are median with [interquartile range]. P-values originate from 2 multivariate models: one with statin use as a group and a second with the different statins separately. [A] adjusted for: age, gender, hypertension, diabetes, history of myocardial infarction.

summarized in Table 2. Simvastatin use was associated with lower cathepsin A levels in univariate (P =.010) but not in multivariate analysis. AAA patients using pravastatin had tendencies for higher levels of active MMP2 (P =.038), higher active MMP9 (P =.040) and higher MMP8 (P =.068) compared with patients not using statins. Multivariate adjustment for differences in risk factors between patient groups revealed pravastatin use to be associated with a trend for higher MMP8 (P =.022), higher active MMP9 (P =.040) and higher cathepsin B levels (P =.056).

Patients using atorvastatin showed no difference on protease level compared to non-users in the multivariate analyses. Statin treatment did not result in differences in the tested cytokines compared with patients not using statins, which holds true for all statins and for the individual statins.

Histology was available for 129 patients (Table 3). We found no differences in histology between statin and no statin users for structural components (elastin, collagen and SMC presence). No differences were observed for CD20-B-lymphocytes, CD138-plasmacells or CD68-macrophages. CD3-T-lymphocytes revealed a higher tendency in the pravastatin group in uni- and multivariate analysis (P =.037 and P =.045, respectively).

To further assess the differences among statins we grouped them based on their pharmacokinetic profiles[25]: lipophilic (simvastatin and atorvastatin) versus hydrophilic (pravastatin). When these groups were compared for proteases (Table 4), MMP8 and cathepsin B showed a higher tendency in the hydrophilic group (P =.019 and P =.027). Multivariate adjustment for cardiovascular risk factors showed MMP8, active MMP9 and cathepsin B to be higher trends in the hydrophilic pravastatin (P =.023, P =.040 and P =.055, respectively). Histological comparison revealed a higher tendency of T-lymphocytes in AAA walls from pravastatin treated patients compared with the combined hydrophilic statins in uni- and multivariate analysis (P =.034 and P =.043, respectively).

Table 3 | Histological characteristics of the AAA walls.

N	No statin 47	Statin 82	P [A]	Simvastatin 37	P [A]	Atorvastatin 33	P [A]	Pravastatin 12	P [A]
Elastin	13(28)	21(26)	.663	5(14)	.067	11(33)	.699	5(42)	.388
Collagen	26(55)	46(56)	.529	20(54)	.280	20(61)	.912	6(50)	.779
Smooth muscle cells	18(38)	37(45)	.913	15(41)	.715	16(48)	.656	6(50)	.820
Macrophages	16(34)	17(21)	.268	10(27)	.933	4(12)	.061	3(25)	.869
T-lymphocytes	16(34)	32(39)	.720	11(30)	.729	13(39)	.770	8(67)	.045
B-lymphocytes	19(40)	36(44)	.860	19(51)	.597	13(39)	.763	4(33)	.613
Plasmacells	16(34)	29(39)	.979	16(43)	.428	10(30)	.605	3(25)	.450

Numbers are absolute and represent moderate to high staining with (percentages) of total. P-values originate from 2 multivariate models: one with statin use as a group and a second with the different statins separately entered. [A] adjusted for: age, gender, hypertension, diabetes, history of myocardial infarction.

Table 4 | Associations among statin groups based on their pharmacokinetic properties.

	Lipophilic statin	**P [A]**	**Hydrophilic statin**	**P [A]**
N	**112**		**23**	
Active MMP 2	0.23[0.11-0.42]	.628	0.36[0.11-0.66]	.452
Total MMP 8	1605[580-4001]	.848	3515[1593-9822]	.023
Active MMP 9	0.24[0.04-0.95]	.721	0.40[0.07-1.74]	.040
Total MMP 9	9837[3501-23291]	.816	14121[7040-60525]	.310
Total Cathepsin A	1482[144-4089]	.365	4546[210-7050]	.846
Total Cathepsin B	13078[7819-18584]	.959	16611[12462-25783]	.055
N	**70**		**12**	
Elastin	16 (14)	.477	5 (42)	.352
Collagen	40 (36)	.516	6 (50)	.805
Smooth muscle cells	31 (28)	.943	6 (50)	.801
Macrophages	14 (13)	.234	3 (25)	.808
T-lymphocytes	24 (21)	.994	8 (67)	.043
B-lymphocytes	32 (29)	.766	4 (33)	.592
Plasmacells	26 (23)	.904	3 (25)	.430

Lipophilic statins are simvastatin and atorvastatin, hydrophilic statin is pravastatin. MMP is matrix metalloproteinase. Total proteases are in arbitrary units and protease activities are in ng/ml/12 ug protein. Values are median with [interquartile range] or N (%). Numbers are absolute and represent moderate to high staining with (percentages) of total. [A] adjusted for: age, gender, hypertension, diabetes, history of myocardial infarction.

Discussion

We found in our large cohort of patients no independent significant effects of statins as a group on proteases, cytokines and histology in harvested AAA walls. Analyzing individual statins after correction for differences in baseline characteristics and cardiovascular risk factors, we found that simvastatin and pravastatin did not stand out in any of the analyses. Surprisingly, pravastatin use was associated with higher trends for MMP8, MMP9 and cathepsin B levels.

As we previously have shown, simvastatin, pravastatin or atorvastatin use was not associated with altered MMP2 or 9 activities in atherosclerotic plaques.[20] In vitro, animal macrophages and SMCs produce less active MMP2 and 9 in response to fluvastatin and simvastatin.[26, 27] In AAA walls, however, lymphocytes outnumber macrophages by far and SMC content is strongly diminished due to apoptosis,[28, 29] indicating that findings in atherosclerotic plaques do not necessarily equal those in AAA walls. In addition, in a large observational study, our group showed that statin use is independently associated with attenuation of AAA growth rate.[8] This finding was also observed in another AAA cohort.[9] The mechanisms responsible for the decreased growth remain to be clarified. Since MMP concentrations are elevated in AAA tissue compared to normal aortic tissue, many researchers focus on the role of MMP in AAA pathogenesis.[16] Relatively small studies have been conducted to determine the effects of statins on MMP activity in AAA walls. Compared to patients without statins, statin use is associated with less active MMP3 and 9 and lower cathepsin H and L concentrations. In addition, no differences were found in total MMP1, 2, 8, 13.[17, 18] A small randomized trial showed less active and total MMP9 in patients treated with simvastatin compared with those given a placebo.[30] Encouraged by these data, we wanted to confirm these results and demonstrate a relation with inflammatory markers in the AAA walls of patients enrolled in our biobank study. We found trends for increased active MMP2 and lower cathepsin A levels in statin treated patients in our cohort, which seemed due to pravastatin use (P =.038). However, in multivariate analyses, these trends disappeared. Higher tendencies of total MMP8 and active MMP9 levels were independently associated with pravastatin use as compared to no statin use. When the different pharmacokinetic properties of the statins were taken into account, we noted the same pattern. Use of the hydrophilic pravastatin was associated with increased trends of MMP8 and cathepsin B compared to lipophilic simvastatin and atorvastatin use. After multivariate adjustment the trends in MMP8 sustained, cathepsin B diminished (likely underpowered) and active MMP9 was added. The hydrophilic molecular structure of pravastatin prevents wide spread tissue distribution, and reduces the potential for uptake by peripheral cells including those in the arterial and AAA wall components. In addition, pravastatin has higher unbound circulating levels and according bioavailability when compared to simvastatin and atorvastatin.[25] These pravastatin-specific properties might contribute to the associations with elevated proteases in this study in contrast with the lack of found associations with wall components and the lipophilic statins, both of which have not been reported before.

We evaluated the cytokine levels in the aneurysmal walls in all groups to exclude the possibility of confounding due to more inflammation-rich aneurysms in the pravastatin group. Analyses showed no difference in inflammatory status between the groups, which was also confirmed by similar serum hsCRP levels in all groups. Histology too showed no differences in structural

components and nearly all inflammatory cells. T-lymphocytes had a higher trend in the pravastatin treated group, also after multivariate adjustment. However, this difference was based on a lower number of patients and needs to be interpreted carefully.

This study has some limitations. The use of statins for indications other than the AAA probably resulted in differences in cardiovascular risk profile and patient characteristics between statin users and non-users. However, none of these differences was associated with the levels of proteases, neither in univariate nor in multivariate analyses. The number of patients was too low to assess dose-response phenomena. Start date of statin use was not known for all patients, but was not likely to have influenced our results, since statins were reported to influence the vessel wall already after 3 weeks of treatment,[30] and in our study nearly all patients used their statins at least one month prior to surgery. Finally, this is an observational cohort study evaluating possible effects of statins on histological characteristics and proteases in AAA walls, and should therefore be considered as hypothesis generating. In order to generate further evidence for effects of statins on AAA wall components confirmative randomized trials are required.

The higher tendencies of MMP8 and 9 associated with pravastatin use indicate possible pleiotropic differences among the different statins. The effects of statins on alterations in AAA pathogenesis and the slowing down of AAA progression may be based on other proteases and inflammation markers than those measured in our study. We could not confirm results from previous findings of statins on AAA wall biology. The mechanisms of actions of statins on various AAA wall components, and biomarkers of those, remain to be unrevealed. Prescribing a low dose statin for stabilizing a patients' AAA is still not warranted when solely based on changes in AAA wall composition. Statin use should be advocated in line with clinical guidelines in this population with many cardiovascular risk factors and co-morbidities. Prospective clinical trials are needed to determine the exact effects of statins on AAA progression and ultimately rupture.

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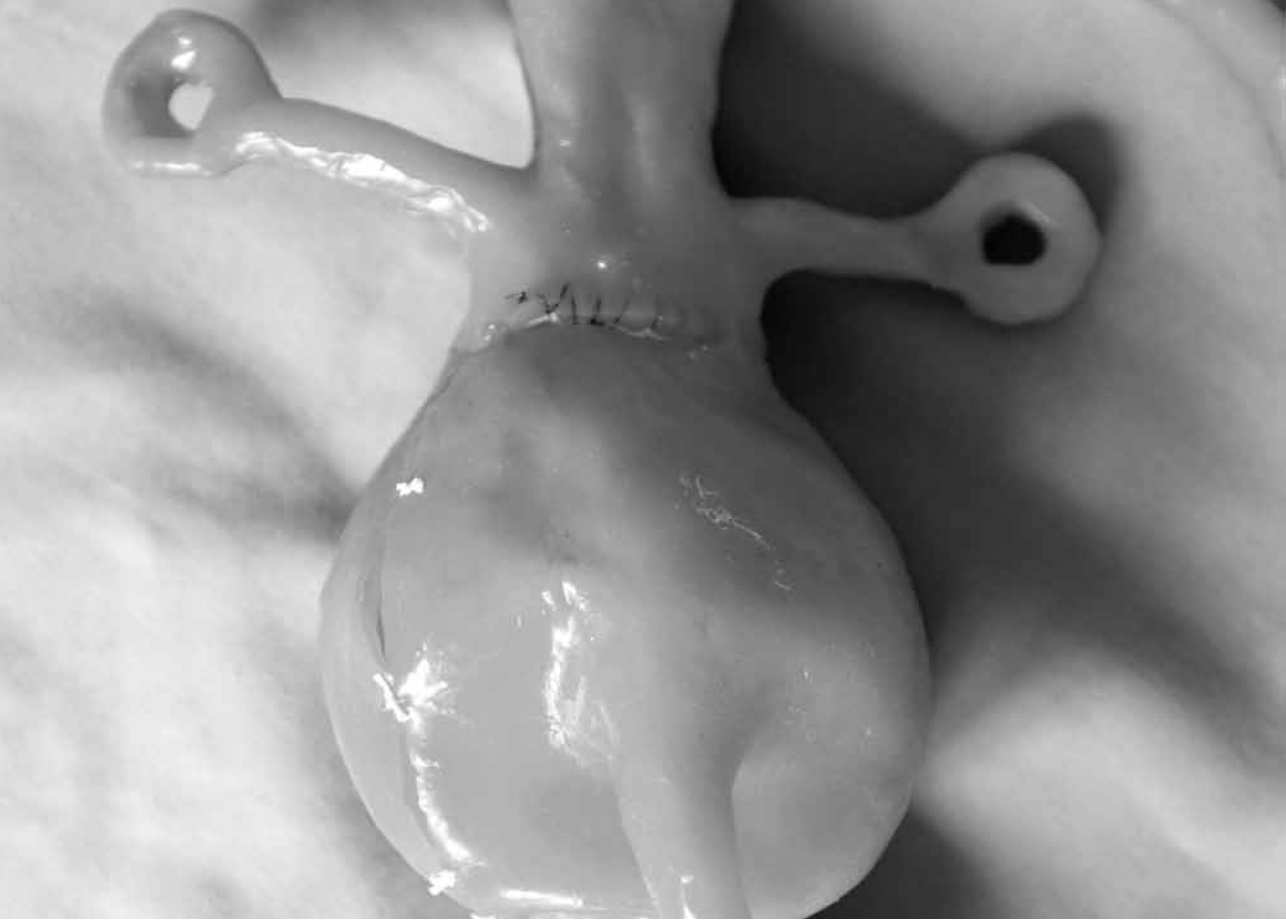

Atherosclerotic risk factors, advanced atherosclerotic lesions, and postoperative events are associated with low inflammation in abdominal aortic aneurysms

7

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Submitted

Abstract

Objective

Evidence is emerging that abdominal aortic aneurysm (AAA) formation cannot completely be explained by atherosclerosis and is in part due to other pathophysiological mechanisms such as local immune reactions. We studied the relation between AAA wall composition and manifestations and risk factors of systemic atherosclerotic disease.

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 low-inflammatory AAAs from 105 high-inflammatory AAAs. The walls of high-inflammatory AAAs had higher concentrations of various inflammatory markers, including interleukin (IL) 6, IL8, matrix metalloproteinase (MMP) 8; however, MMP9 levels were comparable. Patients with low-inflammatory 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 low-inflammatory AAAs (66% vs 52%, P =.026). More postoperative atherosclerotic events occurred in patients with low-inflammatory AAAs (24% vs 14%; HR 2.10 [1.07-3.96]).

Conclusion

Low amount of inflammation in AAAs is associated with more atherosclerotic risk factors, more advanced atherosclerotic lesions and more postoperative atherosclerotic adverse events. This observation supports the view that AAA development cannot completely be explained by atherosclerosis. Other factors such as local inflammatory reaction might play a role, at least in a subgroup of AAA patients.

Introduction

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]

Recent evidence is emerging that the development of AAA cannot completely be explained by 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.[11-13]

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.[14-17] 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.[18] 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.[19]

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 the present study was to study AAA wall inflammation in relation to manifestations and risk factors of systemic atherosclerotic disease.

Methods

Aneurysm-express biobank

Aneurysm-express is a longitudinal vascular biobank study that includes biomaterials 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.[20]

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.[21] 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.[21] Patients completed an extensive questionnaire based on the Rose cardiovascular survey.[22] Inconsistencies were resolved by contacting the patient's referring hospital or general practitioner.

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. Consecutive sections were stained with hematoxylin and eosin (H&E), elastin von Gieson (EvG), and Sirius red, as well as α-actin, von Willebrand factor (vWF), CD68, CD45, CD3, CD20, and CD138 immunostains. 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). Presence or absence of an extracellular lipid core (atheroma) in the intima was scored using the hematoxylin and eosin (H&E) and Sirius red stainings. In the media, the percentage of elastin fiber disruption was scored (the part of the media where no elastic fibers were present). Staining with von Willebrand factor (vWF) was used to assess vessel density, as described previously.[23] Briefly, in the media and adventitia, 5 hotspots were identified and vWF-positive microvessels were counted at 100× magnification. Subsequently, the number of microvessels per square millimeter was calculated.
Infiltration of macrophages (CD68), lymphocytes (CD45), and plasma cells (CD138) were scored as (1) minor or (2) moderate to heavy staining in intima and media combined and in the adventitia separately. Minor staining was defined as fewer than 100 positively stained cells per representative high power field at ×100 magnification, subsequently moderate to heavy staining was defined as more than 100 positively stained cells meeting the same conditions. To assess the composition of the lymphocytic infiltrate, percentages of total lymphocytes were measured for CD3-positive T lymphocytes and CD20-positive B lymphocytes.
The adventitial infiltrate was also quantitatively measured, and for this analysis, we scanned

all H&E and EvG slides using a ScanScope XT scanner (Aperio, Vista, CA, USA). This method of digitalizing slides in high resolution in our institution has been described before.[24] Elastin von Gieson (EvG) staining was used to determine the location of the media and thereby define the inner border of the adventitia, whereas perivascular fatty tissue formed the outer border of the adventitia. Aperio ImageScope software was used to define and measure surface areas of the inflammatory infiltrates and of the total adventitia at ×100 magnification on the H&E slides. The percentage of the adventitia covered with inflammation was quantified by dividing the area covered with inflammatory infiltrates by the total adventitial surface area.

Segments adjacent to those used for histology were snap frozen using liquid nitrogen and stored at - 80°C. These aortic segments were crushed in liquid nitrogen, and protein was isolated using a Tris isolation buffer (Roche, Basel, Switzerland). Samples were filtered via a Spin-X filter column (0.22-µm Nylon pores), and protein concentrations were assessed using the BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL, USA). The isolation method, the filter step, and the protein concentration measurements were performed according to the manufacturers' protocol.

Total amounts of matrix metalloproteinase (MMP) 8 and 9, cathepsin A and S, and CCL5/RANTES were determined via the Bio-Plex system using Luminex multianalyte profiling technology, as described previously.[25] Levels of interleukin (IL) 1β, 2, 3, 4, 5, 6, 8, 10, 12p70, interferon (IFN) γ, and tumor necrosis factor (TNF) α and β were quantified by fluorescent bead immunoassay (Bendermed Systems, Vienna, Austria).

Clinical outcome and follow-up

Cardiovascular follow-up was collected using clinical records for occurring events and interventions during hospitalization and during routine postoperative visits to the vascular surgeon. The patients were also mailed questionnaires at 1, 2, and 3 years after the operation. If the patients did not respond, the general practitioner was contacted by telephone to obtain cardiovascular follow-up. In case of a cardiovascular event, intervention, or death, the referring hospital or general practitioner was contacted to acquire detailed medical records. Reported events were judged by an outcome event committee consisting of 3 physicians (F.M., J.P.d.V. and R.H.) who were blinded to histological results. All events were independently assessed by 2 members of the committee; in case of disagreement, the third member was consulted. The primary outcome measure was a composite of all atherosclerotic events and interventions, including vascular related death, nonfatal myocardial infarction, nonfatal cerebral infarction, and vascular intervention for ischemic events.

Group selection

Adventitial inflammation outnumbers inflammation in other layers of the AAA wall, with lymphocytes being the most widely present inflammatory cell.[12, 26, 27] Because the adventitia is thought to play the most prominent role in AAA pathogenesis,[12] we divided patients according to the extent of adventitial inflammatory infiltrate. A clear variation of this infiltrate inside the aneurysmal wall was demonstrated before.[19] AAAs were considered inflammatory when histologic analyses showed the presence of adventitial lymphocytes as more than 100 positively stained cells per representative high power field at ×100 magnification. Groups of low-inflammatory AAAs (minor lymphocyte staining) and high-inflammatory AAAs (moderate to

heavy lymphocyte staining) were distinguished. To validate this distinction and the scoring method, we quantified the adventitial area covered by total inflammation.

Data analysis

Groups were compared using χ^2 and Mann-Whitney U tests for discrete and continuous variables, respectively. Kaplan-Meier survival analysis was used to estimate cumulative event rates during postoperative follow-up, and single predictor Cox regression analysis was used to measure differences between groups in number and timing of events during follow-up. Probability scores of $P < 0.05$ were considered statistically significant. All analyses were performed with SPSS 15 software (SPSS Inc, Chicago, IL, USA).

Results

Clinical characteristics of the 201 patients are summarized in Table 1. The level of adventitial lymphocytic infiltrate was used to document 105 patients (52%) with high-inflammatory AAAs and 96 (48%) with a low-inflammatory AAA. Assessment of the percentage of adventitia covered with inflammation also confirmed this variance by forming a bimodal distribution consisting of a peak with low-inflammatory and a peak of high-inflammatory AAA (Figure 1). Representative histological photomicrographs are shown in Figure 2.

Patients with low-inflammatory AAA had a higher prevalence of type 2 diabetes (22% vs. 9%, P = .008), hypertension (81% vs. 66%, P = .019), and higher serum cholesterol levels (mean

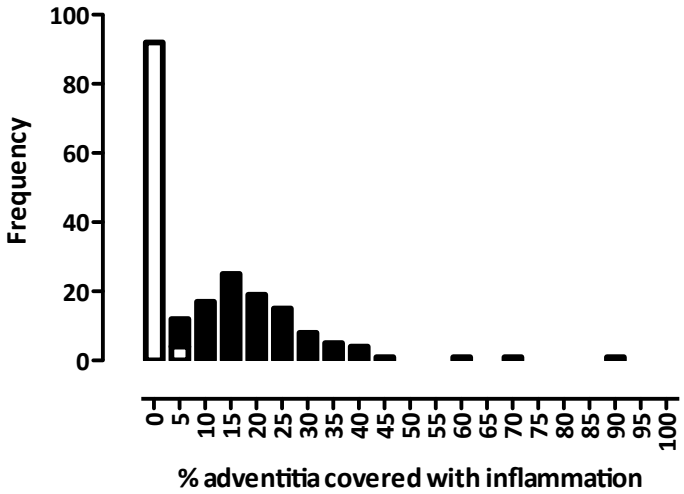

Figure 1 | Distribution of the proportion adventitia covered with inflammation.
This graph shows the bimodal distribution of the percentage adventitia that is covered with inflammation. Low-inflammatory AAAs (white bars, based on lymphocyte content) form a peak in the lower range (0% to 5%), whereas the high-inflammatory AAAs (black bars, based on lymphocyte content) build a second peak in the higher range of inflammation.

[SD]; 5.2 [2.5] vs. 4.2 [1.0] mmol/L; P = .023; Table 1) than patients with high-inflammatory AAA. Use of potential pleiotropic drugs did not differ between both groups (Table 1). No differences in symptomatic AAAs were found between both groups (10% for low-inflammatory and 13% for high-inflammatory AAA, P = .524). AAA diameter was similar in both groups (64.8 [SD, 12.1] for low-inflammation and 62.7 [SD, 2.8] mm for high-inflammation, P = .174).
Patients with low-inflammatory AAA had a more detrimental postoperative follow-up with more atherosclerotic events (hazard ratio, 2.10; 95% confidence interval, 1.07-3.96; Figure 3). This observation could be partly explained by a larger percentage of postoperative in-hospital cardiovascular complications (14% vs. 4%, P = .020), including 9 nonfatal myocardial infarctions, 7 nonfatal ischemic strokes, 2 vascular deaths, and 20 vascular interventions for ischemic events, of which 5 involved coronary arteries and 15 involved peripheral arteries. No aneurysm related events were observed in the follow-up period.
The inflammatory infiltrate in the adventitia mainly consisted of lymphocytes (Table 2). In a

Table 1 | Clinical characteristics of the study population.

Characteristics *	High-inflammatory AAAs (n = 105)	Low-inflammatory AAAs (n = 96)	P Value
Age, mean (SD), y	68.9 (8.1)	70.6 (7.5)	.116
Male sex	82 (78)	82 (85)	.115
Current smoker	52 (50)	40 (42)	.345
Diabetes mellitus type 2	9 (9)	21 (22)	.008 †
Hypertension	69 (66)	78 (81)	.019 †
Coronary artery disease	27 (26)	32 (33)	.344
Peripheral artery disease	15 (14)	19 (20)	.298
Chronic obstructive pulmonary disease	26 (25)	16 (17)	.159
Body mass index, mean (SD), kg/m^2	26.0 (4.1)	26.0 (3.3)	.541
Serum cholesterol, mean (SD), mmol/L	4.2 (1.0)	5.2 (2.5)	.023 †
Serum creatinine, mean (SD), μmol/L	97.4 (40.4)	102.0 (52.0)	.390
Glomerular filtration rate, mean (SD), mL/min/1.73m^2	75.1 (26.1)	74.7 (24.4)	.682
AAA diameter, mean (SD), mm	64.8 (12.1)	62.7 (12.8)	.174
Symptoms attributable to AAA	14 (13)	10 (10)	.524
History of any other aneurysm detected	12 (11)	11 (11)	.986
Statin use	49 (47)	50 (52)	.410
Aspirin use	71 (68)	64 (67)	.944
Angiotensin-converting enzyme inhibitor use	33 (31)	33 (34)	.461
Angiotensin II receptor blocker use	17 (16)	17 (18)	.565
Hospital stay, mean (SD), d	11.8 (6.5)	15.1 (14.3)	.437
Atherosclerotic events during hospitalization	4 (4)	13 (14)	.012 †

* 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. † P <.05.

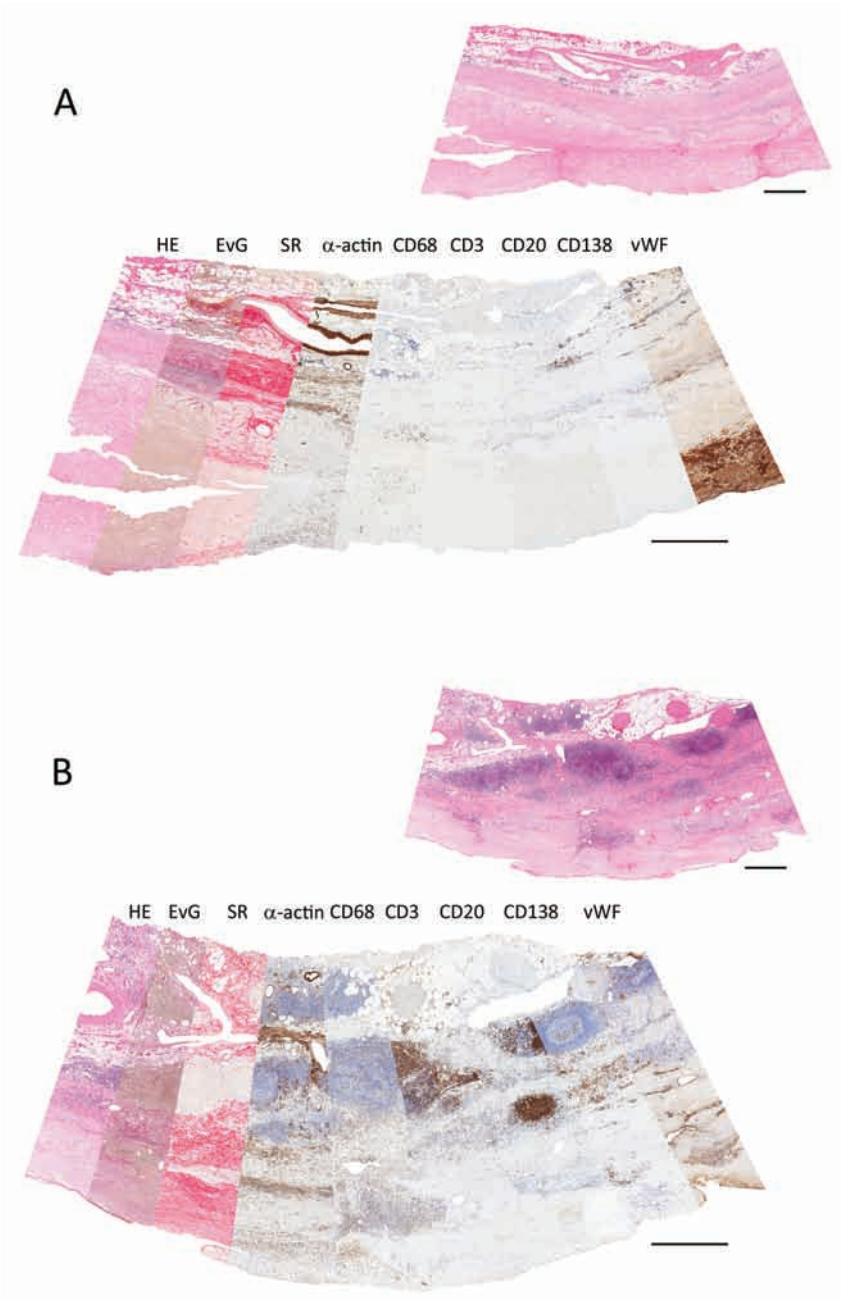

Figure 2 | Histology of low- and high-inflammatory AAA.
A: Representative histology of low-inflammatory AAA. B: Representative histology of high-inflammatory AAA. Hematoxylin and eosin (H&E) stainings of the ventral AAA wall are accompanied by larger composite figures of the same part composed of different stainings on consecutive slides. From left to right: H&E, elastin von Gieson (EvG), Picro Sirius red (SR), α-actin, CD68 macrophages, CD3 T lymphocytes, CD20 B lymphocytes, CD138 plasma cells, and von Willebrand factor (vWF). Scale bars indicate 1 mm and are located at the luminal side of the aneurysm wall. Please note the adventitial inflammation, which is hardly present in A and is abundant in B. The composite picture of B illustrates the distribution of the different types of inflammatory cells in the infiltrate.

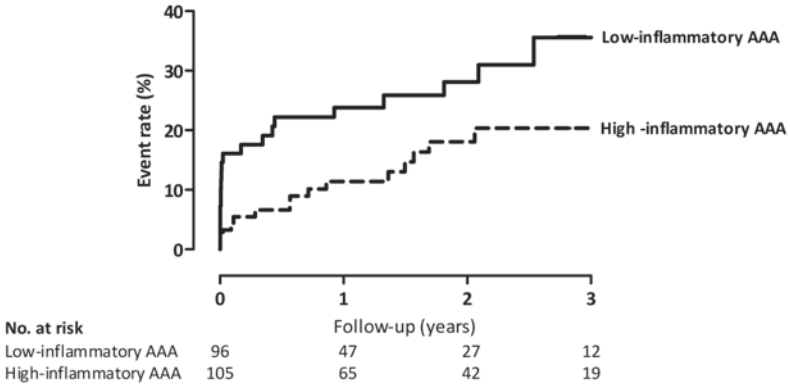

Figure 3 | Atherosclerotic event rate during follow-up in relation to AAA inflammation.
The solid line represents the patients with low-inflammatory AAA, and the dashed line represents patients with high-inflammatory AAA. The number of patients at risk in both groups is included.

number of specimens, heavy macrophage and plasma cell staining was also observed in the adventitia. Lymphocytic infiltrate in the adventitia consisted of 60% B cells, whereas 80% of lymphocytes in the intima and media were T lymphocytes.

Low-inflammatory AAAs revealed more often a cholesterol-rich lipid core in the intima than high-inflammatory AAAs (66% and 52%, respectively; P = .026). Furthermore, a high-inflammatory AAA phenotype was associated with decreased SMC staining in the media (27% vs 43%, P = .023) and an increase in adventitial myofibroblasts (49% vs 26%, P = .001). Microvessels were more abundantly present in the high-inflammatory group (median [interquartile range]; 83 [65-103] vs 67 [55-89], P = .016).

The high-inflammatory phenotype was associated with higher expression levels of several inflammatory markers, such as IL6, IL8, and CCL5/RANTES. Protease levels for cathepsin A, S, and MMP8 were higher in the high-inflammatory group, whereas no significant difference in MMP9 expression was observed between both groups. Measured inflammatory markers and protease levels are reported in Table 3.

Table 2 | Histologic Characteristics of the Study Population.

Aneurysm Wall Characteristics *	**High-inflammatory AAAs (n = 105)**	**Low-inflammatory AAAs (n = 96)**	***P* Value**
Structural components			
Intima			
Cholesterol core, presence, No. (%)	55 (52)	63 (66)	.026 †
Collagen	38 (36)	29 (31)	.367
Smooth muscle cells	24 (23)	22 (23)	.967
Media			
% Elastin disruption, median [IQR]	40 [15-80]	38 [5-80]	.466
Smooth muscle cells	28 (27)	41 (43)	.023 †
Adventitia			
Collagen	82 (78)	68 (71)	.248
Myofibroblasts	51 (49)	25 (26)	.001 †
Microvessels, median [IQR]	83 [65-103]	67 [55-89]	.016 †
Inflammation			
Intima/media			
Lymphocytes	8 (8)	0 (0)	.006 †
% T lymphocytes, median [IQR]	80 [60-100]		
% B lymphocytes, median [IQR]	20 [0-40]		
Plasma cells	5 (5)	0 (0)	.032 †
Macrophages	56 (53)	43 (45)	.252
Adventitia	21.0 [15.1-27.6]	2.2 [1.0-3.6]	<.001 †
% covered by inflammation, median [IQR]	105 (100)	0 (0)	<.001 †
Lymphocytes	40 [30-60]		
% T lymphocytes, median [IQR]	60 [40-70]		
% B lymphocytes, median [IQR]	52 (50)	9 (9)	<.001 †
Plasma cells	24 (23)	11 (11)	.042 †
Macrophages			

* Data are presented as No. (%) of heavy staining vs minor staining unless otherwise indicated. † P <.05.

Table 3 | Inflammatory Markers and Proteases in the AAA Wall.

Aneurysm Wall Characteristics *	High-inflammatory AAAs (n = 105)	Low-inflammatory AAAs (n = 96)	*P* Value
IL 1β	0.04 [0.00-0.92]	0.00 [0.00-0.29]	.039 †
IL 2	1.97 [0.83-4.81]	1.76 [0.65-3.46]	.394
IL 4	0.08 [0.00-1.37]	0.00 [0.00-0.42]	.039 †
IL 5	1.09 [0.00-3.31]	0.57 [0.09-1.33]	.142
IL 6	1.69 [0.40-11.38]	0.72 [0.12-4.36]	.023 †
IL 8	24.17 [12.70-53.47]	15.33 [4.68-41.38]	.015 †
IL 10	0.47 [0.00-1.29]	0.19 [0.00-1.02]	.175
IL 12	0.68 [0.00-2.02]	0.21 [0.00-0.69]	.011 †
TNF-α	0.38 [0.00-1.02]	0.08 [0.00-0.64]	.112
TNF-β	0.62 [0.00-3.70]	0.17 [0.00-1.42]	.095
IFN-γ	0.84 [0.01-2.22]	0.46 [0.00-1.09]	.063
CCL5/RANTES, a.u.	32.06 [16.90-158.84]	22.03 [4.62-60.58]	.009 †
MMP 8, a.u.	23.87 [12.45-40.84]	13.20 [6.03-32.27]	.004 †
MMP 9, a.u.	79.27 [38.49-154.51]	78.03 [29.03-205.52]	.823
Cathepsin A, a.u.	33.30 [15.01-60.20]	19.73 [9.89-30.80]	.003 †
Cathepsin S, a.u.	18.78 [13.97-24.26]	15.10 [11.64-20.48]	.007 †

*Data are presented as median and [interquartile range]. Units are pg/mL unless otherwise indicated. P values compare AAA phenotype. †P <.05.

Discussion

Evidence is emerging that multiple mechanisms are responsible for AAA formation and that the relative importance of these different mechanisms is likely to vary between patients.[4] To our knowledge, this is the first histological study to report associations between clinical atherosclerotic features and AAA wall composition. We found that AAAs with a relatively low amount of inflammation are associated with more cardiovascular risk factors, more cardiovascular events during follow-up, and more frequently show an intimal cholesterol-rich lipid core. This observation suggests a closer relation between generalized atherosclerotic disease and AAA formation in patients with a low amount of inflammation in their AAA than in patients with a high amount of AAA inflammation.

Diabetes, hypertension, and hypercholesterolemia were more prevalent in patients with low-inflammatory AAA. All of these risk factors are strongly associated with atherosclerotic disease but are relatively weak risk factors for AAA formation.[2, 28] Strikingly, diabetes mellitus has previously been described to have an inverse relation with AAA presence.[2] Patients with high- and low-inflammatory AAAs did show differences in cardiovascular risk profile, but not in history of cardiovascular disease. A possible explanation might be the fact that inclusion depends on diagnosis of the AAA. Because AAA is an asymptomatic disease in most cases, diagnosis and subsequent intervention depends on diagnostics for other diseases, predominantly atherosclerosis.

Patients with low-inflammatory AAA revealed more cardiovascular events during follow-up than patients with high-inflammatory AAA. Most of these events occurred in the first days after the operation. Preventive repair is associated with high morbidity and mortality, and this rate rises with increasing atherosclerotic risk factors and accompanying manifested disease.[29, 30] In the present study, the patients in the low-inflammatory AAA were associated with more cardiovascular risk factors and with more atherosclerotic events, suggesting a higher atherosclerotic disease burden in other vascular territories.

Low-inflammatory AAAs more often revealed a cholesterol-rich lipid core in the aortic intimal lesions. The presence of an extracellular lipid core in intimal lesions is a feature of advanced atherosclerotic lesions.[31] This observation suggests that more advanced atherosclerotic lesions were present in low-inflammatory AAAs. In addition to presence of these lesions, low-inflammatory AAA also had higher serum cholesterol levels, confirming a previous report in which high serum cholesterol levels were associated with the presence and size of an atheroma.[32]

The walls of high-inflammatory AAAs revealed higher quantities of cytokines, such as IL6, IL8, and RANTES, and proteases, such as cathepsin A and S and MMP8. This observation suggests that the degenerative process is more active than in the aortic walls of low-inflammatory AAAs. The enhanced activity in inflammatory AAA possibly leads to weakening of the wall of these AAAs with subsequent progression.[33] Together with the demonstrated bimodal distribution of amount of adventitial inflammation in the present study, this might form a possible explanation for a more pronounced expansion rate in half of the small-diameter AAAs that was reported in a recent follow-up study of growth rate.[18]

MMP9 is the most extensively studied and most abundantly present protease in the AAA wall,[34, 35] and did not differ between high- and low-inflammatory AAAs in this study. Previous work has demonstrated that MMP9 expression in aortic aneurysms is not limited to inflammatory cells, because medial SMCs and endothelial cells also express MMP9.[36] The results of the present study support the idea that non-inflammatory cells such as SMCs significantly contribute to MMP9 production.

Patients with relatively high amounts of inflammation had less evidence of systemic atherosclerosis and less atherosclerotic risk factors. This observation might suggest that in patients with high amounts of inflammation other local factors contribute to the pathophysiological development of the AAA. Recent insights in AAA pathogenesis have introduced the concept that autoimmunity may be involved in the pathogenesis of AAA. Substantial evidence has been accumulated suggesting that a specific antigen-driven T-cell response may be responsible for the initiation and/or the propagation of the disease.[12] Although further studies are needed for confirmation, our observation with relatively large amounts of T-cells in the wall of AAAs in patients with a non-atherosclerotic profile supports the hypothesis that a local autoimmune reaction is involved in AAA development in part of the patients.

This study has some limitations. Research on the initiation, progression, and rupture of AAA, including the present study, is hampered by only assessing late-stage disease. Human tissue only becomes available when patients undergo open surgical repair based on current guidelines, predominantly having large diameter AAAs. Therefore, temporal changes cannot be taken into account because tissue collection is only feasible at one moment in time. This infers difficulties in translating knowledge to (early) pathogenesis.

An interesting next step could be to visualize the metabolically active inflammatory infiltrate in the aneurysm wall using fludeoxyglucose F 18 via positron emission tomography/computed tomography (PET/CT). Metabolic activity on PET/CT correlates well with histologic inflammation in the aneurysm wall, and a large variance in FDG uptake has been reported.[37, 38] It would be of great interest to prospectively monitor patients with smaller aneurysms with PET/CT, to translate present findings to a broader group of patients (not limited to patients undergoing open AAA repair), and to assess correlations of wall inflammation with progression and outcome of the AAAs.

In conclusion, low-inflammatory AAAs are associated with more atherosclerotic risk factors, more postoperative atherosclerotic events, and more advanced atherosclerotic lesions. 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. Further research is needed to analyze the relation between AAA wall inflammation and progression and prognosis, and subsequently, whether AAA wall composition should influence treatment strategies.

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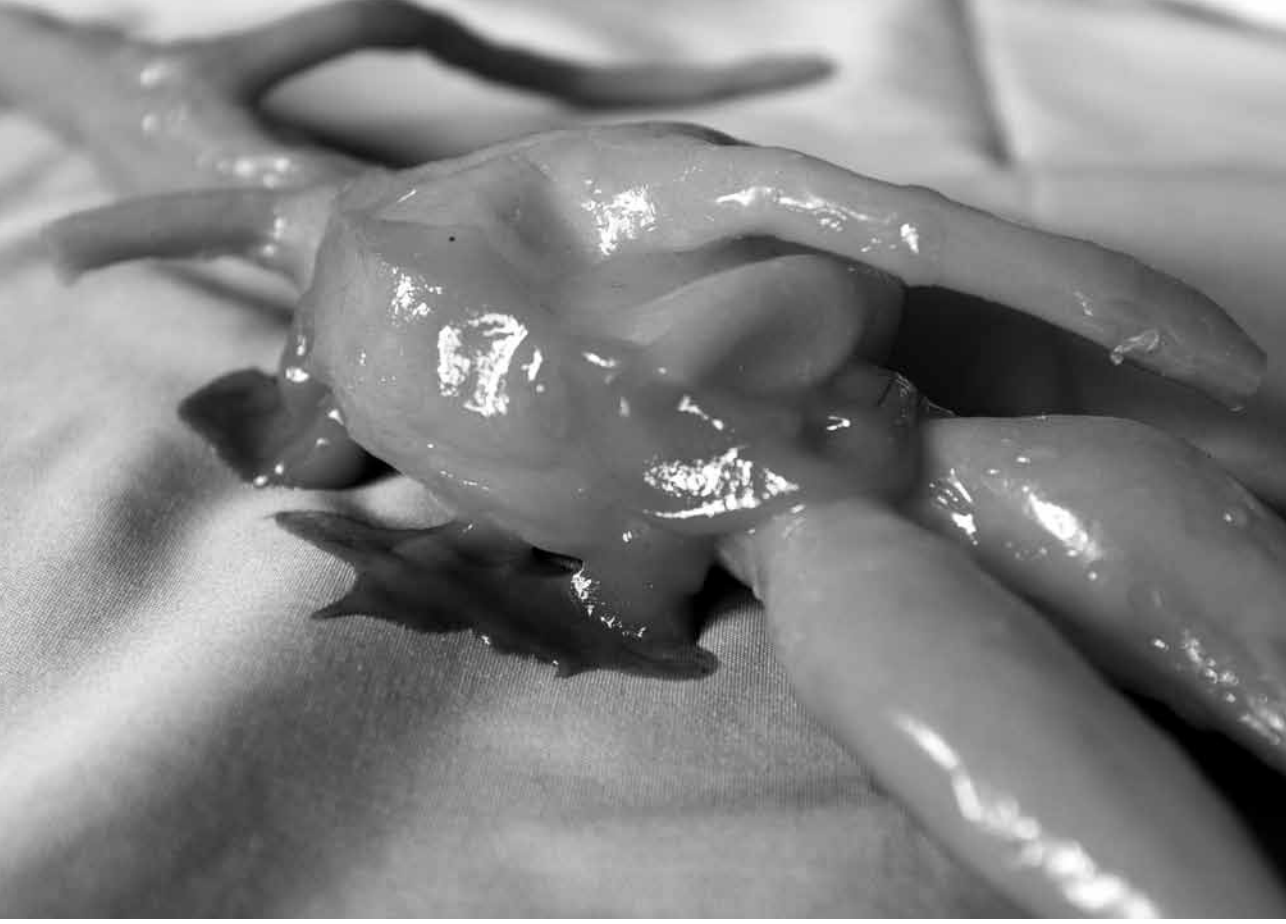

Circumferential heterogeneity in the abdominal aortic aneurysm wall composition suggests lateral sides to be more rupture prone

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Abstract

Objective

We aimed to identify local differences in inflammation and tissue degradation within the circumference of the abdominal aortic aneurysm (AAA).

Background

AAAs have the potential to rupture, and it is unknown why this predominantly occurs at the posterolateral wall. Blood flow dynamics likely influence rupture location but do not explain the whole picture, suggesting that other factors inside the AAA wall have a prominent role.

Methods

As part of the Aneurysm-Express study, full thickness circular biopsy specimens of AAAs from 25 patients were obtained during surgery according to a standardized protocol. Tissue from the dorsal, ventral, and lateral sides was processed for histology and protein extraction. Levels of matrix metalloproteinase (MMP) 2 and 9 and various cytokines were measured.

Results

Lateral AAA sites, when compared with the ventral and dorsal segments, showed more microvessels (median [interquartile range] per mm^2: 91.8 [72.6-124.6] vs 73.9 [63.0-108.0] and 73.6 [52.7-109.5]; P =.013 and P =.005, respectively) and more adventitial inflammation (16.1% [13.5%-24.7%] vs. 5.8% [2.8%-18.6%] and 6.3% [4.3%-13.5%]; P =.001 and P<.001, respectively). We observed a higher active MMP 9 (0.139 [0.059-0.339]ng/mL vs 0.060 [0.000-0.157]ng/mL and 0.045 [0.000-0.147]ng/mL ; P =.001 and P =.014, respectively) and higher interleukin 8 (28.644 [11.921-62.587]pg/mL vs 16.442 [4.300-34.130]pg/mL and 18.258 [8.273-44.989]pg/mL; P<.001 and P =.010, respectively).

Conclusions

Biopsy specimens of the ventral AAA wall do not optimally reflect the magnitude of inflammatory processes in the AAA. The lateral sides of the AAA contain more microvessels, more inflammatory cells, more active proteases, and higher cytokine levels. These results suggest that the lateral aortic regions are more rupture-prone and may better reflect the inflammatory status in histopathologic examinations.

Introduction

Abdominal aortic aneurysm (AAA) rupture has a 30-day mortality rate of more than 60%.[1] Owing to aging of the population in Western countries, AAA disease is becoming more prevalent, with a subsequent rise in associated mortality.[2-4] The pathogenesis of AAA formation still remains to be clarified, which may be partly explained by the limited number of available animal models for AAA formation. In addition, pathologic studies on human aneurysm tissue are restricted to small sample numbers. The availability of AAA tissue samples may become more limited in the near future due to the increasing application of endovascular repair of AAA.[5]

An AAA is a large structure, which by definition exceeds 3 cm but can range up to 17 cm in diameter.[6] Histopathologic research on human AAA has mainly been focused on a paraincisional part of the ventral AAA wall. Despite this focus, it is unknown whether a ventral wall specimen is representative for the entire circumference of the AAA.[7] In addition, aneurysm rupture is a localized process, predominantly occurring at the posterolateral part of the AAA, as reported by autopsy studies.[6, 8]

Our group previously showed that the aortic pulsatile distention is asymmetric, indicating differing function and thereby wall composition for the different regions within the circumference.[9] The morphology of AAAs is asymmetrical. Blood flow patterns and wall stress measurements show pronounced local differences, with subsequent computational models indicating that geometry contributes more to AAA expansion than patient risk profile.[10] On positron emission tomography-computed tomography, metabolic activity inside the AAA wall also shows a heterogeneous pattern,[11] all of which is supportive for presence of regional variance in wall composition within the AAA.

Our aim was to investigate local circumferential differences in aneurysm wall components at the level of the maximal diameter. We had a specific interest in the extent of inflammation and protease levels because this potentially causes wall vulnerability and might accelerate AAA expansion and increase rupture risk. We hypothesized that lateral segments would differ from ventral and dorsal segments given the asymmetric distention, morphology, wall stress, and rupture location.

Methods

Aneurysm-express study

The present work is part of the Aneurysm-express biobank study, a prospective cohort study collecting aneurysm tissue from patients undergoing open AAA repair. Its design was described previously.[12] Briefly, indication for open surgery is set according to international standards.[13] The ethical review boards of the two participating centers approved this study. Consecutive patients scheduled for open AAA surgery were enrolled, and written informed consent was obtained from all patients. Cardiovascular risk factors, medical history, and medication use were recorded at baseline. Aneurysm diameter was determined by computed tomography angiogram. Patients completed an extensive questionnaire, based on the Rose cardiovascular survey.[14]

Tissue collection and processing

During elective open AAA repair, a full thickness circular specimen was obtained at the site of the maximum diameter, shortly after placing the proximal and distal clamps and sewing in the prosthesis, and when local adhesions were absent. This tissue was immediately processed in the operating room according to a strict protocol, as shown in Figure 1. We maintained an equal distance between the different specimens that were designated for histology and protein extraction. Specimens obtained from the ventral, dorsal, and lateral sides were processed separately.

For a previous study, we collected postmortem normal infrarenal aortic specimens during autopsy.[12] Aortas exhibiting aneurysms or rupture were excluded. The entire circumference was used for histology.

The segments designated to histologic assessment were fixed in 4% formaldehyde and embedded in paraffin. Consecutive slides were stained with hematoxylin and eosin (H&E), elastin von Gieson (EvG), picrosirius red, and antibodies against α-actin, von Willebrand factor (vWF), CD68, CD45, CD3, CD20, and CD138. Extracellular matrix components were semiquantitatively scored as (1) minor or (2) moderate to heavy staining in intima, media, and adventitia separately for collagen (picrosirius red) and smooth muscle cells (SMC; α-actin). Elastin degradation was scored as the estimated percentage of disruption of elastin fibers.

Different components of the inflammatory infiltrate were scored as (1) minor or (2) moderate to heavy staining in intima and media combined and in the adventitia separately.

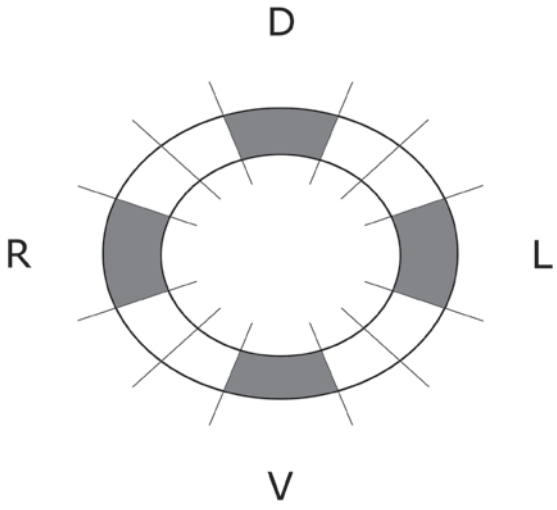

Figure 1 | Schematic overview shows tissue collection and processing.
Schematic overview shows abdominal aortic aneurysm (AAA) tissue collection and processing. A circular biopsy specimen at the site of maximum diameter was collected and cut into segments as illustrated. Dorsal (D), ventral (V), left (L), and right (R) lateral sides were identified. For each side, a segment of full-thickness AAA wall was processed (gray area) for histology and for protein extraction. Other segments were stored for future use.

Minor staining was defined as fewer than 100 positively stained cells per representative high power field at ×100 magnification, and moderate to heavy staining was defined as more than 100 positively stained cells meeting the same conditions. This was performed for macrophages (CD68), total lymphocytes (CD45), T lymphocytes (CD3), B lymphocytes (CD20), and plasma cells (CD138).

Histologic examination was performed by two independent observers (RH, AV), who were blinded from clinical data and laboratory results. In case of discrepancies in judgment, sections were reanalyzed with consensus being reached in all cases.

The adventitial infiltrate was also quantitatively measured. For this analysis, we scanned all H&E and EvG slides using a ScanScope XT scanner (Aperio, Vista, CA, USA). This method of digitalizing slides in high resolution in our institution was published before.[15] EvG staining was used to identify the media that formed the inner border of the adventitia, whereas perivascular fatty tissue defined the outer border of the adventitia. Aperio ImageScope software was used to define and measure surface areas of the inflammatory infiltrates and of the total adventitia at ×100 magnification on the H&E slides. The percentage of the adventitia covered with inflammation was quantified by dividing the area covered with inflammatory infiltrates by the total adventitial surface area.

Staining with vWF was used to measure vessel density, as described previously.[16] Briefly, 5 hotspots were identified in the media and adventitia, and vWF-positive microvessels were counted at ×100 magnification. Subsequently, the number of microvessels per square millimeter was calculated.

Adjacent specimens appointed for protein extraction were snap frozen with liquid nitrogen and stored at -80°C. These segments were later crushed in liquid nitrogen, and protein was isolated as described before.[12]

MMP activities were determined by using the Amersham MMP-2 and MMP-9 Biotrak Activity Assay System (GE Healthcare Ltd, Amersham, UK). Osteopontin (OPN) levels were measured by using enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA). Levels of interleukin (IL) 1β, 2, 4, 5, 6, 8, 10, 12p70, interferon (IFN) γ, and tumor necrosis factor (TNF)α and β were quantified in the aneurysmal wall by fluorescent bead immunoassay (Bendermed Systems, Vienna, Austria). All described procedures complied with the manufacturers' protocols.

Statistical analysis

Discrete variables are shown as frequencies and percentages of the total. Continuous variables are shown as median and interquartile range (IQR). For multiple, paired continuous data, a Friedman test was used to determine differences among groups. If the result was significant, the Wilcoxon sign test was used to determine which pairs differed. Likewise for discrete paired data, first a Cochrane Q test was performed and, if significant, followed by a McNemar χ^2 test. Values of $P < .05$ were considered significant. All analyses were performed with SPSS 17 software (SPSS Inc, Chicago, IL, USA).

Results

None of the patients had previous retroperitoneal surgery. Two patients were excluded due to presence of adhesions. Circular AAA specimens were collected from 25 patients, whose baseline characteristics are reported in Table 1. Symptoms attributable to the AAA were present in 3 patients, however, these patients neither had a thickened AAA wall on CT nor retroperitoneal fibrosis. The transverse diameter exceeded the anteroposterior diameter by 8% (5%-10%). The lateral segments of the AAAs were pooled for histology and protein analyses.

For a previous study, the entire circumference of normal infrarenal aortas was collected in autopsies of 27 patients (median age 54 [40-69] years, 14 males). The different regions within the aorta remained unmarked and systematic localized analyses were hence impossible. However, a histological assessment of the 27 specimens did not show any differences in structural and inflammatory characteristics around the circumference. For illustrative purposes, Figure 2 shows a representative photomicrograph of the circumferential homogenous normal aortic wall.

Detailed histologic characteristics for the different sites and results of the paired analyses of the groups are summarized in Table 2. Lateral and dorsal AAA segments revealed more frequently a cholesterol-rich lipid core compared with the ventral wall (56% and 48% vs 24%;

Table 1 | Baseline demographics

Patient characteristics	
Age, y	66 [64-77]
Male sex	19 (76)
Current smoker	10 (40)
Diabetes type 2	5 (20)
Hypertension	18 (72)
Coronary artery disease	4 (16)
Peripheral artery disease	6 (24)
Chronic obstructive pulmonary disease	2 (8)
Body mass index, kg/m^2	24.7 [22.8-27.1]
Serum creatinine, μmol/L	90 [81-117]
Aneurysm diameter, mm	60 [54-70]
Symptoms attributable to the AAA	3 (12)
History of any other aneurysm detected	2 (8)
Statin use	20 (80)
Aspirin use	16 (64)
ACEI use	8 (32)
Angiotensin II receptor blocker use	4 (16)
Hospital stay, days	10.0 [8.3-13.5]

Data are presented as No. (%) or as median [interquartile range].
Abbreviations: AAA, abdominal aortic aneurysm; ACEI, angiotensin-converting enzyme inhibitor.

P =.016 and P =.031). Elastin content and SMCs in the media were similarly degraded in all groups. Collagen quantity was lower in the adventitia of the lateral wall (P =.065) and the dorsal wall (P =.008) than in the ventral wall. The lateral part of the AAA revealed higher amounts of microvessels per square millimeter compared with the ventral (24% higher; P =.013) and dorsal (25% higher; P =.005) parts.

Table 2 | Histological characteristics

AAA wall characteristics	Ventral	Lateral	Dorsal	*P* value
Structural components				
Intima				
Cholesterol core, presence	6 (24)	14 (56)	12 (48)	.032[a,b]
Collagen	9 (36)	3 (12)	6 (24)	.235
SMCs	6 (24)	3 (12)	3 (12)	.904
Media				
Elastin disruption, %	55.0 [2.5-80.0]	59.5 [41.3-95.0]	60.0 [30.0-96.8]	.475
SMCs	7 (28)	2 (8)	5 (20)	.307
Adventitia				
Collagen	20 (80)	13 (52)	10 (40)	.023[c]
SMCs	10 (40)	8 (32)	7 (28)	.423
Microvessels per mm^2	73.9 [63.0-108]	91.8 [72.6-124.6]	73.6 [52.7-109.5]	.035[a,d]
Inflammation				
Intima/media				
Lymphocytes	0 (0)	0 (0)	0 (0)	>.99
T lymphocytes	0 (0)	0 (0)	0 (0)	>.99
B lymphocytes	0 (0)	0 (0)	0 (0)	>.99
Plasma cells	0 (0)	0 (0)	0 (0)	>.99
Macrophages	7 (28)	8 (32)	8 (32)	.846
Adventitia				
Covered by inflammation, %	5.8 [2.8-18.6]	16.1 [13.5-24.7]	6.3 [4.5-13.5]	<.001[a,d]
Lymphocytes	10 (40)	18 (72)	9 (36)	.001[a,d]
T lymphocytes	9 (36)	14 (56)	6 (24)	.013[d]
B lymphocytes	12 (48)	18 (72)	8 (32)	<.001[a,d]
Plasma cells	4 (16)	4 (16)	5 (20)	.846
Macrophages	1 (4)	4 (16)	5 (20)	.309

Categoric data are presented as No. (%) of heavy staining as opposed to minor staining, unless otherwise indicated. Continuous data are presented as median [interquartile range].
P value results from a paired analysis of the groups, when < .05 a post hoc test was used to determine which groups differed: [a] lateral > ventral; [b] dorsal > ventral; [c] ventral > dorsal; [d] lateral > dorsal;
Abbreviations: AAA, abdominal aortic aneurysm; SMC, smooth muscle cell.

Analysis of infiltration of inflammatory cells found no differences between sites for the intima and media. In the adventitia, total lymphocytes were higher in lateral than in ventral (P =.008) or dorsal (P =.032) sites. An analyses of lymphocyte subgroups showed that the most frequently present cell mainly determined the differences: B lymphocytes were more often observed in lateral compared with ventral (P =.031) and dorsal wall (P =.001; Figure 3). For T lymphocytes, dorsal site staining was lower than in the lateral site (P =.008). These results were also reproduced by the quantitative assessment of the percentage adventitia that was covered by inflammatory cells: In lateral segments, the inflammatory infiltrate covered a 2.8-fold larger area of the adventitia than in ventral segments (P =.001) and a 2.6-fold larger area than in dorsal segments (P < .001; Figure 4).

The histologic differences were accompanied by the measured protease and cytokine concentrations (Table 3). Active MMP2 levels were higher in lateral segments compared with dorsal (P =.032), and active MMP9 was 2.3 times higher in lateral sites than in ventral sites (P =.001) and 3.1 times higher than in dorsal sites (P =.014). OPN concentrations were also elevated in lateral segments compared with ventral (P =.004) and had a higher tendency compared with dorsal (P =.058). The cytokine with the highest measured levels, IL8, was in the lateral sites 74% higher than in ventral and 57% higher than in dorsal sites (P < .001 and P =.010, respectively).

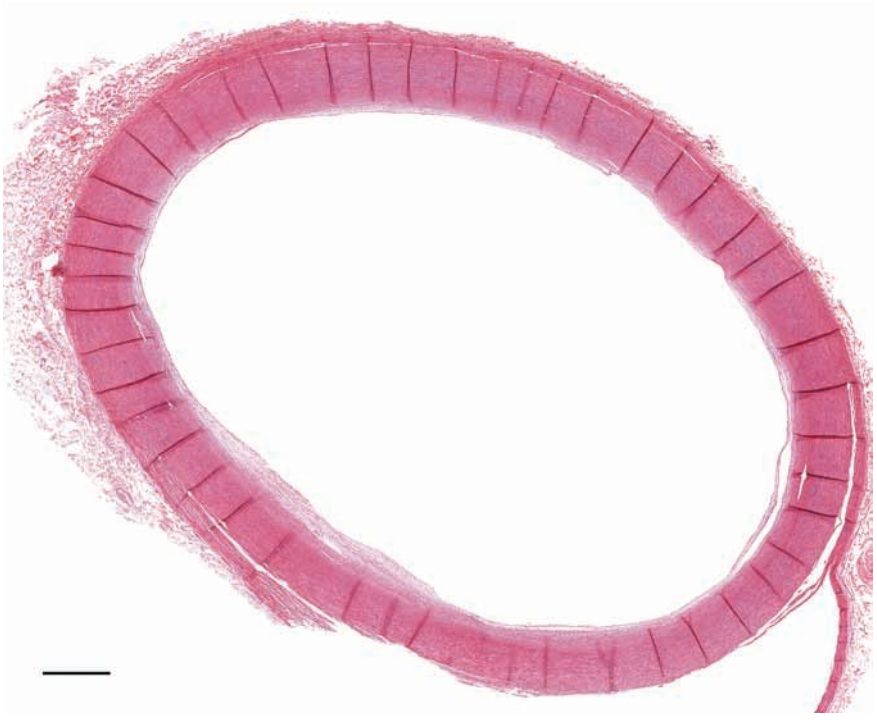

Figure 2 | Overview of the circumference of a normal aorta in H&E staining.
Representative photomicrograph shows the homogenous distribution of structural components and lack of inflammation in the normal infrarenal aorta. The scale bar represents 1 mm.

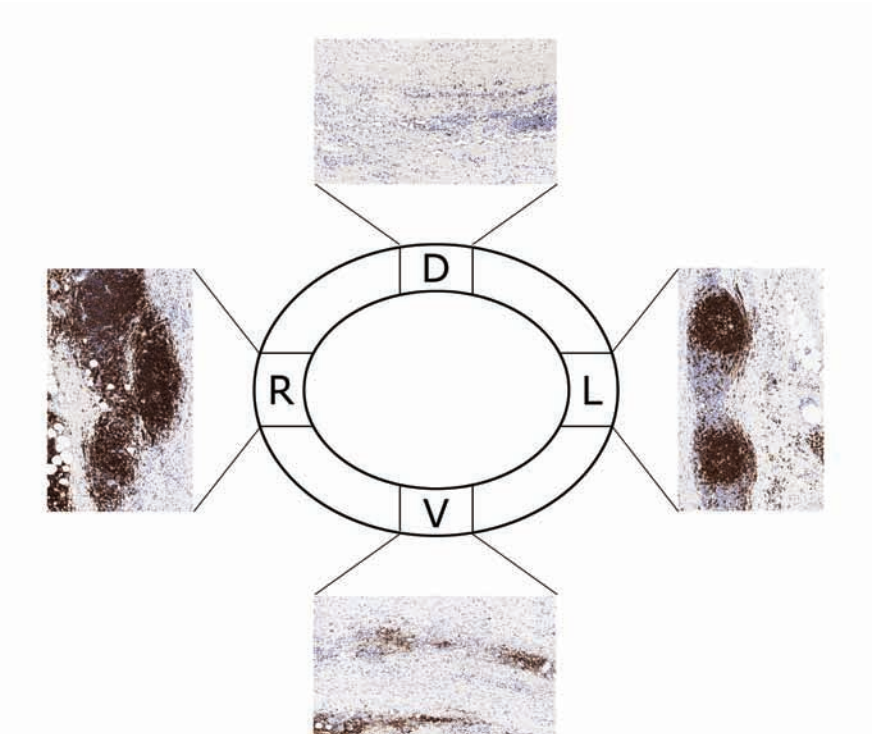

Figure 3 | Histological overview shows the different sites of the abdominal aortic aneurysm in the same patient, stained for CD20-positive B Lymphocytes.
Representative photomicrographs show different sites within one abdominal aortic aneurysm: Dorsal (D), ventral (V), left (L), and right (R) lateral segments surround the lumen. The intima is for all 4 regions located at the luminal side, and the adventitia forms the outer borders of the picture. Note the higher quantity of inflammation in both lateral sides. The scale bar represents 1 mm.

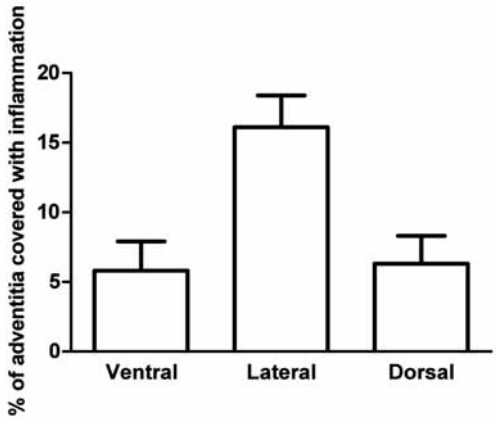

Figure 4 | Distribution of the proportion of the adventitia covered with inflammation in relation to different abdominal aortic aneurysm sites.
This graph shows the distribution of the percentage adventitia that is covered with inflammation. Note that there is a difference among groups (Friedman test $P < .001$), with lateral inflammation being higher than both ventral (Wilcoxon sign test P = .001) and dorsal (Wilcoxon sign test $P < .001$) segments.

Table 3 | Protease and cytokine levels

AAA wall characteristics	Ventral	Lateral	Dorsal	*P* value
MMP 2 active	0.112 [0.041-0.180]	0.133 [0.093-0.200]	0.114 [0.051-0.205]	.037[a]
MMP 9 active	0.060 [0.000-0.157]	0.139 [0.059-0.339]	0.045 [0.000-0.147]	.007[a,b]
Osteopontin	0.635 [0.000-1.083]	0.979 [0.242-4.748]	0.470 [0.000-1.101]	.007[b]
IL 1β	0.022 [0.000-0.936]	0.281 [0.000-0.405]	0.000 [0.000-0.317]	.142
IL 2	2.175 [0.959-5.515]	2.697 [0.682-4.049]	1.401 [0.837-4.188]	.258
IL 4	0.423 [0.000-2.152]	0.767 [0.127-1.454]	0.234 [0.033-1.138]	.192
IL 5	1.500 [0.268-4.449]	1.338 [0.609-3.456]	0.797 [0.253-2.438]	.332
IL 6	0.701 [0.129-1.633]	2.320 [0.680-9.911]	1.227 [0.398-3.525]	.782
IL 8	16.442 [4.300-34.130]	28.644 [11.921-62.587]	18.258 [8.273-44.989]	<.001[a,b]
IL 10	0.458 [0.000-1.377]	0.466 [0.221-0.800]	0.285 [0.000-0.785]	.707
IL 12	0.497 [0.000-2.366]	0.650 [0.113-1.505]	0.210 [0.000-1.103]	.292
TNF-α	0.267 [0.000-1.966]	0.238 [0.079-0.823]	0.128 [0.000-0.636]	.600
TNF-β	1.423 [0.000-4.049]	0.780 [0.106-2.162]	0.151 [0.000-2.711]	.560
INF-γ	0.506 [0.000-2.561]	1.008 [0.471-1.669]	0.533 [0.157-1.184]	.350

Data are presented as median and [interquartile range]. Units are ng/mL for MMP 2 and 8; and pg/mL for the other determinants. *P*-value results from a paired analysis of the groups, when $P < .05$ a post hoc test was used to determine which groups differed: [a] lateral > dorsal; [b] lateral > ventral.
Abbreviations: AAA, abdominal aortic aneurysm; MMP, matrix metalloproteinase; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon.

Discussion

Current research on human AAA tissue is based on specimens collected from part of the ventral wall during open surgical repair. The AAA is a large structure, and it is not known whether its constituents are homogeneously distributed. In addition, it is unknown whether the ventral wall, the most regularly studied part of the circumference, indeed represents the magnitude of inflammatory processes within the AAA. To our knowledge, we are the first to report heterogeneity inside the intact AAA: lateral sides exhibit more active inflammation with more microvessels, more inflammatory cells on histology, and higher protease and cytokine levels than the ventral and dorsal walls.

A previously collected sample of normal infrarenal aortas in which the different regions had not been marked was used to analyze the heterogeneity around the circumference of the vessel wall. We did not find any variation in the structural or inflammatory components. In regard to previously reported pro-inflammatory age-related alterations of the aorta[17] , our results suggest that changes are not necessarily restricted to specific segments. This observation differs substantially from findings in patients with developed AAA, who exhibited more inflammation-related changes in the lateral parts of the aortic wall.

The observed increased quantity of lateral inflammation corresponds well to the most common sites of AAA rupture, which is posterolateral in 80%.[18] Previous work demonstrated that the sites of rupture revealed higher quantities of MMP8 and MMP9 and more neovascularization compared with the intact ventral walls of the same patients.[19, 20] This indicates that the laterally increased quantity of microvessels and active proteases in intact AAAs described in present study predisposes this region of the AAA as being more vulnerable to rupture. In addition, we found the number of inflammatory cells and the concentrations of inflammation-stimulating cytokines OPN and IL8 were all increased at the lateral sites, possibly increasing vulnerability. In previous studies, IL8 was found to be higher in AAA when compared to normal aortas and it was associated with an upregulation of inflammation as well as increased neoangiogenesis.[21, 22] OPN has been described to promote inflammation, proteolysis, and atherosclerosis in experimental studies, which are all integrated processes of AAA development and progression.[23] In addition, an animal model linked OPN directly to AAA development; in humans OPN in serum was associated with AAA growth.[23, 24] A recent study that examined inflammation at the site of rupture reported no difference in IL6, TNF-α, and IL1β with the intact ventral wall.[25] Unfortunately, IL8 and OPN remained unmeasured in that study, which are the most prominent differing cytokines in the present study.

The described vulnerability of the lateral parts of the AAA in this study supports the concept that AAA expansion might be more pronounced laterally, which corresponds with reports in the literature of asymmetrical morphology and differences in flow patterns and wall stress.[10] A recent review on computational models for blood flow emphasized the need for taking AAA wall strength into account when analyzing the ability of wall stress to predict vulnerability and rupture.[26] AAA wall strength varies not only between different individuals (based on, for instance, occurrence of risk factors) but also within the same AAA. Furthermore, wall thickness decrease leads to an increase of peak wall stress, and asymmetry of the AAA is strongly related to wall stress distribution.[26] Just as in the current study, the anteroposterior diameter in most AAAs was smaller than the transverse diameter,[27] which alters the distribution of wall stress.

Combined with our finding of decreased adventitial collagen dorsally, wall stress further increases in the posterolateral part of the AAA. When combined with the increased vulnerability of the lateral regions of the AAA, this may further accelerate lateral expansion.

Serial studies evaluating local expansion within the circumference are lacking, and it would therefore be interesting to monitor patients with small-diameter AAA over time to assess circumferential growth patterns and, eventually, the site of rupture. This finding may also be relevant for the orientation of measuring maximum AAA diameter because large trials used maximum anteroposterior diameter,[28] transverse diameter, or both.[29] It was furthermore described that diameter asymmetry is relevant for determining rupture risk,[27] and current guidelines do not specify a gold standard for measuring AAA diameter.[13, 30]

A specimen of the ventral AAA wall does not necessarily represent the entire AAA wall. Data from small studies using human specimens need to be interpreted carefully, because we have already shown in a limited number of patients that marked regional differences exist. Large human AAA biobanks are necessary to even the effects of heterogeneity and to provide a reliable source of information for investigating pathogenesis.

Because preventive repair of AAA is associated with considerable cardiovascular morbidity and mortality, possible effects of medication on the AAA wall are receiving much attention. Postponing surgery has a substantial effect on diminishing the occurrence of repair-associated cardiovascular events. For statins, described effects are conflicting based on growth rate follow-up studies and ventral AAA tissue assessment in cohort studies.[31, 32] An explorative prospective randomized trial with administration of doxycycline showed a decrease in inflammation and proteases in the ventral wall.[33] The current study, however, raises the question whether the ventral wall alone should be the focus of such trials. The effects of drugs on the lateral sides of the AAA may be more relevant in attenuation of inflammation and protease activity with subsequent growth retardation. This indicates the need for tissue collection at different sites or a more prominent role for imaging of inflammation or proteases as a primary outcome measure.

As with other studies on human AAAs, this study is limited for only being able to assess late-stage disease at one point. However, we aimed to investigate the variance among the different parts within the same AAA and collected these segments at the same time, which makes it unlikely to have influenced present results. The number of patients included in this study did neither allow for reliable analyses of differences in patterns of wall components nor of patient characteristics, such as gender and smoking status.

In conclusion, the constituents of the AAA have a heterogeneous distribution. Commonly used ventral wall specimens do not necessarily resemble the entire AAA wall. Lateral sides of the AAA have more microvessels, more inflammatory cells, higher cytokine levels, and higher levels of active proteases. This infers that these more active regions of the AAA might have a relatively higher contribution to AAA expansion and that these sites are more vulnerable to rupture.

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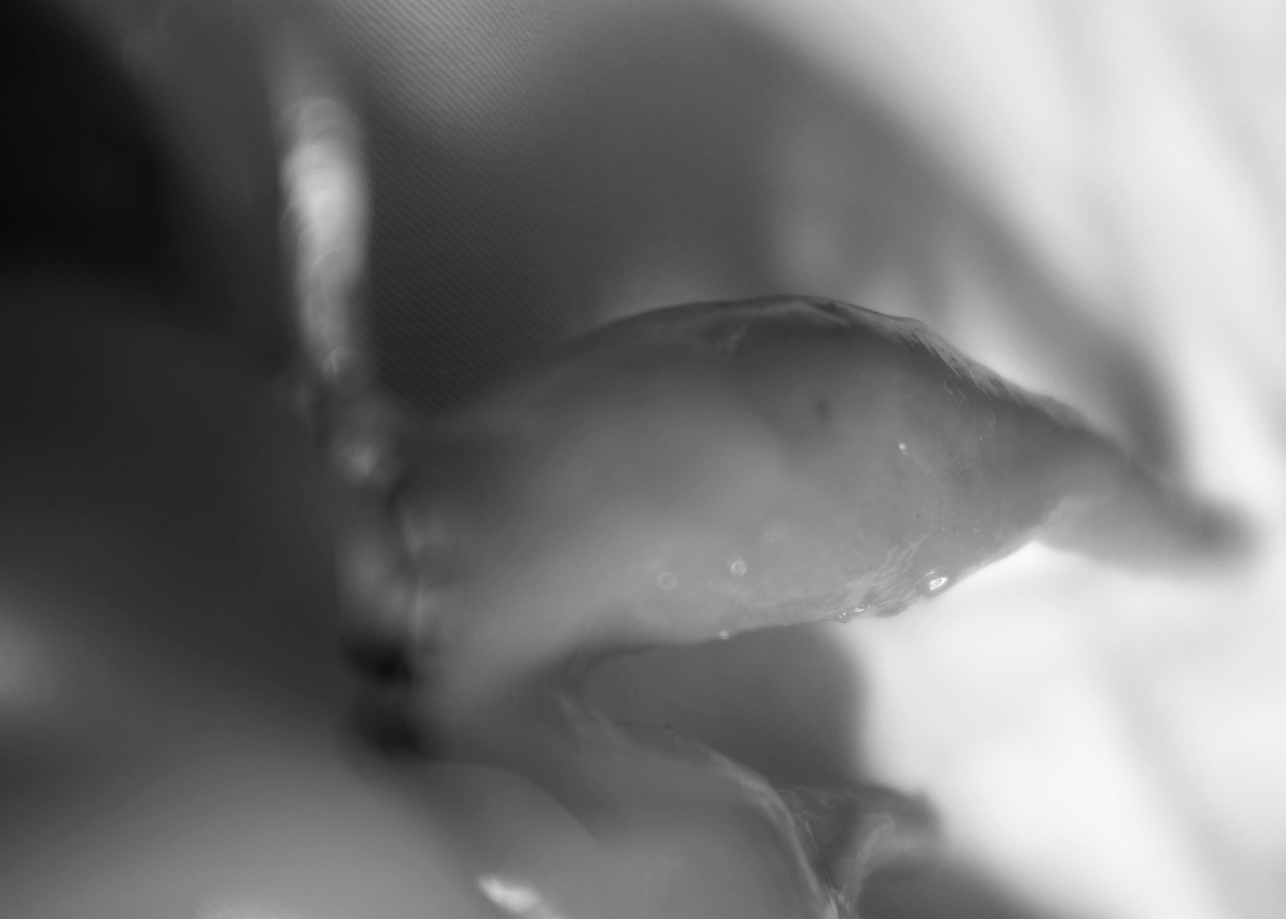

Wall composition of popliteal artery aneurysms differs from abdominal aortic aneurysms

9

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Submitted

Abstract

Objectives

Popliteal artery aneurysms (PAAs) and abdominal aortic aneurysms (AAAs) frequently coincide, however, symptomatology differs. We systematically assessed aneurysm wall composition to compare both anatomic locations.

Methods

Aneurysmal walls of 38 PAAs and 198 AAAs were harvested from patients undergoing elective open surgical repair. Elastin, collagen, smooth muscle cell, iron, and inflammatory cells were quantified by (immune-)histochemistry. In addition, protease and cytokine levels were measured.

Results

Aneurysmal degradation resulted in similarly degraded media. Location of inflammation differed: focus for T and B lymphocytes and plasma cells was the intima in PAAs (all P <.001) and the adventitia for AAAs (all P <.001). Iron was more often observed in PAAs than in AAAs (68% vs. 1%,P <.001), indicating more previous intramural hemorrhages. MMP2 activity was higher in PAAs than in AAAs (median[interquartile range] 0.363[0.174-0.556] vs. 0.187[0.100-0.391] ng/mL ,P =.008), whereas MMP9 showed no difference. Walls of AAAs were richer in tested cytokine levels than walls of PAAs, with the exception of IL6.

Conclusions

PAAs showed more signs of previous hemorrhages compared with AAAs. In addition, inflammation in PAAs is mainly located in the intima, whereas its focus in AAAs is the adventitia. These results suggest important differences in the pathophysiology of aneurysm formation between these locations and might explain the differences in presentation upon diagnosis.

Introduction

Despite different anatomic locations and different wall compositions, the elastic aortic artery and the muscular popliteal artery have comparable elastic artery-like mechanical properties in aged healthy individuals.[1] Both arteries can suffer from aneurysmal degradation; however, aneurysm formation is most commonly found in the abdominal aorta (AAA), with a prevalence of 5% to 10% in men aged 65 to 79 years.[2] Popliteal artery aneurysms (PAAs) form the second most frequently found aneurysm, with a prevalence of 1% in the same age category.[3] Both aneurysms frequently coincide: 5% to 10% of patients with an AAA may be affected with a PAA,[4-6] and about 40% of patients with a PAA also have an AAA.[7, 8] The frequency and nature of symptoms are different: approximately two-thirds of patients with a PAA will be symptomatic at the time of diagnosis, most frequently with thromboembolic complications, and less than 2% of patients present with a ruptured PAA.[7, 9] In contrast, patients with an AAA are mostly asymptomatic at the time of diagnosis, and when symptomatic rupture is the most frequently observed symptom, leaving only 5% of AAAs diagnosed by distal embolization.[10]
AAAs and PAAs are often regarded as the result of similar processes.[11, 12] Given the differences in symptoms, complications, and anatomic location, we aimed at finding an explanation for the described similarities and differences by systematically assessing the aneurysm wall composition of PAAs and AAAs.

Methods

Aneurysm-express biobank
The Aneurysm-express biobank is a prospective cohort study, and its design was described previously.[13] Briefly, all patients scheduled for open aneurysm repair in the University Medical Center Utrecht and St Antonius Hospital Nieuwegein were asked to participate in this study. Indication for open surgical repair was according to international standards and when endovascular treatment was not appropriate.[12] Patients with terminal malignancies were excluded. The medical ethics committees of both centers approved the study, and participants provided written informed consent. Baseline data were obtained from an extensive cardiovascular questionnaire, according to Rose,[14] and from clinical records, including cardiovascular risk factors and medication use. Aneurysm diameter was assessed by computed tomography angiogram or magnetic resonance angiography.

Aneurysm tissue processing
During surgery, a part of the aneurysm wall was collected next to the arteriotomy. The specimen was immediately transported to the laboratory and cut into 5-mm segments. The middle segment was fixated in 4% formaldehyde and embedded in paraffin for histology. A series of stainings was performed on consecutive slides: hematoxylin and eosin (HE) for overview, elastin van Gieson (EvG) for elastin, Sirius red for collagen, Perls for iron, α-smooth muscle actin for smooth muscle cells (SMCs), CD68-macrophages, CD45-lymphocytes, CD3-T-lymphocytes, CD20-B-lymphocytes, and CD138-plasma cells. Extracellular matrix (elastin, collagen) components and iron were assessed on a scale from 0 to 3 (0 = no staining, 1 =

minor, 2 = moderate, and 3 = heavy staining). In the media, the percentage of elastin fibers disruption was scored (the part of the media where no elastic fibers were present). The different inflammatory cells were also scored on a scale of 4 at 100× magnification per representative field: 0 (no), less than 50 positively stained cells; 1 (minor), 50 to 100 cells; 2 (moderate), 100 to 150 cells; and category 3 (heavy), more than 150 cells. Two independent observers (RH, CP), who were blinded from clinical data and laboratory results, scored all stainings separately in the intima, media, and adventitia.

The adjacent segment was used for protein extraction using Tris isolation (Roche, Basel, Switzerland) and subsequent protein concentration assessment with the BCA protein assay kit (Pierce Biotechnology, Rockford, IL, USA) according to the manufacturers' protocol. Matrix metalloproteinase (MMP) activities were determined with the Amersham MMP2 and 9 Biotrak Activity Assay system (GE Healthcare Limited, Amersham, UK). Levels of interleukin (IL) 1β, 2, 4, 5, 6, 8, 10, 12p70, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α and -β were quantified in the aneurysmal wall by fluorescent bead immunoassay (Bendermed Systems, Vienna, Austria).

Statistical analyses

Data are presented as median with interquartile range (IQR) or as number with percentage of total for discrete variables. Continuous variables were compared using the Mann-Whitney test or Spearman's nonparametric correlation, where appropriate, and discrete values were tested using the χ^2 test. Probability values of less than .05 were considered significant. All analyses were performed with SPSS 15 software (SPSS Inc, Chicago, IL, USA).

Results

Arterial tissue from 38 PAAs of 36 patients was collected and from 198 AAAs of 198 different patients. Baseline characteristics of the study groups are reported in Table 1. Most patients were men, which was even more pronounced in the PAA patients (82% vs 95%, respectively; P =.040). The patients with PAA were younger (median [IQR] 65.0 [60.0-73.0] vs 71.0 [64.7-76.0] years, P =.010) and had more previously diagnosed manifestations of aneurysms (53% and 12%, P <.001; Table 1).

Representative histology is shown in Figure 1. Histologic characteristics of the structural wall components for both aneurysm groups are reported in Table 2. The intima of AAAs showed cholesterol cores more frequently than PAAs (55% vs 16%, P <.001). The media layer was similarly degraded in PAAs and AAAs, not showing differences in elastin disruption (25 [IQR, 10-95] and 40 [IQR, 10-80], P =.841) or staining for SMCs (34% and 34%, P =.988).

Focus of inflammation was the intima in PAAs and the adventitia in AAAs, as is shown for the different assessed inflammatory cells in Figure 2. Remarkably, PAAs had far more iron deposits in the adventitia (68% vs 1%, P <.001; Figure 3).

Cytokine levels showed a more pronounced inflammation in AAAs than in PAAs, with the exception of IL6, which showed a weak opposite trend (Table 3). MMP2 activity was higher in PAAs (0.187 [0.100-0.391] vs 0.363 [0.174-0.556] ng/mL, P =.008), whereas MMP9 activity was similar in both aneurysms (0.203 [0.032-0.767] vs 0.258 [0.000-0.637] ng/mL, P =.401).

Table 1 | Baseline characteristics.

Characteristics [a]	AAA (n=198)	PAA (n=38)	P-value
Age, y	71.0 [64.7-76.0]	65.0 [60.0-73.0]	.010 *
Male sex	160 (82)	36 (95)	.040 *
Current smoker	94 (48)	17 (45)	.678
Diabetes type 2	31 (16)	6 (16)	.984
Hypertension	141 (72)	26 (68)	.661
Coronary artery disease	78 (40)	12 (32)	.325
Chronic obstructive pulmonary disease	41 (21)	5 (13)	.282
Body mass index, kg/m^2	25.6 [23.6-28.0]	27.1 [25.6-28.1]	.118
Aneurysm diameter, mm	61 [56-71]	30 [21-46]	<.001 *
Coinciding PAA	4 (2)		
Coinciding AAA		15 (39)	
Coinciding contralateral PAA		17 (45)	
History of any other aneurysm detected	23 (12)	20 (53)	<.001 *
Statin use	119 (61)	21 (55)	.396
Aspirin use	134 (69)	23 (61)	.742
ACEI use	70 (36)	13 (34)	.816
Angiotensin II receptor blocker use	35 (18)	7 (18)	.884
Hospital stay, days	10.0 [8.0-14.0]	6.0 [4.0-9.0]	<.001 *

[a] Data are presented as median [interquartile range] or as No. (%). *p <.05.
Abbreviations: AAA, abdominal aortic aneurysm; ACEI, angiotensin-converting enzyme inhibitor; PAA, popliteal artery aneurysm

Table 2 | Histologic characteristics of structural arterial wall components.

Aneurysm wall characteristics [a]	AAA (n=198)	PAA (n=38)	P-value
Intima			
Cholesterol core, presence, n (%)	108 (55)	6 (16)	<.001 *
Collagen	67 (34)	22 (58)	.010 *
SMC	45 (23)	14 (37)	.076
Media			
Elastin disruption, median [IQR], %	40 [10-80]	25 [10-95]	.841
SMC	68 (34)	13 (34)	.988
Adventitia			
Collagen	148 (75)	18 (47)	.003 *
SMC	75 (38)	5 (13)	.011 *
Iron deposition, presence, n (%)	1 (1)	26 (68)	<.001 *

[a] Data are presented as No. (%) of heavy staining vs minor staining unless otherwise indicated. *p <.05.
Abbreviations: AAA, abdominal aortic aneurysm; IQR, interquartile range; PAA, popliteal artery aneurysm; SMC, smooth muscle cells.

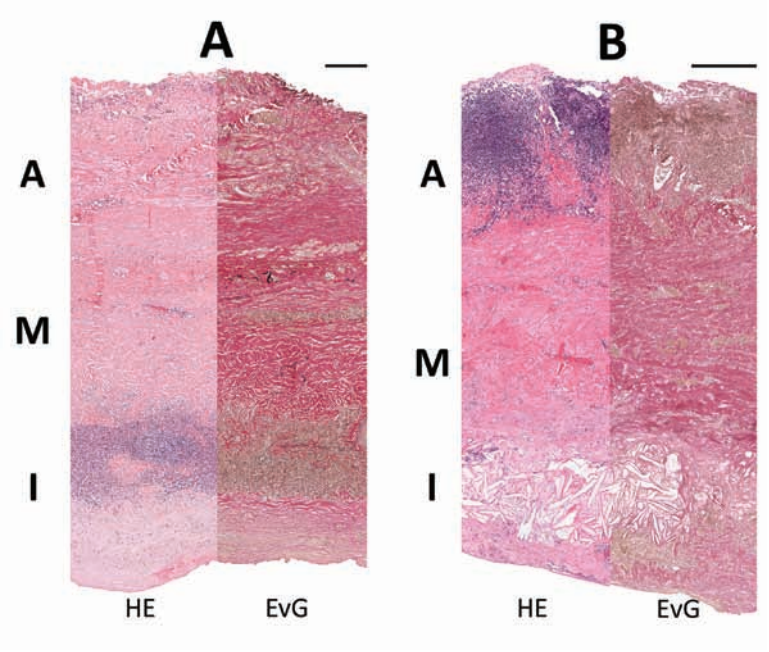

Figure 1 | Representative histology for popliteal artery aneurysm (PAA) and abdominal aortic aneurysm (AAA). Photomicrographs show a composite of hematoxylin and eosin and elastic van Gieson stainings. Location of the different layers are marked: I, intima; M, media; A, adventitia. Note the location of the inflammatory infiltrate, the intima in PAA, and the adventitia in AAA. Scale bars represent 0.5 mm.

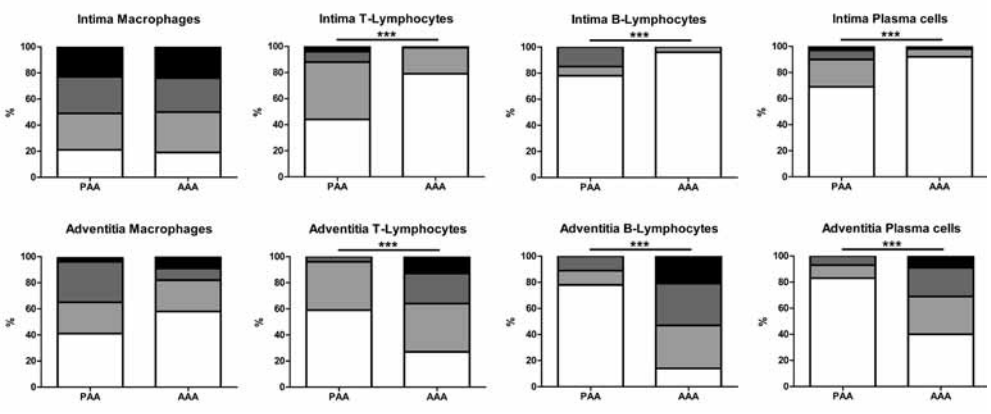

Figure 2 | Comparison of inflammatory cell presence on histology in the two types of aneurysm. Per representative field: white color represents <50 cells, light grey represents 50 to 100 cells, dark grey represents 100 to 150 cells, and black represents >150 cells. PAA is popliteal artery aneurysm, AAA is abdominal aortic aneurysm. *** p <.001

Table 3 | Protease and cytokine levels.

Aneurysm wall characteristics [a]	AAA (n=198)	PAA (n=38)	P-value
IL-1β	0.00 [0.00-0.45]	0.00 [0.00-0.00]	<.001 *
IL-2	1.87 [0.78-3.96]	0.00 [0.00-0.81]	<.001 *
IL-4	0.00 [0.00-0.81]	0.00 [0.00-0.00]	<.001 *
IL-5	0.81 [0.09-2.42]	0.00 [0.00-0.00]	<.001 *
IL-6	1.12 [0.26-6.97]	3.55 [1.11-8.29]	.101
IL-8	22.85 [10.13-50.46]	9.47 [3.09-29.94]	.024 *
IL-10	0.28 [0.00-1.11]	0.00 [0.00-0.33]	.007 *
IL-12	0.35 [0.00-1.24]	0.00 [0.00-0.00]	<.001 *
TNF-α	0.21 [0.00-0.73]	0.00 [0.00-0.06]	<.001 *
TNF-β	0.40 [0.00-2.20]	0.00 [0.00-0.00]	<.001 *
IFN-γ	0.68 [0.01-1.74]	0.00 [0.00-0.00]	<.001 *
Active MMP2	0.187 [0.100-0.391]	0.363 [0.174-0.556]	.008 *
Active MMP9	0.203 [0.032-0.767]	0.258 [0.000-0.637]	.401

[a] Data are presented as median and [interquartile range]. *p <.05. Cytokines are in pg/mL, MMP activities in ng/mL. Abbreviations: IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; MMP, matrix metalloproteinase.

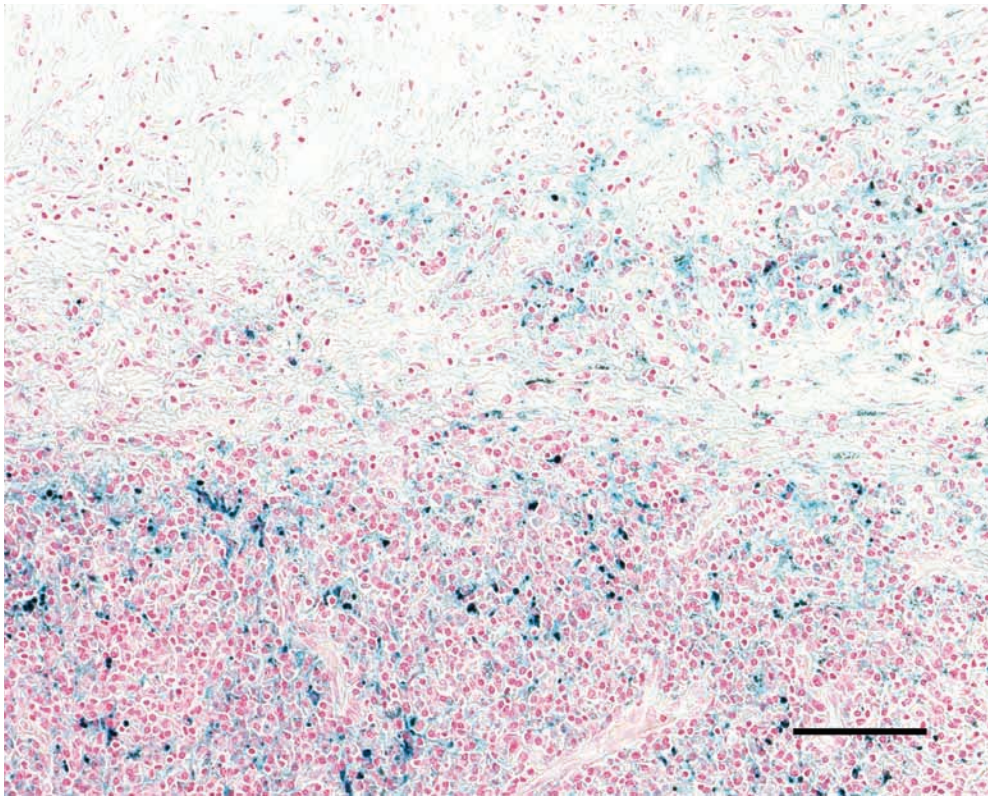

Figure 2 | Representative Perls staining of a popliteal artery aneurysm. Blue staining shows the presence of iron. Scale bar indicates 0.1 mm.

Discussion

To our knowledge, this is the first study to systematically report histologic characteristics in the different layers of the PAA. Given the frequent association with AAA, the coincidence in patients, and the comparison in literature, these aneurysms were used as a reference, and marked differences were found.

As in other muscular arteries, the diameter of the popliteal artery increases with age, being affected by age and body size, and is larger in men than in women. With aging, the popliteal artery also suffers from a decreased distensibility, which is more pronounced in men than in women. These changes do not fit the characteristics of a true muscular artery, because, for example, this does not occur in the common femoral artery but is very similar to the pattern observed in a large elastic artery such as the aorta.[1, 15] Despite original differences in histologic composition between the aorta and the popliteal artery (elastic vs muscular), aneurysm formation resulted in a similar degraded media in our study. Previous smaller studies suggested a common etiology for AAAs and peripheral aneurysms. A central role for SMC apoptosis was proposed, which was also observed in AAAs and other peripheral aneurysms.[16, 17] Another study reported a marked overlap in inflammation between AAA and PAA.[18] Unfortunately, in these studies differences in wall layers remained unreported and focus was the inflammatory infiltrate. The present study shows that compared with AAAs, the media in PAAs is similarly degraded.

The focus on different layers in the arterial wall also proves important in assessing the inflammatory infiltrate. Where previous smaller studies investigated the inflammatory infiltrate in general, we show that the focus of inflammation in the PAA lies in the intima rather than in the adventitia, as in the AAA. Our findings might explain the difference in symptoms between a PAA and an AAA: the intima is adjacent to the mural thrombus or lumen and is likely to influence processes such as thromboembolism or thrombosis as seen in PAAs, whereas the nature of AAAs is one of expansion and rupture.

In addition, the inflammatory reaction in AAAs is far more pronounced than in PAAs, given the higher cytokine levels in AAAs and the larger proportion of high categories histologic inflammation.

The frequent presence of iron depositions in PAAs with virtually no depositions in AAAs also marks an important difference. Iron remnants are long-term results from hemorrhages,[19] and this finding might point to a different etiology. Intramural bleeding can point to a healed dissection or repetitive smaller tears of the connective tissue, or both. This might be explained by structural factors, including turbulent blood flow in association with arterial branching points and wall fatigue secondary to repeated knee flexion.[11]

MMP9 is the most abundantly present protease and the most frequently investigated MMP in AAAs.[20] Given the similar levels in AAAs and PAAs, MMP9 appears to be a common factor and likely is vital for aneurysm formation in general. MMP2 is more active in PAAs, despite lower numbers of inflammatory cells but it can also be produced by SMCs[21], pointing to a more important role in progression of the PAA or a role in a repair mechanism.

Our study design was hampered by not being able to assess temporal changes in the aneurysm walls, which holds true for all current aneurysm-related research on human material. Aneurysmal

disease is characterized by a large proportion of men, which is often described for AAAs but is even more pronounced in PAAs, reaching 94.2% to 99.8%.[9, 22] This is also the case in our study. The lower age in the PAA group might be explained by the occurrence of symptoms leading to an earlier diagnosis and treatment. Both characteristics are not likely to have influenced our results, because the absolute differences are small and no influences on wall composition have been reported.

In conclusion, inflammation in the PAA is mainly located in the intima, whereas the degree of inflammation in the AAA is more pronounced and its focus is the adventitia. In addition, PAAs show more iron compared with AAAs, which might be explained by more (repetitive) external trauma in the PAA through bending of the knee and turbulent flow resulting in tissue tears. Our results suggest differences in the mechanism of aneurysm formation between both localizations, and this might explain the difference in presenting symptoms.

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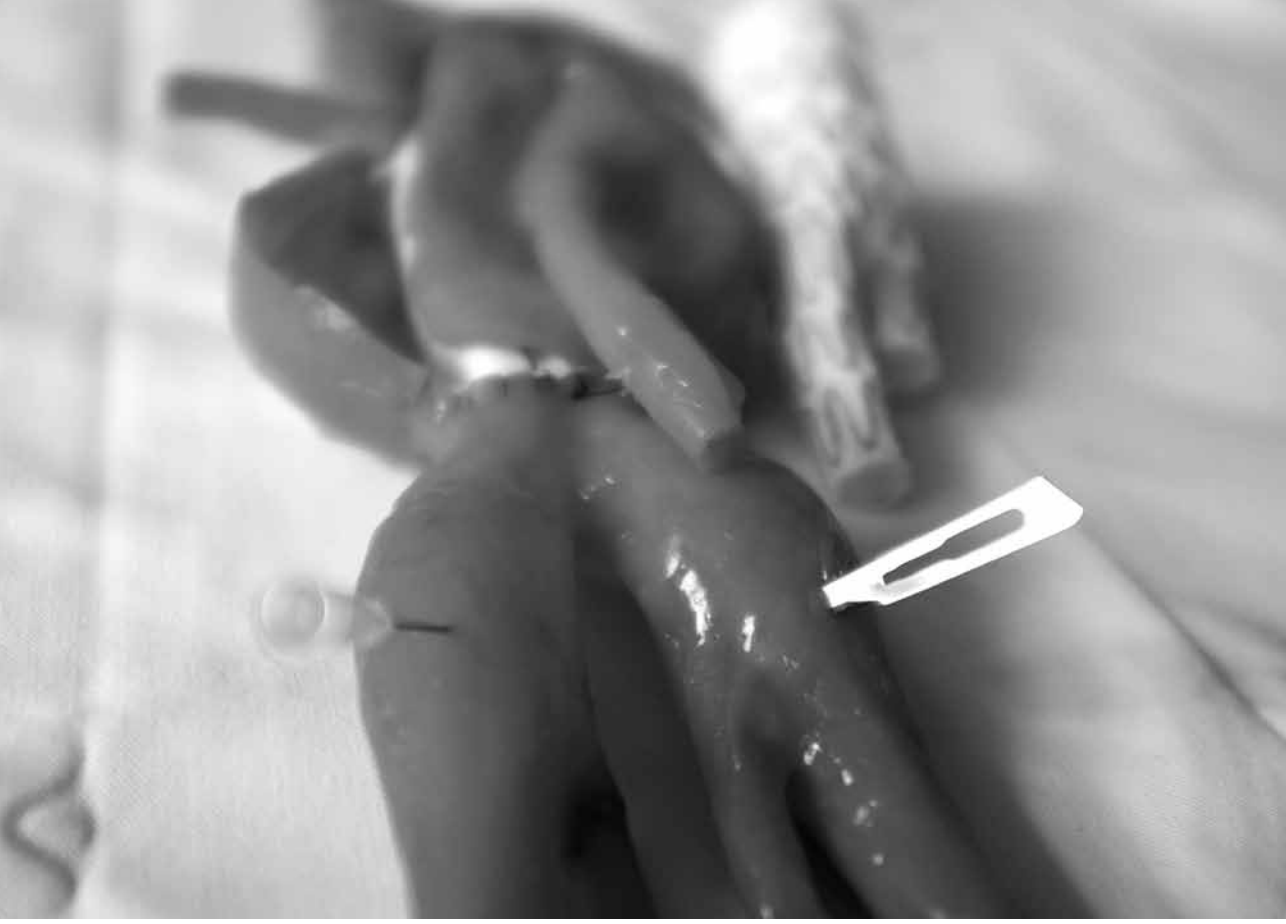

Limited benefit after percutaneous versus femoral cutdown access for endovascular aneurysm repair

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Submitted

Abstract

Purpose

Single center studies suggest that percutaneous access for EVAR (pEVAR) offers significant operative and post-operative benefits compared to femoral cutdown (cEVAR). National data are limited. We aimed to estimate the proportion of EVAR performed with percutaneous access over time and to compare outcomes of pEVAR and cEVAR.

Methods

The ACS NSQIP database was used to identify patients undergoing EVAR from 2005 to 2009. CPT codes were used to distinguish pEVAR from cEVAR. Demographics, operative variables and outcomes were compared between groups.

Results

In the study period, 8236 patients underwent aneurysm repair via cEVAR (57.8%) and pEVAR (42.2%) for intact AAA. pEVAR increased from 36.5% in 2005 to 44.9% in 2009 (P <.001) in NSQIP hospitals. Comorbidities were similar. Surgical vessel repair was necessary in 1.0% of pEVAR. pEVAR without vessel repair did not shorten operative time (mean 150 vs. 154 min; OR 0.93 [0.84-1.02]), decrease the need for intraoperative transfusion (11.0% vs. 10.1%; OR 1.15 [0.99-1.34]) or lower postoperative infection (2.3% vs. 2.5%; OR 0.93 [0.68-1.26]). Undergoing pEVAR increased the risk for DVT (0.7% vs. 0.3%; OR 2.53 [1.27-5.03]) and slightly prolonged hospital stay (3.2 vs. 3.1 days; OR 1.23 [1.08-1.40]). pEVAR that required vessel repair had increased transfusions (27.8% vs. 10.1%, P <.001), operative time (192 vs. 154 min, P =.050) and superficial wound infections (13.9% vs. 2.0%, P <.001) compared to patients with cEVAR or pEVAR without repair.

Conclusions

Percutaneous access for EVAR is increasing nationally. However, potential benefits of decreased wound infections, operative time and length of stay are not being realized nationally. pEVAR was associated with more DVTs. It remains to be seen whether improved patient selection and physician training will lead to improvement in outcome.

Introduction

For patients with an anatomically suitable abdominal aortic aneurysm (AAA), endovascular aortic aneurysm repair (EVAR) has become the preferred treatment during the past decade. Percutaneous access (pEVAR) further minimizes invasiveness compared to femoral cutdown access (cEVAR). When compared to cEVAR, utilization of pEVAR has been reported to reduce the incidence of wound complications,[1] with high success rates even in unselected patients.[2] Additionally, access-related complication rates, reported in several single-institutional studies, are lower with pEVAR.[2-6] Nevertheless, complications are reported and can be significant.[7] Current reports involve limited numbers of patients, and with the increasing and evolving use of pEVAR by academic and community physicians, a larger scale study of contemporary management of AAA comparing pEVAR and cEVAR is warranted. Criteria for percutaneous access have not been established and there are no large trials comparing the relative risks and benefits. We therefore analyzed national trends and outcomes of pEVAR and cEVAR for AAA repair. We aimed to analyze the independent effects of access type on patient outcomes.

Methods

Data source

The American College of Surgeons National Surgical Quality Improvement Program (ACS NSQIP) was used for this study, which includes data from 269 academic and community medical centers throughout the United States. Trained clinical nurse reviewers enter the prospectively recorded data in a standardized fashion in the clinical database; this ensures high accuracy of the available variables.[8] An extensive list of data is collected per hospitalization: clinical characteristics, comorbidities, operative and perioperative variables, and 30 day postoperative outcomes.[9] We used the ACS NSQIP 2005-2009 to identify all patients with a primary ICD-9 (International Classification of Diseases, 9th ed.) diagnosis code of either intact (441.4) or ruptured (441.3) AAA. We isolated, using CPT (Current Procedural Terminology) procedure codes, only those patients who had undergone endovascular AAA repair (34800, 34802-34805, 34812-34813, 34825-34826, 0078T-0081T). Intact and ruptured cohorts were then stratified into subgroups by type of access used; femoral cutdown access (34812), other cutdown access (iliac [34820], iliac with conduit [34833] or EVAR with femoral-femoral bypass [34813]), and percutaneous access (all remaining patients). To obtain a clean comparison we excluded patients with codes for other cutdown access. Patients with codes for brachial access (34834), aorto-aortic-tube prosthesis (34800) or aorto-uni-iliac / uni-femoral prosthesis (34805) or codes for conversion to open repair after failed EVAR (34830-34832) were excluded from the analysis. Concurrent repair of a blood vessel (35226, 35256, 35286) and endarterectomy of local vessels (superficial [35302], profunda [35372] and common [35371] femoral artery, iliac [35351] and iliofemoral [35355] artery) were identified. Per correct coding initiative (CCI) edits the endarterectomy codes also included femoral cutdown and the femoral cutdown code itself included blood vessel repair. NSQIP does not report bilateral coding, which limited us to analyze patients undergoing bilateral percutaneous access versus at least one femoral cutdown access instead of analyzing all groins separately. We aimed to compare pEVAR, pEVAR with surgical vessel repair, cEVAR alone and cEVAR with endarterectomy.

Clinical and outcome variables

Weight classification was assigned based on BMI (kg/m^2) and the National Institutes of Health definitions of underweight (≤18.6 kg/m^2), normal weight (18.7-25 kg/m^2), overweight (25.1-30 kg/m^2), obese class I (30.1-35 kg/m^2), obese class II (35.1-40 kg/m^2), and obese class III (>40 kg/m^2). Renal disease was defined as hemodialysis dependence or a preoperative serum creatinine level exceeding 1.8 mg/dL. Congestive heart failure comorbidity definitions required active symptoms within 30 days before admission.

Outcome variables included intra- and postoperative (<72h) blood transfusion need (units). Deep venous thrombosis (DVT) / thrombophlebitis was defined as the identification of a new blood clot or thrombus within the venous system, which may be coupled with inflammation. This diagnosis needed to be confirmed by a duplex, venogram or CT scan and treatment needed to include anticoagulation therapy and/or placement of a vena cava filter or clipping of the vena cava. Pulmonary embolism was documented if the patient had a V-Q scan interpreted as high probability of pulmonary embolism or a positive CT spiral exam, pulmonary arteriogram or CT angiogram. Presence of wound disruption was scored when there was a separation of the layers of the surgical wound, either partially or complete, with disruption of the fascia. Superficial surgical site infection (SSI) was an infection involving only skin or subcutaneous tissue and at least one of the following: Purulent drainage from the superficial incision; Organisms isolated from a fluid or tissue culture from the superficial incision; At least one symptom of local infection (tenderness, swelling, redness or heat) and superficial incision is deliberately opened by the surgeon (unless incision is culture-negative); Diagnosis of superficial SSI by the surgeon or attending physician. A deep incision SSI involved deep soft tissues (e.g., fascial and muscle layers) of the incision and at least one of the following: Purulent drainage from the deep incision; a deep incision that spontaneously dehisces or is deliberately opened by a surgeon and presence of symptoms (fever, local pain/tenderness) unless site is culture-negative; An abscess or other evidence of infection involving the deep incision; diagnosis of a deep incision SSI by a surgeon or attending physician. Organ/space SSI was defined to include infections of any part of the anatomy (e.g., organs and spaces) other than the incision, which was opened or manipulated during an operation and at least one of the following: Purulent drainage from a drain placed in the organ/space; Isolated organisms from cultured fluid or tissue originating from the organ/space; An abscess involving the organ/space; Diagnosis of an organ/space SSI by a surgeon or attending physician.

Operative time and length of hospital stay were also recorded. All outcome variables, unless otherwise stated, cover the period from the operation until 30 days postoperative.

Statistical analyses

Categorical variables were analyzed using chi-square test or Fisher exact test; continuous variables were compared using Student t tests for parametric data or the Mann-Whitney test for nonparametric data. The predictive value of the available clinical variables for the defined outcome was determined using uni- and multivariable logistic regression analyses. Statistical significance was defined as $P < .05$. Statistical analysis was performed using SAS 9.2 (SAS Institute Inc, Cary, NC) and PASW Statistics 18 (SPSS Inc, Chicago, IL) software.

Results

We identified a total of 8687 patients with AAA who underwent EVAR, of which 8382 (96.5%) were intact AAA repairs and 305 (3.5%) were ruptured AAA repairs. Of the intact repairs, 3479 (41.5%) were performed with pEVAR and 4757 (56.8%) with cEVAR. One hundred-forty-six (1.7%) had other cutdown access (these patients were excluded from our analysis). Of ruptures, 134 (43.9%) were performed utilizing pEVAR, 157 (51.5%) with cEVAR and 14 (4.6%) with other cutdown. Given the limited number of patients in NSQIP with ruptured EVAR, we chose to focus all further analyses on patients with intact AAA. In 36 cases (1.0%) pEVAR was complicated by surgical vessel repair. For cEVAR, 191 (4.0%) had additional femoral endarterectomies. The number of participating centers in NSQIP increased over time and consequently the absolute number of EVAR increased as well. The proportion of pEVAR increased significantly from 36.5% in 2005 to 44.9% in 2009 (P <.001; Figure 1).

Demographics and comorbidities

For intact AAA repairs, patients in the cEVAR group were older (mean ± SD; 74.1 ± 8.3 vs. 73.5 ± 8.3 years; P =.001), were more often identified as white (88.5% vs. 83.3%; P <.001) and more frequently had a history of previous cardiac surgery (25.0% vs. 23.0%; P =.034). Other previous interventions and comorbidities were similar for both access types (Table 1). The method of anesthesia for pEVAR and cEVAR differed (P <.001): general 84.6% vs. 80.2%; spinal/epidural 9.2% vs. 13.7%; and local 6.2% vs. 6.0%.

Patients with both pEVAR and vessel repair had a higher prevalence of hypertension (91.7% vs. 79.9%, P =.075), were more often current smokers (44.4% vs. 28.3%, P =.033), and had undergone more prior cardiac surgery (38.9% vs. 22.8%, P =.023) and percutaneous coronary intervention (33.3% vs. 20.7%, P =.062) compared to patients with uncomplicated pEVAR. Other comorbidities were similar. After multivariable analysis, current smoking (OR 2.23 [1.12-4.44]) and previous cardiac surgery (OR 2.39 [1.19-4.80]) were independent predictors for the need for vessel repair after pEVAR.

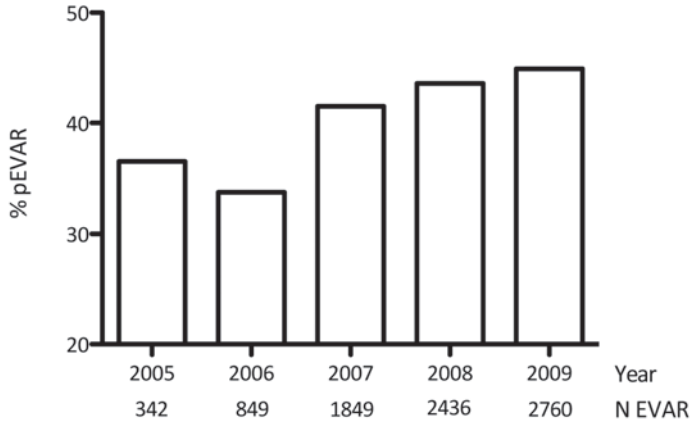

Figure 1 | Trends in proportion percutaneous access of total EVAR in intact abdominal aortic aneurysm repair. X-axis shows the total number of endovascular repairs (EVAR) per year.

Table 1 | Clinical characteristics of the endovascular repaired intact AAA.

Characteristic*	Percutaneous	Femoral cutdown	P-value
N	3479	4757	
Age, mean (SD), y	73.5 (8.4)	74.1 (8.3)	<.001
Age, y			.007
<=67	24.0	21.8	
68-72	18.9	18.2	
73-77	22.8	21.8	
78-82	19.3	21.6	
83+	14.9	16.5	
Female gender	15.6	16.8	.139
Non-white race	16.7	11.5	<.001
Hypertension	79.8	78.5	.132
Diabetes mellitus	15.0	14.8	.748
Current smoker <1y	28.5	28.3	.873
Alcohol >2 units/d in <2w	4.1	4.6	.355
BMI, kg/m^2			.258
<18.6	1.8	2.2	
18.6-25	26.9	27.0	
25-30	40.1	39.9	
30-35	21.0	21.1	
35-40	8.1	7.1	
>40	2.2	2.7	
Renal function			.592
Normal	94.0	93.4	
Insufficiency	5.0	5.5	
Dialysis	1.0	1.0	
Cerebrovascular disease	14.5	14.3	.814
PVD	5.1	5.0	.862
Prior MI <6 months	1.1	1.0	.604
CHF <30 days	1.4	1.3	.917
Previous cardiac surgery	23.0	25.0	.034
Previous PCI	20.8	20.5	.716
COPD	19.3	18.5	.358

* Data are presented as % unless otherwise indicated. Abbreviations: AAA, abdominal aortic aneurysm; BMI, bodymass index; PVD, peripheral vascular disease; MI, myocardial infarction; CHF, congestive heart failure; PCI, percutaneous coronary intervention; COPD, chronic obstructive disease.

Outcomes

Outcome variables for the groups are shown in Table 2 and Table 3 lists the uni- and multivariable model analyses for pEVAR and cEVAR. When comparing successful pEVAR to straightforward cEVAR, both intraoperative and postoperative transfusion administration was similar. Although infrequent, DVT occurred more often in the successful pEVAR group (0.7% vs. 0.3%, P =.022), and this type of access was a strong (OR 2.53 [1.27-5.03]) predictor for this complication after multivariable correction. Pulmonary embolism did not differ between groups. Pneumonia was infrequent (1.4% vs. 1.1%, P =.195), however, after multivariable correction pEVAR proved to be a risk factor (OR 1.56 [1.01-2.39]). Postoperative wound infections were comparable in both uncomplicated access types (2.3% vs. 2.5%, P =.465).

Mean operative time was similar among uncomplicated pEVAR and cEVAR (150 vs. 154 minutes, P =.080) and patients with pEVAR had a slightly longer median length of hospital (3.2 vs. 3.1 days, P <.001), which persisted after multivariable correction (OR 1.23 [1.08-1.40]).

Complicated pEVAR lead to more intraoperative transfusions (27.8% vs. 11.0%, P =.001), more wound disruptions (2.8% vs. 0.2%, P =.003) and more superficial SSI (13.9% vs. 1.6%, P <.001) compared to uncomplicated pEVAR. With the addition of vessel repair, the mean operative time increased (192 vs. 150 minutes, P =.010). In addition, patients with vessel repair had more intraoperative transfusions than those with straightforward cEVAR (27.8% vs. 10.1%, P <.001) as well as more wound disruptions (2.8% vs. 0.1%, P <.001) and more superficial SSI (13.9% vs. 2.0%, P <.001).

Table 2 | Outcome parameters for the different groups.

	Percutaneous			Femoral cutdown				
Outcome*	**Uncomplicated**	**Vessel repair**	**P-value**	**Straightforward**	**Endarterectomy**	**P-value**	**P-value**	**P-value**
Groups	**1**	**2**	**1 vs. 2**	**3**	**4**	**1 vs. 3**	**3 vs. 4**	**2 vs. 3**
N	3443	36		4566	191			
Intraoperative transfusion	11.0 (379)	27.8 (10)	.001	10.1 (460)	25.7 (49)	.177	<.001	<.001
Postop transfusion <72h	0.6 (20)	0 (0)	.647	0.4 (19)	2.6 (5)	.297	<.001	.698
DVT/ Thrombophlebitis	0.7 (24)	0 (0)	.615	0.3 (15)	1.6 (3)	.022	.006	.731
Pulmonary embolism	0.2 (6)	0 (0)	.802	0.2 (8)	0 (0)	.992	.563	.802
Pneumonia	1.4 (48)	2.8 (1)	.483	1.1 (49)	3.7 (7)	.195	.001	.326
Total wound infection	2.3 (78)	13.9 (5)	<.001	2.5 (115)	4.2 (8)	.465	.154	<.001
Wound disruption	0.2 (8)	2.8 (1)	.003	0.1 (6)	0.5 (1)	.291	.166	<.001
Superficial SSI	1.6 (54)	13.9 (5)	<.001	2.0 (91)	3.1 (6)	.159	.271	<.001
Deep incisional SSI	0.4 (13)	0 (0)	.712	0.4 (18)	0.5 (1)	.905	.781	.706
Organ/space SSI	0.1 (5)	0 (0)	.819	0.1 (4)	0 (0)	.451	.682	.859
Total operation time, mean ± SD, m **	150 ± 70	192 ± 101	.010	154 ± 62	226 ± 97	.080	<.001	.050
Total hospital stay, mean ± SD, d **	3.2 ± 2.6	3.4 ± 6.4	.087	3.1 ± 4.3	4.6 ± 7.9	<.001	<.001	.122

* Data are presented as % (n), unless otherwise stated. ** Continuous variable converted to binary using the median. P-values represent differences between groups within types of access. Abbreviations: AAA, abdominal aortic aneurysm; DVT, deep venous thrombosis; SSI, surgical site infection.

More patients received transfusions after cEVAR with endarterectomy compared to straightforward cEVAR, both intraoperative (25.7% vs. 10.1%, P <.001) and postoperative (2.6% vs. 0.4%, P <.001).
Postoperative DVT (1.6% vs. 0.3%, P =.006) and pneumonia (3.7% vs. 1.1%, P =.001) were higher in the endarterectomy group, however, wound disruption (0.5% vs. 0.1%, P =.166) and superficial SSI (3.1% vs. 2.0%, P =.271) were comparable. Patients with endarterectomy had a prolonged operative time (226 vs. 154 minutes, P <.001) and hospital stay (4.6 vs. 3.1 days, P <.001).

Table 3 I Outcome for pEVAR when compared to cEVAR.

Outcome	Univariable		Multivariable	
	OR [95% CI]	P-value	OR [95% CI]	P-value
Intraoperative transfusion	1.10 [0.96-1.28]	.177	1.15 [0.99-1.34]	.076
Postop transfusion <72h	1.40 [0.75-2.62]	.297	1.37 [0.72-2.61]	.332
DVT/ Thrombophlebitis	2.13 [1.12-4.07]	.022	2.53 [1.27-5.03]	.008
Pulmonary embolism	1.00 [0.35-2.87]	.992	1.08 [0.36-3.26]	.879
Pneumonia	1.30 [0.87-1.95]	.195	1.56 [1.01-2.39]	.043
Total wound infection	0.90 [0.67-1.20]	.465	0.93 [0.68-1.26]	.618
Wound disruption	1.77 [0.61-5.11]	.291	2.01 [0.69-5.89]	.204
Superficial SSI	0.78 [0.56-1.10]	.159	0.82 [0.57-1.17]	.263
Deep incisional SSI	0.96 [0.47-1.96]	.905	1.00 [0.47-2.10]	.990
Organ/space SSI	1.66 [0.45-6.18]	.451	1.34 [0.32-5.54]	.691
Total operation time, mean ± SD, m *	0.92 [0.84-1.01]	.080	0.93 [0.84-1.02]	.122
Total hospital stay, mean ± SD, d *	1.25 [1.11-1.41]	<.001	1.23 [1.08-1.40]	.002

* Continuous variable converted to binary using the median. Odds ratios with 95% confidence intervals compare successful percutaneous access to straightforward femoral cutdown access. Abbreviations: AAA, abdominal aortic aneurysm; DVT, deep venous thrombosis; SSI, surgical site infection.

Discussion

Over the study period, pEVAR for intact AAA repairs increased from 36.5% in 2005 to 44.9% in 2009. When compared to cEVAR, pEVAR was not associated with differences in prevalence of postoperative wound infections or operative time. Instead, its use was a predictor for DVT, which was not described before. The need for vessel repair after pEVAR substantially increased the need for blood transfusion, operative time and postoperative superficial infections compared to successful pEVAR or straightforward cEVAR. Endarterectomy was associated with longer operative time and hospital stay and more transfusion need than straightforward cEVAR.
Access related complication rates for pEVAR varied from 3.9% in the largest series to date[10] to 4.4% (3.5-5.3, 95% CI) in a recent systematic review.[11] In the current study, the minimum complication rate was 1.0%, which included vessel repairs but not failed percutaneous access requiring femoral endarterectomy as this can't be identified in NSQIP. We chose to consider patients with an endarterectomy as a separate group as we expect many of these to be

planned. If every endarterectomy was due to a failed pEVAR, the failed pEVAR rate would be 6.2%. Endarterectomy had a similar negative effect on OR time, bleeding, length of stay and wound infection as did vessel repair. In some studies previous interventions/scars in the groin had a detrimental effect on access complications[10, 12], another study found no differential effect of scarred groins[13], furthermore some studies excluded these patients.[4, 14] The presence of scars was not recorded in NSQIP.

Prior reports showed significantly decreased operative time in the group utilizing pEVAR.[2, 13-15] The current study shows similar operative times between uncomplicated pEVAR and cEVAR. A recent large study showed that operative time is mainly determined by age, gender and BMI.[16] Access type in the present study showed no beneficial effect on operative time in addition to these known predictors. While local anesthesia use is considered a significant advantage associated with pEVAR,[4, 6] we noted that general anesthesia was very frequently applied and its use was even higher than in the group undergoing cEVAR. While percutaneous access offers a theoretical advantage in ability to avoid increased morbidity associated with general anesthesia, it is not often being performed nationwide. Application of local anesthetic techniques could also reduce operative time.

Intraoperative blood loss has been described to be less when using pEVAR[14], while other studies report similar blood loss.[2, 6, 17] The present study showed both intra- and postoperative transfusion need to be similar in both groups. However, when the level of complexity of both access types increased by either concurrent surgical vessel repair or endarterectomy, transfusion need increased dramatically.

To our knowledge, an increased risk of postoperative DVT was not described before in relation to type of access. Although DVT was an infrequent complication, it was significantly higher in the group undergoing uncomplicated pEVAR. This difference might be caused by postprocedural compression or a hematoma causing compression of the adjacent vein, which might warrant an alternating technique and checking common femoral vein compression on the table. Pulmonary embolism was rare and similar in both groups.

While prior reports associate pEVAR with shorter lengths of stay,[1, 4, 6] we found no such difference. On the contrary, pEVAR was associated with a slightly longer hospitalization.

One small study described fewer infections with pEVAR1, however, this was not confirmed by other studies that described an association between obesity and wound infections instead in these patients.[2, 5, 7] In this study it was also confirmed in a large series that type of access did not influence occurrence of systematically scored wound infection but obesity did. Success in obtaining percutaneous access was very relevant in this regard, as pEVAR requiring surgical vessel repairs had more postoperative superficial SSI than uncomplicated pEVAR or cEVAR.

In addition to the increase in superficial wound infections, the combination of pEVAR with vessel repair also required more frequent administration of blood intraoperatively and a longer operative time when compared to straightforward cEVAR. This finding indicates that patients at risk for failed pEVAR might benefit from cEVAR instead. In an attempt to identify this group of patients, we found that current smoking and previous cardiac surgery were independent predictors for utilization of vessel repair. This is likely a marker for advanced atherosclerotic occlusive disease.

This study had some limitations. Anatomic information is not included in this database, which prevented us from correcting outcome parameters for this in multivariable analyses. However,

for uncomplicated pEVAR this was not likely to introduce bias. We were unable to determine when ultrasound guidance was available, which has recently been suggested to reduce access-related complications.[18] Our determination of pEVAR was based on lack op CPT codes for cutdown. It is possible that surgeons performed cutdown but forgot to bill for this, thereby overestimating the proportion pEVAR. We think this is unlikely as it impacts reimbursement, which may in fact influence the decision to perform cEVAR. It is perhaps more likely that surgeons may incorrectly label vessel repair after failed pEVAR as cutdown (open femoral artery for delivery of endovascular prosthesis) despite the relative financial loss. This would underestimate the rate of pEVAR failure and bias outcomes in favor of pEVAR. We could not exclude other potential benefits of pEVAR such as postoperative pain, paresthesia and time to return to normal activity. Patient preference could also play a role.

Utilization of pEVAR for intact AAA is expanding nationally when compared to cEVAR. It was not associated with a decreased operative time, but the opportunity for applying local anesthetic techniques was under-utilized. pEVAR was not associated with shorter operative time or shorter hospitalization, and no difference in wound infections was observed. pEVAR was associated with an limited increase in DVT. When pEVAR was complicated by the need for open vessel repair: operative time, need for blood transfusion and postoperative superficial infections all substantially increased to levels higher than cEVAR. Similarly, performing endarterectomy lead to more transfusions and longer operative times and hospital stays. This indicates that patients who are at risk for pEVAR failure might benefit from a planned cEVAR. The study suggests that the reported benefits in single center studies are not being realized nationally. With little difference in outcome patient preference is likely to drive further expansion, given the problems with failure there is a need to better identify those at risk of failure.

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Ultrasound guided percutaneous endovascular aortic aneurysm repair can be performed routinely with high success and minimal complications

11

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Submitted

Abstract

Introduction

Ultrasound guided access allows for direct visualization of the access artery during percutaneous endovascular aortic aneurysm repair. We hypothesize that the use of ultrasound guidance allowed us to safely increase the utilization of percutaneous endovascular aortic aneurysm repair and to benefit from its lower rates of wound complications.

Methods

A retrospective chart review of all elective endovascular aortic aneurysm repairs, both abdominal and descending thoracic, from 2005-2010 was performed. Patients were identified using ICD9 codes and stratified based on access type: percutaneous vs. cutdown. We examined the success rate of percutaneous access and the cause of failure. Sheath size was large (≥18 Fr) or small (12-16 Fr). Minimum access vessel diameter size was also measured. Outcomes were wound complications (infections or clinically significant hematomas), operative and incision time, length of stay, and discharge disposition. Predictors of percutaneous failure were identified.

Results

168 patients (296 arteries) had percutaneous access (pEVAR) while 131 patients (226 arteries) had femoral cutdown access (cEVAR). Ultrasound guided access was introduced in 2007. pEVAR increased from zero in 2005 to 92.3% in 2010. The success rate with percutaneous access was 96%. Failures included 7 for hemostasis and 6 for flow limiting stenosis or occlusion of the femoral artery. pEVAR had fewer wound complications (0.7% vs. 7.4%, P =.001) shorter operative time (153.3 vs. 201.5min, P <.001) and larger access vessel size (6.7mm vs. 6.1mm, P <.01). Patients with failed percutaneous access had smaller access vessels when compared to successful pEVAR (4.9mm vs. 6.8mm, P <.001). More failures occurred in small sheaths than large ones (7.4% vs. 1.9%, P =.02). Vessel size <5mm is predictive of percutaneous failure (OR 7.3 [1.58-33.8]).

Conclusion

pEVAR can be performed in the vast majority of patients with a high success rate, shorter operative times, and fewer wound complications. Access vessel diameters less than 5mm are at greater risk for percutaneous failure and should be treated selectively.

Introduction

Endovascular aortic aneurysm repair has replaced open surgical repair as the preferred method for treating intact infrarenal abdominal aortic aneurysms (AAA) and descending thoracic aortic aneurysms (DTA) in the elective setting.[1] Endovascular repair requires the placement of large diameter sheaths into the common femoral arteries (CFA) for stent graft deployment. Access to the CFA has typically been achieved by open femoral cutdown incisions. Complications related to femoral cutdown include wound infections, lymphoceles, and hematomas. With the development of suture mediated closure devices and the "preclose" technique[2] for closure of large femoral arteriotomies, some vascular surgeons are now performing percutaneous EVAR. Among prior reports including 50 or more patients, the technical success rates of percutaneous access have ranged between 76% and 96%.[3-6] The utilization of ultrasound guidance has been suggested to improve accuracy and lower the complication rate associated with percutaneous access.[7, 8]

Several patient factors have been stated to be relative contraindications for percutaneous arteriotomy closure. Some studies have demonstrated that morbid obesity (body mass index >35kg/m^2), femoral artery calcification, groin scars from prior interventions, and large sheath size are more commonly associated with percutaneous failure and wound complications.[3, 9-15] Other studies have demonstrated no association between sheath size, morbid obesity, and femoral artery calcification with percutaneous failure and wound complications.[7, 16, 17] Percutaneous access has been suggested to decrease operating room (OR) time as well as patient length of stay (LOS) in the hospital.[9, 11] The majority of the data are from single institution case series and small single center randomized trials. The purpose of this study is to describe our experience with percutaneous endovascular aortic aneurysm repair and to compare our outcomes with the published literature.

Methods

Overview

We performed a retrospective study of all elective endovascular aortic aneurysm repairs (abdominal and thoracic) from January 1, 2005 to December 31, 2010 at a single tertiary care medical center. Patients were stratified based on their type of access for stent graft delivery: percutaneous (pEVAR) vs. femoral cutdown access (cEVAR). Only limbs accessed with a 12 Fr sheath size or larger were included in the study. Sheaths were categorized as small (12-16 Fr) or large (18 Fr or larger). Most cases were performed in an operating room endovascular suite and most patients received general anesthesia. All patients were anticoagulated with heparin during the procedure and received protamine reversal at the end of the case. Patients were seen for follow up typically at 1 month where each CFA puncture site and cutdown incision was assessed via clinical examination. A pulse examination was performed as well as a history taken to identify symptoms of claudication. Demographic data were recorded as well as BMI. Intraoperative variables analyzed included sheath size and minimum access vessel diameter (the minimum diameter of the CFA or external iliac artery as determined by the PEMS M2S Patient Evaluation and Management System).

Outcome measures evaluated were technical success rate of percutaneous access, conversion to open femoral artery repair, cause of percutaneous failure, LOS, discharge disposition, and wound complications. Technical success was defined as a successful arterial closure without the need for conversion to open femoral artery repair as well as no change in the patient's baseline pulse examination. Percutaneous failures were due to either hemorrhage or flow limiting stenosis or occlusion of the CFA. Hemorrhage was defined as persistent bleeding or an expanding hematoma that required surgical exploration after tying the perclose sutures. Flow limitation was identified immediately in the operating room based on a change in the patient's baseline pulse exam at the end of the procedure or at the 1 month follow-up visit if the patient had symptoms of claudication or a change in the pulse examination. OR time (time from entry into the OR until discharge to the recovery room) and incision time (time from initial groin puncture or skin incision to the end of the procedure) were measured as well. OR and incision time were measured in uncomplicated AAA repairs only (patients who underwent DTA repair or concomitant procedures such as hypogastric coiling and renal and iliac stents were excluded from this measurement). Total hospitalization costs were analyzed as well. Wound complications included documented wound infections requiring antibiotic treatment, seromas, and clinically significant hematomas that delayed routine discharge from the hospital. This study was approved by the Institutional Review Board at Beth Israel Deaconess Medical Center and Harvard Medical School.

Statistical analysis

We calculated the total number of patients and arteries that underwent percutaneous or femoral cutdown access and how the proportion of each has changed over time. Analyses of mean sheath size, proportion of large sheaths, and technical success of percutaneous access were performed on a per limb basis. Analyses of wound complications and other outcomes were calculated on a per patient basis. Preoperative characteristics and outcomes are reported as proportions of the sample and mean ± standard deviation. Patient variables were compared utilizing univariate analysis. Categorical variables were analyzed using chi-square and the Fisher exact test where appropriate. Continuous variables were compared using the Wilcoxon rank sum test. A test of trend over time was used to test for significance in changes in the minimum access vessel diameter in pEVAR patients over the study period. Multivariable logistic regression was used to identify predictors of percutaneous failure. Statistical significance was defined as $P < .05$. All statistical tests were performed using STATA 12 software (StataCorp, College Station, TX).

Patient Selection

Early in our experience, the decision to perform endovascular aortic aneurysm repair via percutaneous access varied by attending. Percutaneous access was initially performed on the contralateral 12 Fr side when deploying the Gore Excluder stent graft (WL Gore & Associates, Flagstaff, AZ). Percutaneous access was generally avoided in patients with small arteries and femoral artery calcification in our early experience. Ultrasound (US) guidance for percutaneous access was introduced and routinely used beginning in 2007. As our experience broadened with the utilization of ultrasound guidance, patient selection for percutaneous access expanded rapidly and now virtually every EVAR is performed with percutaneous access.

Preclose Technique

All patients receive preoperative intravenous antibiotics. After sterile drapes are placed, the US probe is used to identify the CFA and the femoral bifurcation in all patients. The best location for CFA puncture is determined by the extent of calcification on the anterior wall and plaque both anteriorly and posteriorly. A small stab incision is made in the skin inferior to the expected arterial puncture site. Blunt dissection is then carried down through the subcutaneous tissue to the anterior wall of the CFA under US guidance. A micropuncture needle is inserted into the CFA under direct visualization with the US probe. Fluoroscopy is used to confirm puncture over the femoral head. A microsheath and 0.035 inch wire are inserted into the CFA followed by a 7 Fr dilator. After dilation of the artery and subcutaneous tissue a 6 Fr Perclose Proglide device is inserted over the wire into the CFA. Pulsatile blood flow from the marker lumen confirms proper positioning of the Proglide device within the CFA. The Proglide device is then fired and the wire is replaced before the device is withdrawn from the CFA. The sutures are not tied down as they are secured to the sterile drapes with a Kelly clamp. A second Proglide device is inserted into the same artery, confirmation of intra-arterial placement is obtained, the device is fired, the wire is replaced, and the sutures are secured with a Crile clamp to distinguish the second knot from the first. A 7 Fr 25cm sheath is then inserted into the CFA. The above steps are repeated on the contralateral groin leaving two sets of untied Proglide sutures in each groin. Initially, we deployed the two devices at 10 and 2 o'clock and changed over time to 11 and 1 o'clock and currently aim for 11:59 and 12:01 with the goal of slightly offsetting the devices without puncturing the sidewall of the CFA to avoid narrowing the vessel or failure to puncture the vessel wall. The patient is then heparinized. If there is hemorrhage around the 7 Fr sheath a 12 Fr sheath is inserted over an Amplatz or Lunderquist wire. Serial dilations are employed with Coons dilators before placement of the large sheath or device.

Standard EVAR then commences. The majority of the stent grafts placed at our institution are Zenith (Cook, Bloomington, IN) and Gore stent grafts. At the completion of the operation the previously placed perclose sutures are irrigated to remove any debris that may be present. It is important to maintain wire access at all times. Gentle manual pressure is applied to the proximal CFA as the sheath is removed over a wire. After removal of the sheath, manual pressure is released to allow the knot pusher to advance properly through the subcutaneous tissue and cinch down the preformed knot of the perclose suture. If hemostasis is adequate the wire is removed and the suture is locked and cut. The second suture is then tied down, locked and cut. Manual pressure is then held for 5-10 minutes. If hemostasis is inadequate after tying the two knots, a third perclose device is inserted over the wire and deployed and this usually results in adequate hemostasis. If hemostasis is still not achieved, a sheath is then introduced over the wire to provide hemostasis while the CFA is surgically explored. The pedal pulses are always checked at the end of the operation and compared to the preoperative pulse exam. Duplex examination of the puncture site may be performed to assess the patency of the femoral vessels. If pulses are lost or the duplex examination shows significant stenosis, the ipsilateral groin is then explored surgically.

Results

We identified 294 patients who underwent elective endovascular repair of their aortic aneurysm between 2005 and 2010 at our institution; 267 had AAA and 27 had DTA. 163 patients underwent percutaneous access (pEVAR) while 126 underwent femoral cutdown access (cEVAR). Five patients had combined access with one groin accessed via percutaneous technique and the other accessed via cutdown. Only limbs accessed with 12 Fr sheaths or larger were identified: 168 patients (296 arteries) underwent pEVAR and 131 patients (226 arteries) underwent cEVAR. Mean sheath size was similar between pEVAR and cEVAR patients (17.0 Fr vs. 17.2 Fr, P =.58). Similarly, the proportion of sheaths that were considered large (≥18 Fr) was similar between pEVAR and cEVAR patients (54.1% vs. 60.6%, P =.13).

Percutaneous vs. cutdown

We performed zero percutaneous repairs in 2005 and two in 2006 (6.7% of all endovascular repairs in 2006). After the introduction of ultrasound in 2007, the proportion of cases performed via percutaneous access increased from 39.3% in 2007 to 92.3% in 2010 (Figure 1). Four of the 5 femoral cutdowns performed in 2010 were for planned femoral-femoral bypasses in patients with severe occlusive disease of one of their iliac arteries while the remaining femoral cutdown was performed in a patient with distal embolization of debris from the aneurysm sac. Femoral cutdown was performed in this patient to isolate the femoral vessels and prevent further lower extremity embolization during wire manipulation and device delivery.

Demographic and comorbidities

The majority of patients were white males with a similar mean age in the pEVAR and cEVAR patients (75.0 years vs. 75.7 years, P =.47; Table 1). Comorbidities were similar between the pEVAR and cEVAR patients. The only significant differences were a higher rate of hypertension and hyperlipidemia in the pEVAR group. Mean BMI was similar (27.0 vs. 26.7 kg/m^2, P =.70) as well as the proportion of obese patients, BMI >30 kg/m^2 (28.2% vs. 19.4%, P =.11). The proportion of patients with prior groin operations was similar as well, 6.8% in the pEVAR group vs. 11.1% in the cEVAR group (P =.21).

Minimum access vessel diameter

The mean minimum access vessel diameter was significantly larger in the pEVAR group (6.7mm vs. 6.1mm, P <.01). The change in minimum vessel diameter over time in arteries undergoing pEVAR is shown in Figure 2. The mean diameter was 7.3mm in 2006 and this decreased by over 1mm to 6.2mm in 2010 (P =.01, test of trend).

Percutaneous failure

There were 13 percutaneous failures (12 patients) in 296 arteries requiring conversion to open surgical exploration and repair of the artery, giving a technical success rate of 96%. Eleven failures occurred immediately in the operating room at the conclusion of the case, one failure occurred 12 hours post-operatively when the patient was on the vascular ward, and one failure was discovered on post-operative day 20 at the patient's follow-up clinic visit (Table 2). A greater proportion of women experienced failure compared to men, but this was not significant (12.5%

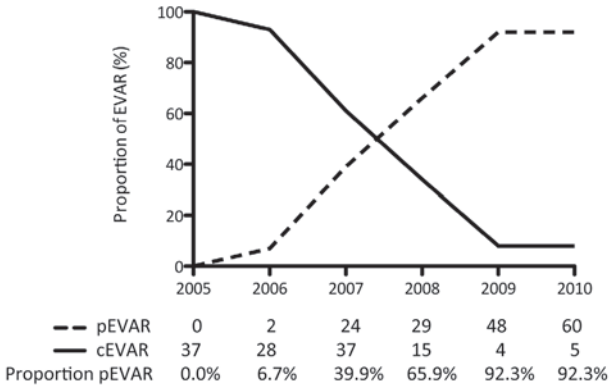

Figure 1 | Proportion of PEVAR over time, 2005-2010

Table 1 | Demographic and comorbidities.

	pEVAR	cEVAR	P-value
Male	81.0	77.0	.41
Age, y	75.0 ± 8.8	75.7 ± 8.9	.47
White race	85.9	89.7	.33
Coronary artery disease	45.4	43.7	.77
Myocardial infarction	25.2	27.0	.73
Percutaneous coronary intervention	17.2	13.5	.39
Coronary artery bypass graft	19.0	21.4	.61
Hypertension	82.8	69.1	<.01
Atrial fibrillation	15.3	19.8	.32
Valvular heart disease	8.0	12.7	.19
Congestive heart failure	7.4	11.9	.19
Hyperlipidemia	74.2	57.9	<.01
Diabetes	19.6	22.2	.59
Renal insufficiency	19.0	23.8	.32
COPD	25.2	31.8	.22
Stroke	11.7	10.3	.72
Peripheral vascular disease	19.6	19.1	.90
BMI, kg/m^2	27.0 ± 5.3	26.7 ± 5.7	.70
% BMI >30 kg/m^2	28.2	19.4	.11
Prior groin operation	6.8	11.1	.21
Minimum access vessel diameter, mm	6.7 ± 1.6	6.1 ± 1.4	<.01

Data are presented as % or as mean ± sd. Abbreviations: pEVAR, percutaneous endovascular aneurysm repair; cEVAR, femoral cutdown endovascular aneurysm repair; COPD, chronic obstructive pulmonary disease; BMI, bodymass index.

vs. 5.9%, P =.18). One failure occurred in an obese patient and one occurred in a patient with groin scar tissue. Figure 3 shows the absolute number of arteries by each millimeter of size along the left horizontal axis (48 diameters are missing) and the proportion of arteries that failed percutaneous access at each millimeter of size along the right horizontal axis (1 diameter is missing). The majority of the access vessels in our study had minimum diameters between 5 and 8.9 mm (78.2%), while a small amount (4.8%) were larger vessels (≥9mm) and the remaining 16.9% were small vessels between 3 and 4.9 mm. The percutaneous failure rate was 16.7% for all access vessels <5mm, 6.3% for vessels 5-5.9 mm, 2.1% for vessels 6-7.9mm, and 0% in vessels ≥8mm. The minimum access vessel diameter was significantly smaller in patients who failed percutaneous access (4.9 mm vs. 6.8 mm, P <.001). Most failures (58.3%) in our study occurred in arteries smaller than 5 mm. Figure 4 shows the absolute number of sheaths by Fr size along the left horizontal access and the proportion of failures with each sheath size along the right horizontal axis. More failures occurred in small sheaths than large ones (7.4% vs. 1.9%, P =.02). Percutaneous failures have decreased over time: 9.1% in 2007, 6.8% in 2009, and 2.7% in 2010 (there were zero failures in 2008).

Postoperative outcomes

There were no deaths. More pEVAR patients were discharged home (91.2% vs. 84.4%, P =.11). Mean LOS was 1 day shorter in pEVAR patients, which exhibited a trend towards significance (3.1 days vs. 4.1 days, P =.08). Sixteen pEVAR patients and 31 cEVAR patients had no 30-day follow up and were excluded from the analysis of wound complications. There was one wound complication (0.7%) in the pEVAR group (hematoma that delayed discharge)

Table 2 | Causes of percutaneous failure.

Immediate failures
Hemostasis
1. Longitudinal tear in the anterior wall of artery
2. Disrupted plaque
3. Perclose suture tied in subcutaneous tissue
4. Failed perclose deployment due to calcification
5. Failed perclose deployment due to calcification
6. Failed perclose deployment due to scar tissue
7. Failed perclose deployment, unknown
Flow limiting stenosis/occlusion
1. Disrupted plaque
2. Disrupted plaque
3. Disrupted plaque
4. Thrombosis from posterior intimal damage
Delayed failures due to flow limiting stenosis/occlusion
1. Disrupted plaque (12 hours post-operatively)
2. Disrupted plaque (discovered on post-op day 20)

and 7 wound complications (7.4%) in the cEVAR group (3 wound infections, 2 hematomas that delayed discharge, 1 pseudoaneurysm, and 1 seroma), P <.01. There were no wound complications in the 12 patients with failed percutaneous access.

OR time, incision time, and cost

When examining straight forward EVAR cases only (excluding patients with concomitant procedures such as hypogastric embolization (n=11) and renal (n=6) or iliac stents (n=11) for

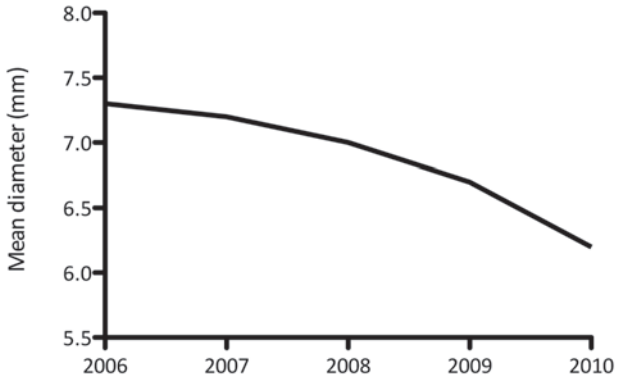

Figure 2 | Change in PEVAR minimum access vessel diameter over time, 2005-2010

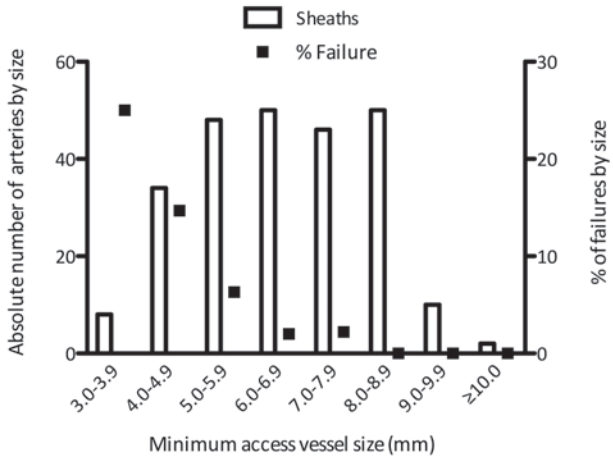

Figure 3 | Number of arteries based on size per millimeter (left axis) and proportion of percutaneous failures based on size per millimeter (right axis).

stenosis), percutaneous access significantly reduced total OR time and incision time when compared to femoral cutdown access: OR time (153.3 minutes vs. 201.5 minutes, P <.001) and incision time (97.3 minutes vs. 139.2 minutes, P <.001). Hospitalization costs were slightly more expensive in the PEVAR group ($66,114 vs. $62,694, P =.52). Failed percutaneous access negated the improvement in OR and incision times when compared to patients with successful percutaneous access: OR time (263.0 minutes vs. 159.6 minutes, P <.001) and incision time (198.6 minutes vs. 102.8 minutes, P <.001).

Predictors of percutaneous failure

Multivariable logistic regression was performed to identify predictors of percutaneous failure. Due to the low rate of percutaneous failures (n=13), a limited multivariable model was run. After controlling for age, female gender, and the presence of peripheral vascular disease, small vessel size (<5mm, dichotomous variable) is predictive of percutaneous failure (OR 7.3 [1.58-33.8], P =.01).

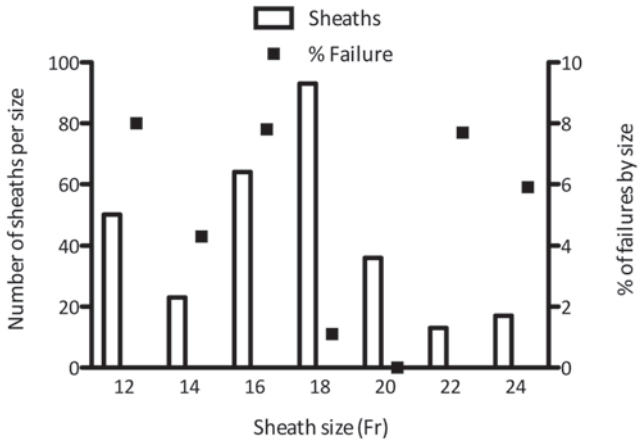

Figure 4 I Number of sheaths based on Fr size (left axis) and proportion of percutaneous failures based on sheath size (right axis).

Discussion

We found a high technical success rate with ultrasound guided percutaneous access and that access vessel diameter rather than sheath size predicts failure. In our experience, we started simple, and as we gained experience and familiarity with percutaneous access we expanded this technique to virtually all patients. In addition to experience, we feel that ultrasound allows us to more accurately puncture the CFA rather than the superficial femoral and profunda femoris arteries. Ultrasound also allows us to find the best puncture site on the CFA and to avoid any anterior calcification or plaque if present. Our study is the first to incorporate the minimum access vessel diameter as a potential predictor of percutaneous failure and found this to be the only predictor of failure even with 24 Fr sheaths.

The majority of the access vessels in our study had minimum diameters between 5 and 8.9mm (78.3%), while a small amount (4.8%) were larger vessels (≥9mm) and the remaining 16.9% were small vessels between 3 and 4.9mm. As we have expanded pEVAR to nearly all patients who present for elective AAA repair, it is of no surprise that the minimum access vessel diameter of patients undergoing pEVAR has decreased over time from 7.3mm in 2006 to 6.2mm in 2010 (P =.01, test of trend). Of the 12 percutaneous failures with documented minimum vessel diameters, 7 (58.3%) were in vessels smaller than 5mm (16.7% of all vessels <5mm failed). The minimum access vessel diameter was significantly smaller in patients who failed percutaneous access (4.9mm vs. 6.8mm, P <.001). Women typically have smaller arteries than men, and a greater proportion of women in our study experienced percutaneous failure when compared to men (12.5% vs. 5.9%, P =.19), but this was not significant. When performing multivariable logistic regression and controlling for age and gender, access vessel size <5mm predicts percutaneous failure. This does not mean that patients with small access vessels should not be offered percutaneous repair, but rather that care must be exercised when deciding which small vessels upon which to attempt percutaneous repair. More data is needed regarding small access vessels and percutaneous repair. Small diseased CFAs may not allow adequate room for function of the perclose ProGlide device.

The technical success rate in our study reached 96%. This compares favorably to the 76% to 96% success rates of other studies with >50 patients.[3-6] Since 2007, we have used ultrasound guidance when performing all percutaneous EVARs and the percutaneous failure rate per artery has decreased despite expanding pEVAR to more patients and smaller diameter arteries. Starnes et al demonstrated, in a study of 59 and 93 arteries accessed with and without ultrasound respectively, that ultrasound guidance significantly decreased their conversion to open repair in sheaths >20 Fr (0% vs. 14%, P <.05) as well as significantly increased their technical success rate in sheaths >20 Fr (100% vs. 82%, P <.05).[7] In a study of 52 patients and 85 arteries by Oquzkurt et al, technical success was achieved in 49 patients (94%) and there were no wound complications at the end of the case and at their 30 day follow-up visit.[8]

Femoral cutdown access for EVAR has complications that include wound infections, lymphoceles, and hematomas. These complications can occur after percutaneous access as well. Morasch et al reported a wound complication rate of 22.8% in 35 patients who received femoral cutdown vs. zero complications in 47 patients who received percutaneous access.[11] Dalainas et al, in a study of 186 patients undergoing femoral cutdown for EVAR, reported an

8% wound infection rate and a 6.5% rate of wound necrosis and dehiscence.[18] There was no percutaneous comparator group. Our results demonstrate that patients undergoing percutaneous access have significantly fewer wound complications than those undergoing femoral cutdown as well. One patient (0.6%) in the pEVAR group (hematoma) and 7 patients (6.5%) in the cEVAR group (3 wound infections, 2 hematomas, 1 pseudoaneurysm, and 1 seroma) experienced wound complications, P <.01.

Several studies have demonstrated that morbid obesity[6, 7, 11-15], large sheath size[11-13, 15], and femoral artery calcification[10, 15] were associated with failed percutaneous access. Our study revealed no such findings. We looked at obesity (BMI >30) rather than morbid obesity (BMI >35) in our study and found that 1 obese patient experienced failure (2.7%) versus 11 non-obese patients (8.7%), P =.22. Large sheath size (≥18 Fr) was not associated with percutaneous failure in our study. In fact, we found the exact opposite. There was a significant increased risk of percutaneous failure with small sheaths in our study (7.4% vs. 1.9%, P =.02). It is hard to draw any concrete conclusions from this other than to reiterate that even small sheaths can cause serious complications when placed into diseased arteries and care needs to be taken when performing percutaneous access. We made no effort to quantify femoral artery calcification in this study and we do not exclude patients with calcification from receiving percutaneous access. With ultrasound guidance we avoid heavily calcified areas of the CFA when locating the best puncture site. In the 5 years that we have been performing pEVAR, we have only had 2 failures (out of 11 total failures) attributed to femoral artery calcification.

Limitations to this study include its retrospective design. Certainly, earlier in the study period there was a selection bias as patients with small vessels and calcification were not offered percutaneous access. As virtually all patients who now undergo elective AAA repair are doing so via percutaneous access, the earlier selection bias has been eliminated. The low number of events can lend itself to misinterpretation of the data as well as limit the amount of multivariable modeling one can perform.

Percutaneous EVAR can be performed in the vast majority of patients with a high success rate, shorter operative times, and fewer wound complications. Access vessel diameters less than 5mm are at greater risk for percutaneous failure and should be treated selectively.

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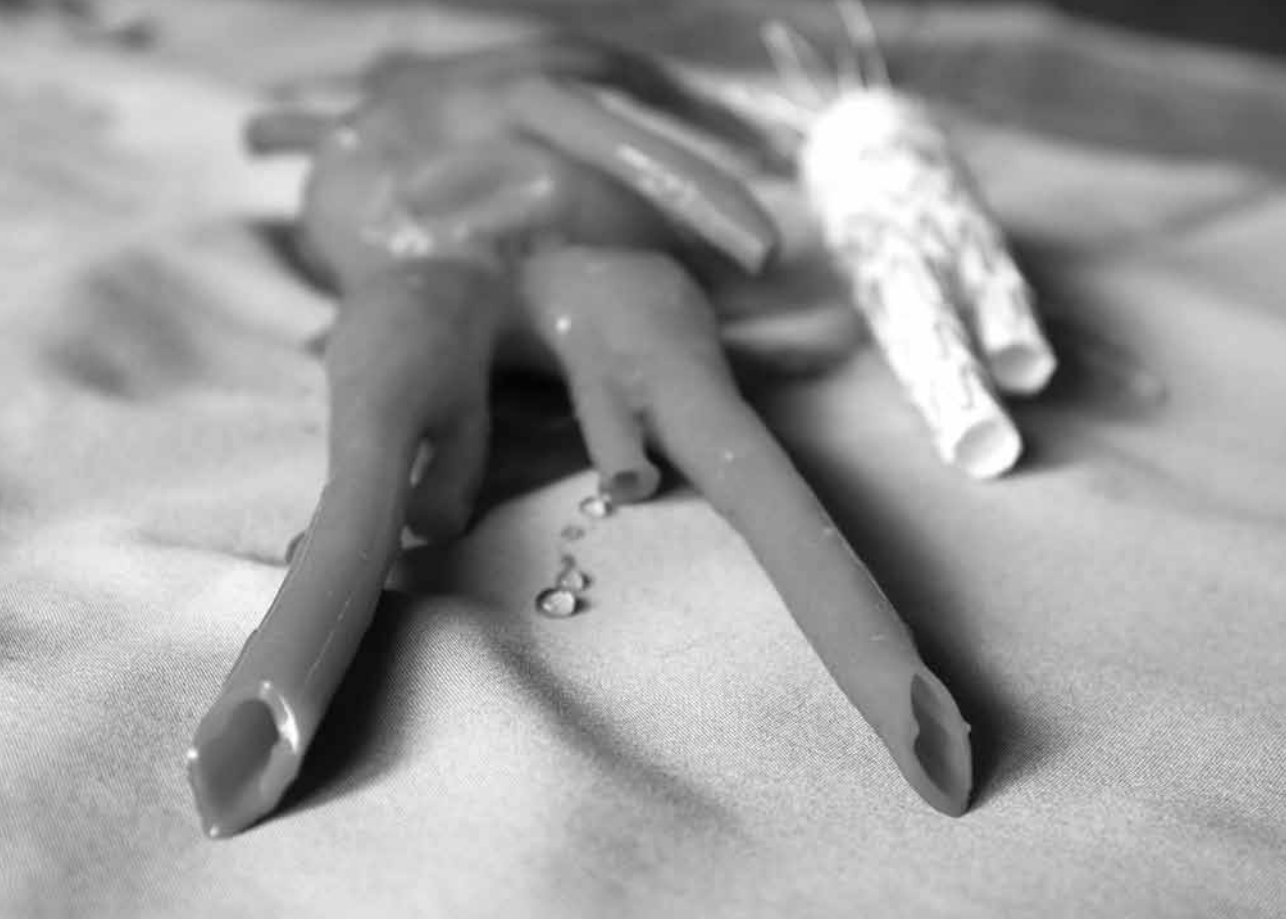

Vascular surgeons repair an increasing majority of abdominal aortic aneurysms, where volume load changes over time and determines outcome

12

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Submitted

Abstract

Introduction

Outcome after abdominal aortic aneurysm (AAA) repair relies on level of experience and volume of the treating physician. Multiple specialties perform these repairs, however, distribution of proportions remain unknown, as are interactions with surgeon and hospital volume, especially for endovascular aneurysm repair (EVAR). We aimed to address these issues in a national setting and analyze quality of care as measured by inpatient mortality.

Methods

The Nationwide Inpatient Sample was queried from 2001 through 2009 for intact and ruptured AAA and for EVAR and open repair. Specific procedures were used to identify cardiac (CS), general (GS) and vascular surgeons (VS) as well as interventional cardiologists (IC) and radiologists (IR) for states that reported unique treating physician identifiers. Annual surgeon and hospital volumes were calculated.

Results

We identified 193,668 AAA repairs in the studied period. VS performed an increasing proportion of AAA repairs (from 44% to 64% in the studied period) driven by the increased utilization of EVAR as VS perform 67% of all EVARs while GS perform 23% and CS 15%. Open intact AAA were mostly performed by high volume VS in high volume hospitals. For GS, most open intact repairs were performed by low volume surgeons in low volume hospitals. After adjustment, treatment by CS was beneficial (OR 0.61 [0.54-0.69]) for open but detrimental (1.26 [1.04-1.53]) for EVAR in terms of mortality. Differences between specialty (GS and VS) proved less relevant for outcomes than high surgeon volume (open repair 0.72 [0.64-0.82] and EVAR 0.58 [0.45-0.74]) and patient characteristics. Median annual surgeon volume decreased over time for open (8 to 4) but increased for EVAR (9 to 12). Treatment by CS (0.89 [0.86-0.92]) and emergent type admission (0.75 [0.72-0.77]) decreased the likelihood of receiving EVAR. High hospital volume (1.29 [1.24-1.34]), but mainly high surgeon EVAR volume (14.63 [14.03-15.25]) increased the chance of receiving EVAR.

Conclusions

VS performed an increasing majority of AAA repairs, driven by an increased utilization of EVAR. Mortality was mainly driven by low surgeon volume and patient characteristics, less by differences between specialties (GS and VS). The number of high volume surgeons for open repair has decreased and low volume surgeons had worse outcomes. This study emphasizes the need for appropriate training and volume load for these procedures and supports regionalization.

Introduction

Abdominal aortic aneurysm (AAA) repair is associated with significant mortality and morbidity.[1] Level of training and experience likely has a direct influence on outcome after this procedure. Quality of care may be affected by the frequency that a physician performs a procedure in a specific part of the body, treats a disease, and performs a certain intervention. High hospital volume[2, 3] and high surgeon volume[4, 5] have been suggested to influence patient outcomes. Both endovascular aneurysm repair (EVAR) and open repair are being performed by physicians with different specialist backgrounds and different annual volume loads. For open AAA repair it was shown that in the US in 1996 39% of elective open AAA repairs were performed by vascular surgeons (VS), 33% by cardiac surgeons (CS) and 28% by general surgeons (GS).[6] A study in Ontario, covering 1992-1996, reported that the respective proportions were 75%, 5% and 20% for the same procedure.[7] Changes over time remain unknown, and the effect of the introduction of EVAR on this distribution among specialties has not been documented. We aimed to analyze national trends over time for specialties performing EVAR and/or open AAA repair and their differences in volume over time as we expected those to change. In addition, we also wanted to report differences in mortality by specialty and procedure.

Methods

Database

For this study, the Nationwide Inpatient Sample (NIS) was used from 2001 to 2009. The NIS is maintained by the Healthcare Cost and Utilization Project of the agency for Healthcare Research and Quality. This database includes a 20% all-payer sample of hospital stays.

The database was queried using ICD-9 codes to identify patients with diagnosis codes for ruptured AAA (441.3) and intact AAA (441.4). Patients with open AAA repair (38.44, 39.25) and EVAR (39.71) were selected. Patients with procedural codes for both open repair and EVAR were considered as EVAR patients as they likely result from coding errors or conversions to open repair. To increase the study population homogeneity, patients with codes for thoracic aneurysm (441.1 or 441.2), thoracoabdominal aneurysm (441.6 or 441.7) or aortic dissection (441.00-441.03) were excluded.

Demographic and operative characteristics were captured within NIS. Patient comorbidities were identified using the Elixhauser definitions.8 Primary outcome was in-hospital mortality. Mortality was defined as death from any cause prior to discharge regardless of time from operation.

Specialty algorithm

For AAA repair, we were interested in the following specialties: VS, GS, CS, interventional cardiologists (IC) and interventional radiologists (IR). As of 2001, NIS provided unique physician identifiers per state that allow tracking of procedures that were performed by that physician during that specific year. Of the available states, 27 provided 2 unique physician identifiers, of them 22 states specifically stated which identifier correlated with the primary procedure performed. For the remaining 5 states the identifiers were only used when both were identical

to make sure that it involved the physician that performed the primary procedure (the identifiers were identical in 75% of cases). We composed a list of specialty specific procedures (Appendix Table 1) that we used to label the specialty of each physician (VS, GS, CS, IC, or IR). Similar approaches were performed before.[9-11] Subsequently we created a hierarchical model: each physician that performed >10 cardiac surgery procedures was labeled a CS; the remaining physicians that performed >10 interventional cardiac procedures were labeled IC; physicians with >10 interventional radiology procedures were identified as IR; the remaining physicians that performed 75%-100% vascular surgery procedures (of vascular and general surgery codes combined) and >10 in number were classified as VS; physicians with 0%-75% vascular procedures and >10 general surgery procedures were classified as GS.
Annual volume data was calculated and subsequently divided into terciles to create equal groups for comparison. This was undertaken for hospital, VS, GS and CS; separately for EVAR and open repair. Because of low absolute numbers these volume comparisons were not conducted for the interventional specialists.

Statistical approach

Statistical analyses were performed using SAS 9.2 software (SAS Institute, Cary, NC) and PASW Statistics 18 (SPSS Inc., Chicago, IL). Mean and standard deviation are reported for parametric data. Variables were compared using Chi-square tests or t-tests where appropriate. Multivariable logistic regression models were utilized for the prediction of mortality and for the likelihood of receiving EVAR. Analyses were considered statistically significant when P <.05.

Table 1 | Baseline characteristics per specialist.

	Open				EVAR						Open	EVAR	
	VS	GS	CS	P-value	VS	GS	CS	IC	IR	P-value	Overall		P
N	45804	21625	17651		72489	17500	14033	3056	1510		85080	108588	
Age, y	71.3±8.5	71.9±8.4	71.0±8.6	<.001	73.7±8.4	74.0±8.3	72.8±8.3	73.1±8.2	74.1±8.1	<.001	71.4±8.5	73.6±8.4	<.001
Female gender	25.5	23.6	23.0	<.001	18.3	15.4	16.9	16.8	17.0	<.001	24.5	17.7	<.001
Non-white race	9.6	9.3	9.0	.051	8.5	8.8	7.8	12.7	21.3	<.001	9.4	8.8	<.001
Hypertension	62.5	57.6	64.9	<.001	69.1	66.6	70.4	72.9	69.9	<.001	61.7	69.0	<.001
Diabetes	11.7	11.1	11.1	.022	15.9	14.9	16.6	17.7	17.6	<.001	11.4	15.9	<.001
COPD	27.1	29.1	28.4	<.001	22.5	22.9	24.5	21.6	22.2	<.001	27.9	22.8	<.001
Obesity	3.8	3.8	4.7	<.001	5.7	5.0	6.8	5.1	7.3	<.001	4.0	5.8	<.001
CHF	10.2	12.9	8.1	<.001	5.5	4.6	5.6	7.1	4.2	<.001	10.4	5.4	<.001
Prior MI	10.6	7.9	9.9	<.001	14.4	13.5	12.3	12.8	11.9	<.001	9.8	13.9	<.001
Renal failure	7.3	6.1	6.2	<.001	7.5	6.4	6.4	7.4	8.9	<.001	6.8	7.2	<.001

Data are presented as % or as mean ± sd. Abbreviations: VS, vascular surgeon; GS, general surgeon; CS, cardiac surgeon; IC, interventional cardiologist; IR, interventional radiologist; COPD, chronic obstructive pulmonary disease; CHF, chronic heart failure; MI, myocardial infarction. P-values compare differences within the treatment groups.

Results

Overall, 108,588 EVAR and 85,080 open AAA repairs were identified in the studied period. Baseline characteristics are reported in Table 1. The top 15 procedures of the different specialists are listed in appendix Table 2. Of all AAA repairs, 61% were performed by VS, 23% by GS, 15% by CS, 1% by IC and 1% by IR. Figure 1A illustrates changes over time. The increase of the proportion intact AAA repairs treated with EVAR is shown in Figure 1B. The majority of open repairs of intact AAA were performed by VS (55%) and the remaining proportion was equally divided between GS (24%) and CS (22%), Figure 1C. The proportions for specialists performing EVAR for intact AAA remained fairly constant over time (VS 67%, GS 16%, CS 13%, IC 3% and IR 1%; Figure 1D), however the absolute number of EVAR increased from 5,906 in 2001 to 16,252 in 2009. Since VS performed a greater proportion of EVAR, this had led to VS performing an increasing majority of overall intact AAA repairs. For repair of rAAA, a dramatic increase in proportion treated with EVAR was seen, most pronounced for VS (4% to 46%, Figure 1E). CS repaired a smaller proportion of rAAA with an open procedure (16%), GS repaired 35% and VS 49%, Figure 1F. This was a decrease in proportion of open rAAA repairs for VS and CS when compared to intact AAA repairs, while GS were repairing a greater proportion of rAAA compared to intact. VS were dominant in performing EVAR for ruptured AAA (73%), the remainder were performed by GS (15%), CS (9%), IC (1%) and IR (2%), Figure 1G.

Table 2 lists mortality after intact AAA repair (open and EVAR) for urgent and non-urgent admissions per tercile of hospital- and surgeon volume by specialty. For non-urgent open intact AAA repair, most procedures were carried out by high volume VS at high volume hospitals. To a lesser extent this also held true for CS but not for GS, where most procedures were done by low volume surgeons at lower volume hospitals. Mortality was higher with low-volume surgeons, most pronounced in the low- and medium volume hospitals. VS and GS had similar mortality rates among the different hospital volume terciles, but mortality decreased with higher surgeon volume. For CS the mortality rates were lower in high volume hospitals.

Overall, for non-urgent EVAR for intact AAA, the numbers of repairs were higher but similarly distributed among hospital and surgeon volumes compared with open repair. Mortality rates appeared similar between the different terciles of hospital volume, however, the low volume GS and CS had higher mortality rates at both high- and low volume hospitals. Figure 2A demonstrates the decrease in patients treated by high volume surgeons over time for open AAA repair, whereas Figure 2B shows fewer patients receive EVAR by low volume surgeons over time. Prominent predictors of mortality after open and EVAR were renal failure (3.19 [2.81-3.62] and 4.20 [3.54-5.01]) and CHF (1.67 [1.50-1.86] and 3.14 [2.61-3.78]). Female gender (1.28 [1.17-1.40] and 2.76 [2.41-3.18]) and emergent admission (1.74 [1.59-1.90] and 2.73 [2.36-3.15]) were more detrimental in EVAR (Table 3). High annual procedure-specific surgeon volume proved to be relevant for both open and EVAR when compared to the lowest tercile (0.72 [0.64-0.82]) and 0.58 [0.45-0.74]). Treatment at high volume hospitals was protective for mortality with open repair (0.77 [0.68-0.87]) but not for EVAR (0.82 [0.66-1.01]), although close to being a significant benefit. Factors predicting the likelihood of receiving EVAR are listed in Table 4. Female sex (0.55 [0.54-0.57]) was the strongest predictor of receiving open repair. Additionally, treatment by CS (0.89 [0.86-0.92]) and emergent admission (0.75 [0.72-0.77])

decreased the likelihood of EVAR. Advancing age (1.46 [1.44-1.46]) and treatment by GS (1.23 [1.19-1.28]) predicted EVAR. High hospital volume (1.29 [1.24-1.34]), but mainly high surgeon EVAR volume (14.63 [14.03-15.25]) increased the chance of receiving EVAR. Over the years the probability for receiving EVAR increased (1.21 [1.20-1.21]). Treatment by cardiac surgeons was associated with lower mortality for open repair (0.61 [0.54-0.69]) but higher mortality with EVAR (1.26 [1.04-1.53]).

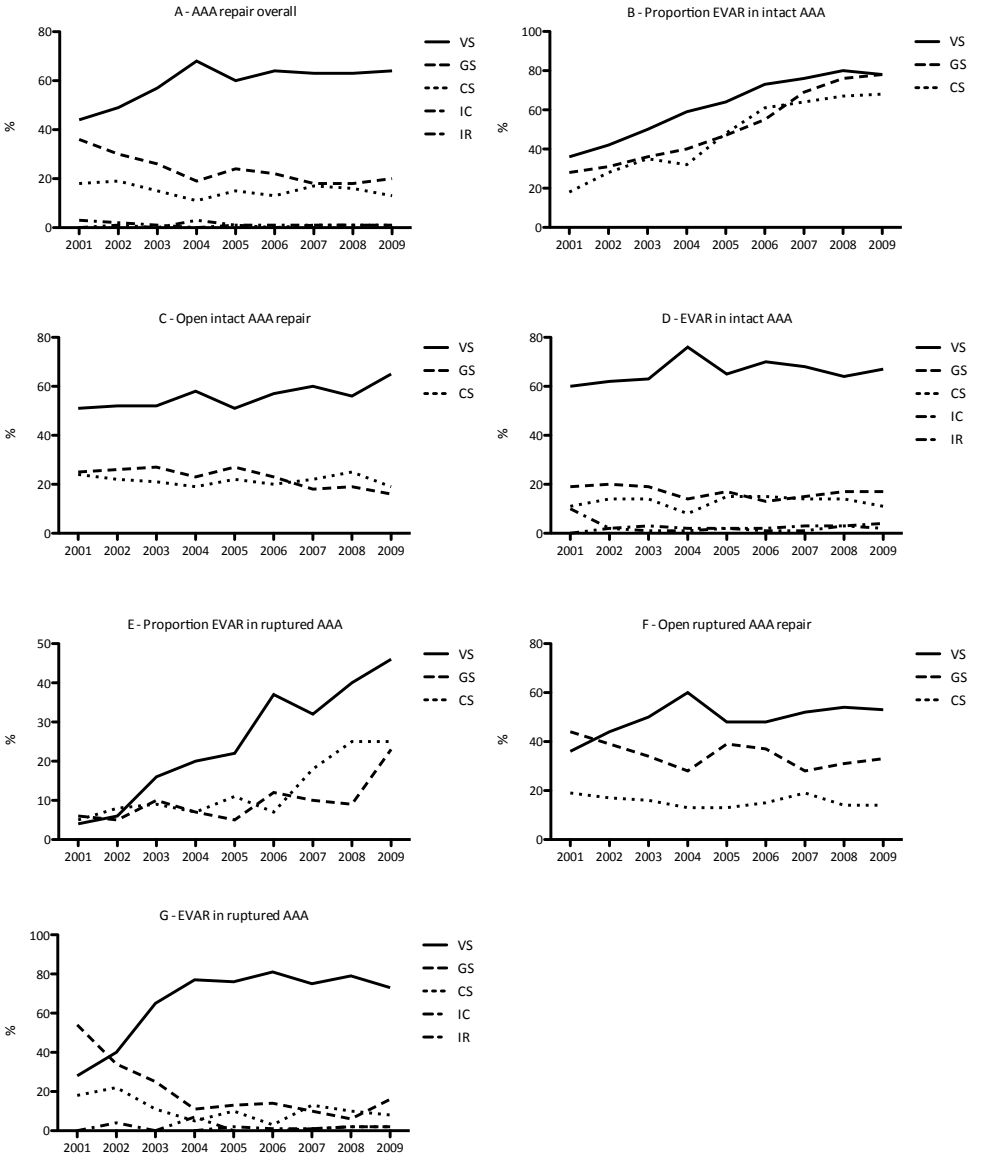

Figure 1 A-G | Proportion of specialty types performing AAA repair from 2001-2009.
Abbreviations: AAA, abdominal aortic aneurysm; EVAR, endovascular aneurysm repair; VS, vascular surgeon; GS, general surgeon; CS, cardiac surgeon; IC, interventional cardiologist; IR, interventional radiologist.

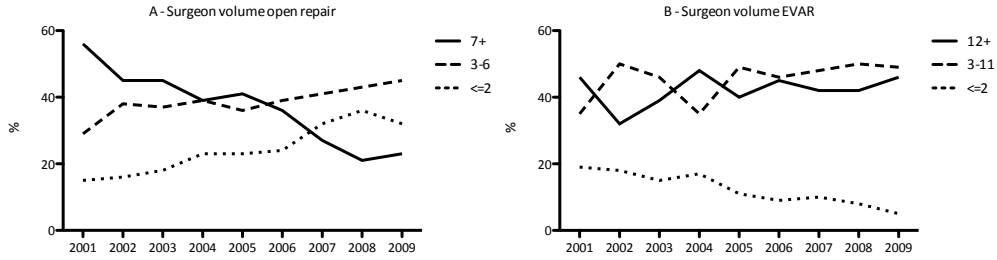

Figure 2 A-B l Tertiles surgeon volume for AAA repair from 2001-2009.
Abbreviations: AAA, abdominal aortic aneurysm; EVAR, endovascular aneurysm repair.

Table 2A l Annual volume of hospital and surgical specialty vs. percentage mortality after open repair of intact AAA for non-urgent admissions.

		LV Hospital (≤9)			**MV Hospital (10-21)**			**HV Hospital (22+)**		
		ST1	ST2	ST3	ST1	ST2	ST3	ST1	ST2	ST3
		≤2	3-6	7+	≤2	3-6	7+	≤2	3-6	7+
VS	N	2275	3473	591	866	3731	5336	706	3439	12245
	Mort	5.1	4.5	2.5	6.4	3.6	2.0	3.4	3.7	3.1
GS	N	2420	2392	276	981	1888	1440	586	1725	1909
	Mort	5.1	3.5	3.6	7.8	3.1	3.4	8.2	3.0	3.8
CS	N	854	1126	302	785	1917	2053	450	1286	3295
	Mort	2.2	2.2	3.3	5.5	2.8	2.5	1.1	1.1	1.6
Total	N	5549	6991	1169	2632	10168	8829	1742	6450	17449
	Mort	4.7	3.8	3.0	6.7	3.3	2.4	4.4	3.0	2.9

Abbreviations: AAA, abdominal aortic aneurysm; EVAR, endovascular aneurysm repair; LV, low volume; MV, medium volume; HV, high volume; ST, surgeon volume tercile; VS, vascular surgeon; GS, general surgeon; CS, cardiac surgeon; IC, interventional cardiologist; IR, interventional radiologist.

Table 2B I Annual volume of hospital and surgical specialty vs. percentage mortality after intact EVAR for non-urgent admissions.

		LV Hospital (≤13)			MV Hospital (14-35)			HV Hospital (36+)		
		ST1	ST2	ST3	ST1	ST2	ST3	ST1	ST2	ST3
		≤2	3-11	12+	≤2	3-11	12+	≤2	3-11	12+
VS	N	1565	6308	709	791	8707	10732	541	7236	25135
	Mort	0.9	0.9	0.7	0	0.8	0.6	0	0.8	0.5
GS	N	1337	3207	141	662	3762	2163	215	1603	2102
	Mort	2.6	0.4	0	0.8	1.1	0.7	2.3	0	0.7
CS	N	469	1179	150	482	2444	2480	182	1437	2506
	Mort	2.1	0.5	0	1.0	1.1	1.2	2.7	0.3	0.8
Total	N	3371	10694	1000	1935	14913	15371	938	10276	29743
	Mort	1.8	0.7	0.5	0.5	0.9	0.7	1.1	0.6	0.6

Abbreviations: AAA, abdominal aortic aneurysm; EVAR, endovascular aneurysm repair; LV, low volume; MV, medium volume; HV, high volume; ST, surgeon volume tercile; VS, vascular surgeon; GS, general surgeon; CS, cardiac surgeon; IC, interventional cardiologist; IR, interventional radiologist.

Table 3 I Multivariable predictors for mortality in intact AAA repair.

	Open repair		EVAR	
	OR [95%CI]	P-value	OR [95%CI]	P-value
Vascular surgeons	Ref		Ref	
General surgeons	1.09 [0.99-1.21]	.083	1.07 [0.89-1.29]	.473
Cardiac surgeons	0.61 [0.54-0.69]	<.001	1.26 [1.04-1.53]	.019
Emergent admission	1.74 [1.59-1.90]	<.001	2.73 [2.36-3.15]	<.001
Age (per 10 y)	1.82 [1.73-1.91]	<.001	1.86 [1.71-2.02]	<.001
Female sex	1.28 [1.17-1.40]	<.001	2.76 [2.41-3.18]	<.001
Non-white race	1.45 [1.28-1.64]	<.001	0.99 [0.79-1.23]	.899
Hypertension	0.33 [0.31-0.36]	<.001	0.31 [0.27-0.35]	<.001
Diabetes	1.05 [0.92-1.20]	.501	0.85 [0.69-1.05]	.123
COPD	1.08 [0.99-1.19]	.074	1.11 [0.95-1.29]	.185
CHF	1.67 [1.50-1.86]	<.001	3.14 [2.61-3.78]	<.001
Prior MI	0.50 [0.41-0.60]	<.001	0.65 [0.51-0.83]	<.001
Renal failure	3.19 [2.81-3.62]	<.001	4.20 [3.54-5.01]	<.001
Low volume surgeon	Ref		Ref	
Medium volume surgeon	0.84 [0.75-0.94]	.002	0.71 [0.56-0.89]	.003
High volume surgeon	0.72 [0.64-0.82]	<.001	0.58 [0.45-0.74]	<.001
Low volume hospital	Ref		Ref	
Medium volume hospital	0.91 [0.82-1.02]	.108	0.99 [0.81-1.20]	.903
Large volume hospital	0.77 [0.68-0.87]	<.001	0.82 [0.66-1.01]	.064
Year of treatment (per y)	1.00 [0.98-1.02]	.887	1.01 [0.98-1.05]	.364

Abbreviations: EVAR, endovascular aneurysm repair; COPD, chronic obstructive pulmonary disease; CHF, chronic heart failure; MI, myocardial infarction.

Table 4 | Multivariable predictors for treatment with EVAR (OR >1 predicts EVAR).

	OR [95%CI]	P
Vascular surgeons	Ref	
General surgeons	1.23 [1.19-1.28]	<.001
Cardiac surgeons	0.89 [0.86-0.92]	<.001
Emergent admission	0.75 [0.72-0.77]	<.001
Age (per 10 y)	1.46 [1.44-1.48]	<.001
Female sex	0.55 [0.54-0.57]	<.001
Non-white race	1.13 [1.08-1.19]	<.001
Hypertension	1.12 [1.09-1.15]	<.001
Diabetes	1.28 [1.23-1.33]	<.001
COPD	0.84 [0.81-0.86]	<.001
CHF	0.60 [0.57-0.63]	<.001
Prior MI	1.34 [1.29-1.40]	<.001
Renal failure	0.70 [0.67-0.74]	<.001
Low EVAR volume surgeon	Ref	
Medium EVAR volume surgeon	6.75 [6.51-6.99]	<.001
High EVAR volume surgeon	14.63 [14.03-15.25]	<.001
Low EVAR volume hospital	Ref	
Medium EVAR volume hospital	1.23 [1.19-1.27]	<.001
Large EVAR volume hospital	1.29 [1.24-1.34]	<.001
Year of treatment (per y)	1.21 [1.20-1.21]	<.001

Abbreviations: EVAR, endovascular aneurysm repair; COPD, chronic obstructive pulmonary disease; CHF, chronic heart failure; MI, myocardial infarction.

Appendix table 1 | Specialist type specific procedures.

Vascular surgeon		General surgeon		Cardiac surgeon		Interventional cardiologist		Interventional radiologist	
ICD 9	Description	ICD 9	Description	ICD 9	Description	ICD 9	Description	ICD 9	Description
38.12	Carotid endarterectomy	17.11-24,53.00-9	Hernia repair	36.10-19	Coronary artery bypass grafting	00.66, 36.01-02,36.05	Percutaneous transmural coronary angioplasty	33.26	Closed lung biopsy
39.29	Peripheral vascular bypass	47.01-19	Appendectomy	35.20-28	Heart valve replacement	36.04	Intracoronary thrombolysis	39.1	Transjugular intrahepatic portosystemic shunt
84.15	Below knee amputation	51.21-24	Cholecystectomy	39.61	Cardio-Pulmonary Bypass	36.06-07	Intracoronary stenting	50.11	Closed liver biopsy
84.17	Above knee amputation					37.21-23	Heart catheterization	55.03-04	Percutaneous nephrostomy
								78.49, 81.65	Percutaneous vertebroplasty
								99.25	Chemoembolization

Appendix table 2A-E I Top 15 procedures per classified specialty.

A		**Vascular surgeon**
ICD9	**%**	**Description**
38.12	17.64	Carotid endarterectomy
39.29	9.95	Peripheral vascular bypass
39.50	7.70	Angioplasty or atherectomy of other non-coronary vessel(s)
39.71	5.24	Endovascular implantation of graft in abdominal aorta
38.7	3.41	Interruption of the vena cava
39.49	3.34	Revision of anastomosis of blood vessel or vascular proc
39.27	3.00	Arteriovenostomy for renal dialysis
38.44	2.75	Resection of vessel with replacement, aorta, abdominal
86.22	2.53	Excisional debridement of wound, infection, or burn
39.25	2.36	Aorta-iliac-femoral bypass
84.15	2.23	Other amputation below knee
84.17	2.16	Amputation above knee
38.95	2.14	Venous catheterization for renal dialysis
38.18	1.77	Endarterectomy, lower limb arteries
84.11	1.68	Amputation of toe

B		**General surgeon**
ICD9	**%**	**Description**
51.23	10.23	Laparoscopic cholecystectomy
38.12	8.63	Carotid endarterectomy
39.29	4.10	Peripheral vascular bypass
47.09	3.82	Other appendectomy
47.01	3.13	Laparoscopic appendectomy
45.73	2.54	Open and other right hemicolectomy
38.93	2.46	Venous catheterization
86.22	2.44	Excisional debridement of wound, infection, or burn
45.76	2.11	Open and other sigmoidectomy
39.50	2.02	Angioplasty or atherectomy of other non-coronary vessel(s)
51.22	1.89	Cholecystectomy
39.27	1.76	Arteriovenostomy for renal dialysis
38.7	1.67	Interruption of the vena cava
86.04	1.61	Other incision with drainage of skin and subcutaneous tissue
54.59	1.57	Other lysis of peritoneal adhesions

C		Cardiac surgeon
ICD9	%	Description
38.12	9.96	Carotid endarterectomy
36.12	9.08	(Aorto)coronary bypass of two coronary arteries
36.13	8.90	(Aorto)coronary bypass of three coronary arteries
36.14	4.22	(Aorto)coronary bypass of four or more coronary arteries
36.11	3.52	(Aorto)coronary bypass of one coronary artery
36.15	3.18	Single internal mammary-coronary artery bypass
39.29	3.12	Peripheral vascular bypass
35.22	2.91	Other replacement of aortic valve
35.21	2.85	Replacement of aortic valve with tissue graft
38.44	1.70	Resection of vessel with replacement, aorta, abdominal
32.4	1.67	Lobectomy of lung
39.71	1.48	Endovascular implantation of graft in abdominal aorta
39.50	1.33	Angioplasty or atherectomy of other non-coronary vessel(s)
32.29	1.27	Other local excision or destruction of lesion or tissue of lung
35.12	1.10	Open heart valvuloplasty of mitral valve without replacement

D		Interventional cardiologist
ICD9	%	Description
00.66	21.42	Percutaneous transmural coronary angioplasty
37.22	13.73	Left heart cardiac catheterization
39.50	11.66	Angioplasty or atherectomy of other non-coronary vessel(s)
36.01	11.55	Percutaneous transmural coronary angioplasty
39.71	2.52	Endovascular implantation of graft in abdominal aorta
36.05	2.31	Percutaneous transmural coronary angioplasty
00.61	2.14	Percutaneous angioplasty or atherectomy of precerebral (extracranial) vessel(s)
37.23	2.05	Combined right and left heart cardiac catheterization
37.72	1.85	Initial insertion of transvenous leads [electrodes] into atrium and ventricle
37.61	1.18	Implant of pulsation balloon
88.72	1.06	Diagnostic ultrasound of heart
64.0	1.04	Circumcision
57.94	0.92	Insertion of indwelling urinary catheter
35.52	0.88	Repair of atrial septal defect with prosthesis, closed technique
88.56	0.72	Coronary arteriography using two catheters

E		Interventional radiologist
ICD9	**%**	**Description**
38.93	18.34	Venous catheterization, not elsewhere classified
39.50	11.90	Angioplasty or atherectomy of other non-coronary vessel(s)
38.7	7.03	Interruption of the vena cava
54.91	6.84	Percutaneous abdominal drainage
34.91	6.07	Thoracentesis
38.95	2.94	Venous catheterization for renal dialysis
55.03	2.43	Percutaneous nephrostomy without fragmentation
50.11	2.10	Closed (percutaneous) [needle] biopsy of liver
88.41	2.05	Arteriography of cerebral arteries
39.71	2.03	Endovascular implantation of graft in abdominal aorta
33.26	1.95	Closed [percutaneous] [needle] biopsy of lung
81.66	1.65	Percutaneous vertebral augmentation
34.04	1.33	Insertion of intercostal catheter for drainage
8842	1.32	Aortography
9929	1.24	Injection or infusion of other therapeutic or prophylactic substance

Discussion

Surgeon specialty has been reported to influence outcome after open AAA repair.[6, 7] Since its introduction, the increase in utilization of EVAR has been widely reported. It was reported to comprise 78% of all intact AAA repairs in 2008.[12] Despite this, the proportions of the different specialists performing these repairs have not been elucidated, neither have their association with treatment volume and outcome. The present study describes that VS have gained a substantial increase in the proportion of AAA repairs performed from 2001 to 2009, mainly caused by the increase of EVAR of which VS perform the vast majority. Mortality was higher with lower surgeon volumes, especially for open repairs. After multivariable correction, treatment by a CS was beneficial for open repair but detrimental for EVAR. In addition, patients treated by CS were less likely to receive EVAR, where hospital volume but mainly high surgeon EVAR volume had a dramatic effect on the likelihood of undergoing this procedure.

It was previously reported that in US Medicare beneficiaries in 1996, VS performed 39% of intact open AAA repairs, GS 28% and CS 33%.[6] We show, that overall, VS have increased their proportion of AAA repairs from 44% to 64% from 2001 to 2009, while GS and CS specialties decreased. Open intact AAA repair showed a steady increase in proportion treated by VS (51% to 65%), in contrast, the proportion of intact EVAR performed by VS remained stable but high (60% to 67%).

EVAR for ruptured AAA were almost exclusively performed by VS. For ruptured open AAA repairs, in 1996 GS performed the most procedures (39%), VS 33% and CS 29% in the US.[6]

In the present study, which covered 2001 to 2009, VS treated half of the patients receiving open ruptured AAA repair, leaving one third for GS and the remainder for CS. The relative high contribution of GS compared to the other treatment groups could be due to geographic location where no larger centers with VS were present.

We observed that in each tercile of hospital volume, an overall increase in surgeon volume led to a decrease in mortality, predominantly for open repair. The highest mortality rates were found in patients treated by low volume surgeons. Interestingly, for VS and CS both hospital and surgeon volume appeared to influence mortality, whereas for GS only surgeon volume showed differences in outcome. Multivariable analyses revealed that for mortality, being treated by a CS was beneficial for open repair but not for EVAR. After correction, differences between VS and GS were of less importance than surgeon volume and patient characteristics.

Higher mortality rates for general surgeons were noted before for open repair, both uncorrected6 and corrected for comorbidities via the Charlson score.[7] Overall mortality did not differ much after correction (6.5% unadjusted, 6.2% adjusted). We also noted higher mortality rates, which might be due to unmeasured patient characteristics or differences in setting. Furthermore, after multivariable correction the difference between GS and VS proved less important than other variables in the model.

High surgeon volume was analyzed before and was proved to be inversely related with outcome after AAA repair.[5, 13] In one of these studies, however, the cut off for hospital volume was chosen in such a way that it was impossible to have a high volume surgeon at a low volume hospital.[5] We show that those surgeons do better than their medium and low volume counterparts within the low volume hospitals. Suggesting that these low volume centers should attract high volume surgeons to improve their quality of care, as an alternative to centralizing all AAA repair.

It was previously reported that high volume hospitals have substantially lower mortality rates for both intact and ruptured open AAA repair.[14] When analyzed per type of repair, it was shown that with open repair a steady inverse relation between hospital volume and mortality exists. For EVAR, however, after a relatively low volume threshold mortality only increases slightly.[3] We confirmed these findings, also demonstrating in multivariable analyses that hospital volume is less relevant in predicting mortality after EVAR when compared to surgeon volume and patient characteristics. For open repair, being treated in a high volume hospital was beneficial in addition to surgeon volume.

The likelihood of receiving EVAR as opposed to open repair was greater with GS and lower with CS. High hospital volume increased the chance of receiving EVAR, but EVAR volume of the surgeon was the prominent determining factor for receiving EVAR. Except for higher age, the presence of severe comorbidities (female gender, COPD, CHF, renal failure and emergent admission) increased the chance for open AAA repair. This might be due to patient selection, where the vast majority of the patients received EVAR and the remaining patients who were unsuitable for EVAR had more challenging anatomy, were sicker, and underwent open repair. The likelihood for EVAR increased over the years.

This study was subject to certain limitations. Claims based databases do not list clinical data such as anatomical information, which could influence choice of procedure and outcome. The standard NIS database does not include the specialty of the treating physician. Determining specialty in NIS using an algorithm that incorporates specialty specific procedures has been

described before.[10, 15, 16] We think that our choice of procedures used for physician labeling was optimal for identifying specialists performing AAA repair. This assumption seemed valid as the top 15 procedures per identified specialist appeared appropriate with procedures that matched expectations for those specialists: in addition to the predefined procedures, other commonly performed interventions for each specialist appeared in the list. Secondly, the proportions for open AAA repair in the earliest analyzed years were similar to a previously published large study.[6]

Outcome differs substantially after AAA repair. After multivariable correction CS do better for open AAA repair but slightly worse after EVAR. The difference between VS and GS proved to be of less importance than surgeon volume and patient characteristics.
Generally, low volume hospitals need high volume surgeons, who could potentially do procedures in multiple centers. Low volume surgeons had higher mortality rates, most pronounced for open AAA repairs. In the open group, the proportion of high volume surgeons decreased substantially in the studied period suggesting that it will be increasingly hard to deliver good quality care without centralizing treatment. For EVAR, the opposite trend was observed. More physicians have a medium or large annual volume, thereby significantly improving outcome.

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Management of small AAA

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The introduction and propagation of screening programs for abdominal aortic aneurysm disease and the more frequent application of cross sectional imaging has resulted in an increased detection of patients with small diameter AAA. Consequently, the management of these small aneurysms is becoming increasingly relevant to improve outcomes in this group of patients. AAA repair is a prophylactic procedure performed to prevent AAA rupture and its high mortality. Many patients with small AAA will die of other causes before rupture. Ultimately, one would repair AAA electively just before rupture. Recent large screening studies and RCTs of small AAA management have improved our knowledge of this topic and informed out management of these patients.

Rupture risk

The first step in decision-making in a patient with an asymptomatic AAA is to estimate the risk of aneurysm rupture. Of the factors known to increase rupture risk, diameter is the most prominent and most useful. AAAs with diameters ranging from 3-4 cm have an annual rupture risk of 0%; 4-5 cm 1%; 5-6 cm 1-11%; 6-7 cm 10-22% and >7cm AAAs are at a 30-33% risk of rupture per year.[1-3] The wide range of these estimates reflects variation between patients as well as imprecise data. Observational studies can rarely analyze risk of aneurysm rupture as most AAAs are repaired when they are still intact. Since all AAA do not rupture at the same diameter other factors are clearly involved. Other factors known to increase rupture risk include: rapid expansion, eccentric aneurysm shape, female gender, smoking hypertension and COPD.[4-12] Peak wall stress was described to be higher in patients who required subsequent emergency AAA repair and predicted rupture risk at diameters below 5.5cm, which demonstrates its potential usefulness in smaller AAA.[13, 14] At any given diameter, women are more likely to rupture than men, likely due in part to smaller stature.[11]

Table 1 | Life expectancy in years for patients with AAA.

	Normal population				AAA patients			
	Male		Female		Male		Female	
Age, y	White	Black	White	Black	White	Black	White	Black
60	21.0	18.3	24.0	22.4	17.8	15.5	20.0	18.7
65	17.3	15.2	19.9	18.7	14.7	12.9	16.6	15.6
70	13.8	12.4	16.0	15.2	11.7	10.5	13.3	12.7
75	10.6	9.9	12.4	12.1	9.0	8.4	10.3	10.1
80	7.9	7.7	9.3	9.4	6.7	6.5	7.7	7.8
85	5.7	6.0	6.8	7.1	4.8	5.1	5.7	5.9
90	4.1	4.6	4.8	5.3	3.5	3.9	4.0	4.4
95	2.9	3.5	3.3	3.9	2.5	3.0	2.7	3.2
100	2.0	2.6	2.2	2.8	1.7	2.2	1.8	2.3

Adjusted from: U.S. National Center for Health Statistics, National Vital Statistics Reports (NVSR), Deaths: Final Data for 2007, Vol. 58, No. 19, May 2010.

Unfortunately, there is no accurate algorithm that incorporates all of these factors to estimate rupture risk for an individual patient.

Life expectancy

If AAA rupture risk is average or high, the next step in decision-making should be to estimate the patient's life expectancy. Patients with a short life expectancy, due to comorbid conditions, are less likely to benefit from AAA repair unless the risk of AAA rupture is very high. AAA patients frequently have multiple co-morbid diseases such as coronary artery disease (CAD) and hypertension, therefore, the late survival rate for patients after elective AAA repair is significantly less than age-matched and sex-matched patients without AAAs (60% versus 79% at 6 years).[15] In the United Kingdom Small Aneurysm Trial (UKSAT)[16], it was shown that during a mean of 8 years surveillance of small AAA 38% died due to non-AAA related causes, before their AAA reached 5.5 cm, expanded rapidly or became tender. Table 1 provides an overview of life expectancy by age, sex and race for patients with AAA. Estimates for individual patients must be refined by an assessment of their overall health, especially for factors that have substantial influence such as malignancies. In general, the lower the rupture risk, the longer life expectancy should be to justify AAA repair. Patients with short life expectancies are best managed conservatively unless the risk of rupture is very high.

Operative risk

In the US Medicare population perioperative mortality was 1.2% after EVAR and 4.8% after open repair in 45,000 matched patients undergoing AAA repair.[17] Other prominent perioperative complications were higher after open repair: myocardial infarction (7.0% and 9.4%), pneumonia (9.3% and 17.4%), acute renal failure (5.9% and 10.9%) and need for dialysis (0.4% and 0.5%).

In most analyses the strongest predictors of perioperative mortality are older age, renal disease and heart failure. Other predictors include female gender and evidence of atherosclerotic disease. Operative mortality with open repair with concomitant renal bypass was reported to be 30% higher than open repair without renal revascularization.[18] Table 2 provides a valuable tool to preoperatively estimate a patient's risk for mortality.[19] The scoring system stratifies patients into three risk classes (depending on the procedure) that can be easily calculated using clinical or administrative data.

To be considered for EVAR patients must have appropriate anatomy, which is primarily determined by an adequate infrarenal neck. The use of EVAR in those with suboptimal anatomy is associated with an increased rate of subsequent reinterventions. In 2008, 74% of AAA were repaired with EVAR in the Medicare population. At 4 years, reinterventions related to AAA were more common after EVAR (9.0%) than after open repair (1.7%). Most of these procedures were minor (7.8% vs. 1.3%, for EVAR and open repair), however major procedures (conversions to open repair, infection related reconstructions) also occurred (1.6% vs. 0.6%). Laparotomy-related reinterventions, such as abdominal-wall hernia and

bowel obstruction, at 4 years occurred in 4.1% of the EVAR and 9.7% of the open repaired aneurysms. Similarly, hospitalizations for these bowel obstructions without operative intervention were 8.1% and 14.2% in the 4 years following the aneurysm repair and were also associated with 11% 30-day mortality.[17]

The introduction of EVAR has led to an increase in AAA repairs, primarily in the elderly, a decrease in AAA ruptures and a decreased procedure related mortality for both intact and ruptured AAA.[20] The overall operative risk associated with AAA repair is improving, mainly caused by the increased use of endovascular techniques.

Operative decision making

Consensus exists that small fusiform aneurysms, less than 4.0 cm in diameter, are at low risk of rupture and should be monitored while a fusiform aneurysm greater than 5.4 cm in diameter or a saccular aneurysm of any size should be repaired in an otherwise healthy patient.

Two RCTs examined patients presenting with AAAs between 4.0 and 5.4 cm comparing immediate surgical treatment or surveillance and selective repair for those aneurysms that subsequently enlarge beyond 5.4 cm, grow >1 cm/year or become tender. In both the UKSAT[16] and Aneurysm Detection and Management (ADAM) trial[21], the 30-day operative mortality rate in the immediate surgery group (5.5% UKSAT, 2.1% ADAM) led to an early disadvantage in survival. No significant difference in long-term survival between the immediate surgery and surveillance groups was found.

Table 2 | Scoring method to predict mortality after EVAR or open AAA repair.[19]

Risk factor		**Score**	**OR [95% CI]**
Age	>80	+11	3.1 [2.4-4.2]
	76-80	+6	1.9 [1.4-2.5]
	71-75	+1	1.2 [0.9-1.6]
Female		+4	1.5 [1.3-1.8]
Renal failure	Dialysis dependent	+9	2.6 [1.5-4.6]
	No dialysis	+7	2.0 [1.6-2.6]
CHF		+6	1.7 [1.5-2.1]
PVD or CBVD		+3	1.3 [1.2-1.6]
	Total score	____	
Risk	**Score range**	**Open**	**EVAR**
		Predicted mortality	**Predicted mortality**
High	>11	>6.3%	>2%
Medium	3-11	2.8-6.3%	0.9-2.0%
Low	<3	<2.8%	<1%

CHF, Congestive heart failure; PVD, peripheral vascular disease; CBVD, cerebrovascular disease.

Selected subgroups might have potential benefits of early surgery. In the UKSAT, the estimated adjusted hazard ratios were in the direction of greater benefit of early surgery for younger patients and those with larger AAA, but statistical significance was not demonstrated.[16] In both studies, it was found that 62% of the AAA in the surveillance group had undergone repair after 5 years. The proportion of repairs in this group increased with a larger initially measured diameter: 27% were repaired for AAAs that measured 4.0 – 4.4 cm; 53% for 4.5 – 4.9 cm and 81% for 5.0 – 5.4 cm.[21] Rupture risk during surveillance was 1% per year in the UKSAT and 0.5% per year in the ADAM trial. Neither study was designed or powered to examine the question of whether immediate surgery might be helpful or harmful for patients with aneurysms between 5.0 and 5.4 cm. Moreover, differential effects for older or younger cohorts or those of exceptional physiologic fitness could not be addressed. Women were not well represented in the ADAM trial and had an increased rupture risk during surveillance in the UKSAT. Consequently, most would have a lower threshold for intervention in women. Uncertainty regarding the potential benefit of early repair in selected patients with small AAA is further magnified by the demonstration that EVAR is associated with reduced perioperative mortality.[17] Consequently, the introduction of EVAR substantially changed the operative risk-benefit tradeoff as it might be offered to patients with a high-risk profile.

The CAESAR[22] and PIVOTAL[23] trials compare immediate EVAR with surveillance and selective EVAR in 360 and 728 patients respectively. Both studies reported similar rates of aneurysm rupture and aneurysm-related mortality between the investigated groups. However, neither trial was designed or had sufficient power to determine whether immediate EVAR might be beneficial or harmful for specific AAA diameter ranges or age subgroups. The results from CAESAR suggest that 16.4% of patients with small AAA may lose their initial suitability for EVAR during the period of surveillance. This fact alone is not likely sufficient to justify EVAR on small AAA.

The 4 RCTs together demonstrate that in general it is safe to wait until AAA diameter reaches 5.5 cm for most patients. However, selected patients with an increased risk of rupture, low operative risk, and long life expectancy may wish to consider repair at a slightly lower threshold. Finally, patient preferences should be considered regarding the upfront risk of surgery versus the ongoing risk of rupture.

AAA growth and medical management

Brady et al. reported that the growth rate is higher in patients with larger aneurysms and in patients using tobacco products, whereas it appeared to be lower in patients with diabetes or with low ankle-brachial indexes. Interestingly, growth did not appear to be a continuous process as there were periods of rapid growth as well as episodes without any increase in diameter.[24] Furthermore, growth rate analyses of small AAAs in a screening study of 1231 patients showed that initial AAA diameter followed a unimodal distribution that in 5 years evolved into a bimodal distribution: half of the small AAAs remained quiescent with only little growth, whereas the other half expanded substantially, leading to either surgical repair or rupture.[25]

Reducing the aneurysm expansion rate could delay AAA-related events and the need for AAA repair. Extrapolation of currently available growth rate data indicate that a modest (41%)

reduction of growth can postpone treatment for a 3.5 cm AAA for 5 additional years.[25] A number of approaches have been proposed to prevent aneurysm expansion during the period of aneurysm surveillance including hemodynamic control, as well as inhibition of inflammation and protease activity.[26] Several studies have demonstrated that tobacco use is associated with an increased rate of AAA expansion and smoking cessation is likely the most important recommendation that can be made to a patient with a small AAA.[24, 27, 28] Evidence from two RCTs indicate that propranolol does not inhibit aneurysm expansion.[29, 30] However, these results were compromised by low compliance, with 20-40% of patients discontinuing propranolol during the study period. Animal studies have demonstrated that angiotensin-converting-enzyme (ACE) inhibitors or losartan, an angiotensin receptor blocker, decrease the rate of AAA expansion. Hackam et al. recently reported in an analysis of 15,326 patients in linked administrative databases that use of ACE inhibitors within the prior 3 to 12 months was less frequent among those patients with AAA rupture. Beta-blockers, lipid-lowering agents, and angiotensin receptor blockers showed no relation to rupture.[31] Further, Lederle and Taylor observed an increased risk of aneurysm rupture among those patients who discontinued ACE inhibitors within the past 3 to 12 months.[32] Doxycycline has been described to inhibit aneurysm formation in animal models. Lindeman et al. showed in a trial with 60 patients that AAA wall inflammation was reduced in patients treated with doxycycline for 2 week prior to open repair, regardless of the dosage (50, 100 or 300 mg/d).[33] Morosin et al. randomized 32 patients with AAA to doxycycline (150 mg/d) or placebo for 3 months and showed a trend towards lower AAA growth rates in the group receiving the drug.[34]

Small observational studies suggest that statins inhibit AAA expansion.[35, 36] A recently published large growth rate follow-up study did not confirm these findings.[37] This study included 652 patients with small AAA and after adjustment for risk factors, statins were not found to influence AAA growth. A randomized study of simvastatin (UK heart protection study) included 6,748 patients with peripheral arterial disease. Patients randomized to statins had a reduction in all major cardiovascular end points, but there was no reported reduction in the frequency of AAA repair or AAA-related mortality.[38] In addition, a follow-up study of 4345 individuals for the detection of AAA failed to show a protective effect of statins on AAA development.[39] Also at the tissue level, attenuation of AAA wall inflammation and protease activity after utilization of statins remains controversial.[40] Additional studies are required to clarify the potential role of doxycycline and statin therapy in prevention of aneurysm expansion. In the meantime, with limited detrimental effects of statins described, their use in this group of patients with many cardiovascular risk factors is often indicated, in accordance with clinical guidelines.

In summary, during the surveillance period, patients should be counseled to cease smoking when tobacco products are being utilized. Patients should be encouraged to seek appropriate management for hypertension, hyperlipidemia, diabetes, and other atherosclerotic risk factors. A statin and an ACE inhibitor should be considered given their broad potential benefits and acceptable risk profile. Insufficient data exist to recommend the use of doxycycline. Patients should be counseled that moderate physical activity does not precipitate rupture and may limit AAA growth rate and it will help cardiovascular fitness.[7] Screening of family members should be recommended given the increased prevalence among first-degree relatives.

Surveillance recommendations

The optimal frequency of surveillance of AAA has not been defined by a randomized clinical study.[41] Some authors have suggested that there is no need to follow aortas less than 3 cm in diameter given their low risk of rupture.[42, 43] However, McCarthy et al. determined in a 12 year analysis of 1121 small AAA in 65 year-old men that 13.8% of aortas with an initial diameter of 2.6 to 2.9 cm exceeded 5.5 cm at 10 years. Among patients with an aortic diameter between 3.0 and 3.4 cm, 2.1% had reached 5.5 cm at 3 years and of those with a diameter between 3.5 and 3.9 cm, within 2 years 10.5% exceeded 5.5 cm or required surgery and 1.4% had ruptured.[44]

Two randomized controlled trials, the UKSAT[45] and the ADAM trial[46], as well as a follow-up study of patients detected in the Multicenter Aneurysm Screening Study (MASS)[47] demonstrated that a policy of surveillance until aneurysm diameter exceeds 5.5 cm was safe and associated with a very low rate of rupture (~1%/y). However, surveillance frequency differed among these studies. The MASS trial scanned patients with diameters between 4.5 and 5.0 cm at 3-month intervals, whereas the UKSAT used 6-month intervals for these patients. Patients in the ADAM study with an AAA diameter of 5.0 to 5.5 cm underwent surveillance every 6 months, compared with every 3 months in the UK. In an analysis of expansion rates among 1743 patients over a mean interval of 1.9 years, Brady et al. noted that AAA growth rate increased with aneurysm size and among current smokers, was lower in those with low ankle-brachial index and diabetes, and unaffected by lipids and blood pressure.[24] The authors estimated that for a patient with a 4.5 cm AAA, the risk of enlargement to 5.5 cm was less than 1% during a 12-month interval.

In summary, follow-up surveillance is recommended at 12-month intervals for patients with an AAA of 3.5 to 4.5 cm in diameter. Surveillance imaging at 6-month intervals is recommended for those patients with an AAA diameter between 4.6 and 5.4 cm. For otherwise healthy patients, follow-up imaging is recommended at 3 years for those between 3.0 and 3.4 cm in diameter and at 5 years for aortas between 2.6 and 2.9 cm in size. It is of importance that the patients are closely followed up with ultrasound, a physical exam and for occurring AAA tenderness to minimize rupture risk during surveillance.

Summary & conclusions

For the majority of patients it is safe to defer AAA repair until the diameter exceeds 5.5 cm, grows rapidly or becomes tender. The majority of patients with an initial diameter of 4.5 cm and higher will ultimately come to repair within 5 years, whereas only a minority of those AAA that are initially less than 4.5 cm require repair within 5 years.

Meanwhile, patients should be followed closely through surveillance with ultrasound and physical exam. To diminish rupture risk, risk factors should be managed: Counseling for smoking cessation and control of hypertension.

Selected patients should be offered repair at a lower threshold: women, young and healthy patients, irregular shaped AAA, those with rapid growth, symptoms attributable to the AAA and tenderness. Patients with short life expectancies are best managed conservatively unless the risk of rupture is very high. At the current time, medical treatment should be focused on risk factor management.

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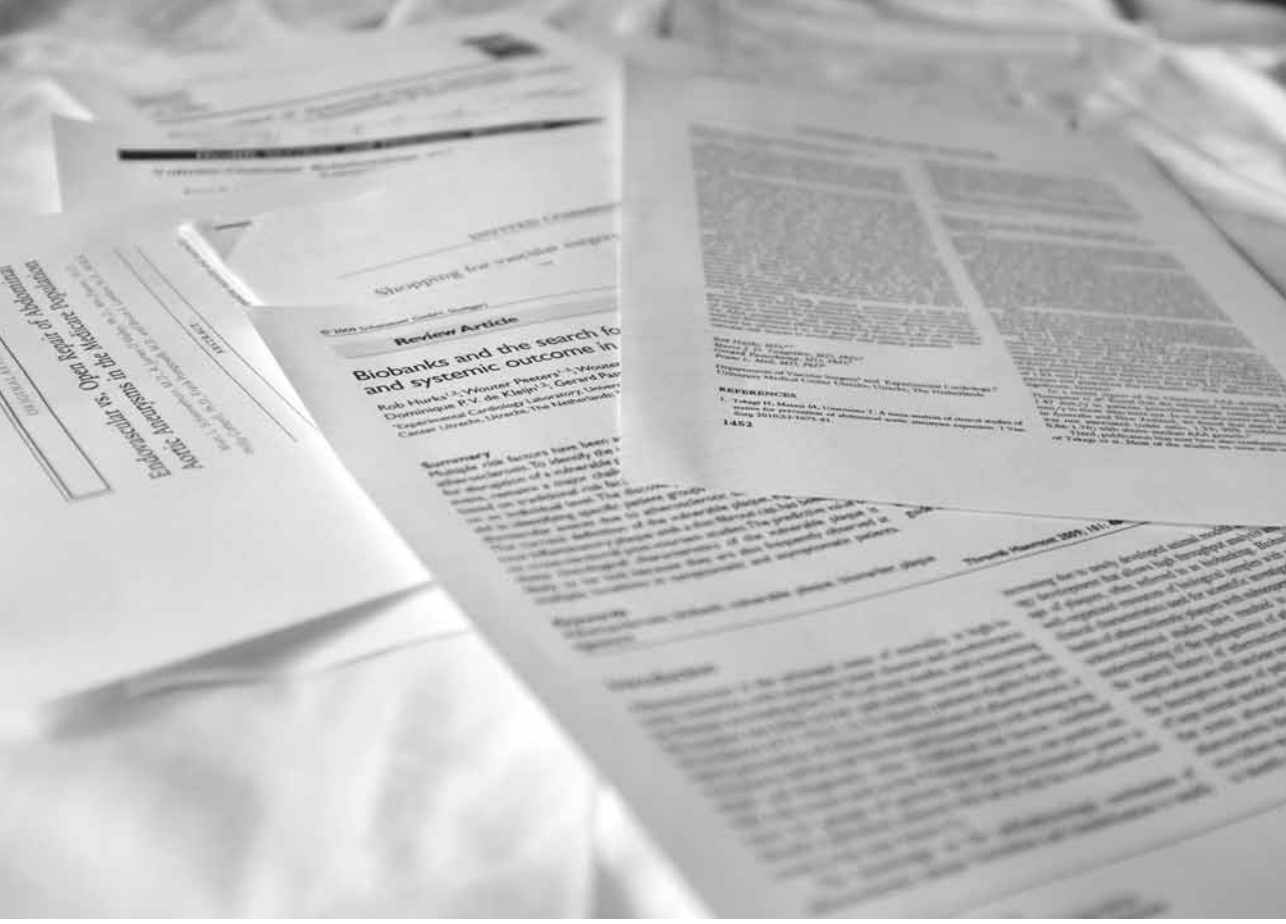
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General discussion, summary and perspectives

14

Abdominal aortic aneurysm (AAA) disease is a growing healthcare burden. With a larger elderly population and a prolonged life expectancy the true prevalence will increase consequently. Since AAAs are mostly asymptomatic, current prevalence numbers will be an underestimation. Because of increased detection, mainly due to screening programs and increased awareness, the reported prevalence will increase and further approach the true prevalence.
Most risk factors for AAA development overlap with those for other cardiovascular diseases. For instance, smoking, advancing age, male gender and hypertension increase the risk for AAA.[1, 2] As a consequence, AAA patients frequently have comorbid conditions such as coronary artery disease and hypertension, which means that they have a different life expectancy than age-matched and sex-matched patients without AAA (60% vs. 79% survival rates after 6 years).[3]
In addition to this burden for these patients, the AAA itself also poses a risk in 2 fashions. First, the AAA could rupture, which is associated with high mortality and morbidity. Rupture risk is influenced by a variety of factors, but increases most with larger diameters.[4, 5]
This thesis focused on the second, more indirect, hazard the AAA forms for the patient: the operative risk. To prevent AAA rupture, interventions are undertaken to exclude the AAA from the circulation. However, these procedures are associated with a significant mortality and morbidity. In patients undergoing AAA repair perioperative mortality was 1.2% after endovascular aneurysm repair (EVAR) and 4.8% after open repair. Prominent other perioperative complications were also higher after open repair: myocardial infarction (7.0% and 9.4%), pneumonia (9.3% and 17.4%), and acute renal failure (5.9% and 10.9%).[6]

Our strategy to meet our aim of improving patient outcome was to seek *discrepancies in AAA expressions and repair*. Two approaches were explored: assessing variance and variants in AAA and improving outcome after repair.

Ameliorate patient outcome - variance and variants in AAA

EVAR comprised 78% of all intact AAA repairs in 2008.[7] Tissue harvesting is limited to patients undergoing open AAA repair. This is the main reason for current AAA research to be hampered by low sample numbers, which does not allow correction for confounding factors. To analyze variance and variants in AAA, large sample numbers are required as differences might be relatively subtle. In Utrecht we have a unique setting for conducting this type of research. First, there is quite some experience with a large biobank containing atherosclerotic plaques and an accompanying organizational structure (Athero-express).[8] Second, because this vascular tissue biobank is up and running, the threshold of also collecting AAA tissue was low and it was commenced in 2003. This resulted in the start of the Aneurysm-express biobank. With information and material of over 300 patients, this is currently the largest reported AAA tissue biobank.

Predictive capacity vessel wall
Screening for patients at risk for adverse events due to advancing atherosclerotic or aneurysmal disease is currently based on traditional risk factors, which does not allow individual risk

stratification to predict the likelihood of occurring cardiovascular events such as myocardial infarction. Several studies showed the instability of the vascular wall being a systemic process instead of only local inflammation and that a part of the atherosclerotic vascular wall at 1 site might hold information on the stability of the whole system.[9, 10] As described in **Chapter 2**, one of the strengths of the Athero-express biobank is its follow-up, which allows coupling with tissue characteristics. The Athero-express follows a new concept to search for the atherosclerotic patient who may suffer from adverse events. The plaque could be a concentrated expression of systemic atherosclerosis and hide predictive value for adverse events in other vascular territories. Results from this longitudinal biobank study show that the local plaque hides strong predictive value for cardiovascular events elsewhere in the vascular tree.[11-13] Atherosclerosis is a systemic inflammatory disease and shares many factors with aneurysmal disease.[14] When an atherosclerotic plaque, only being composed of intima and media, hides predictive information it is likely that a sample of an AAA wall, including all 3 layers of the artery, has even more predictive capacity. To analyze the predictive value of the AAA wall, large prospective tissue biobank studies are essential. In **Chapter 3**, we describe the design of the Aneurysm-express study. This biobank stores AAA tissue, blood, clinical characteristics and postoperative follow-up from patients originating from 2 medical centers: University Medical Center Utrecht and St Antonius Hospital Nieuwegein.

An important focus for predictive capacity is inflammation as it is a prominent and consistent finding in AAA.[15] Inflammation originates from the Latin word *Inflammare* (to set on fire), and is believed to be part of the non-specific immune response that occurs in reaction to any type of injury, not restricted to exogenous pathogens.[16, 17] Osteopontin (OPN) is an interesting protein in this regard. It is pro-inflammatory, plasma levels were associated with AAA presence and most importantly, its predictive capacity was demonstrated in atherosclerotic plaques in our lab.[12, 18] In **Chapter 4**, we described the value of OPN as an independent predictor of adverse cardiovascular outcome. High OPN levels, as present in the AAA wall at the time of surgery, were predictive of postoperative events. Events were predominantly myocardial infarctions in the weeks following surgery and minor vascular interventions occurring years after surgery. Intriguingly, high levels of OPN appear to have identified a high-risk group of patients. This could potentially be useful in two settings. First, if it is known shortly after surgery who will be at risk for adverse events, then these patients can be subject to increased surveillance and receive more aggressive treatment. Second, if a method was to be developed, such as a targeted imaging approach, which would allow preoperative screening that could influence both the choice and timing of AAA treatment. The choice for OPN was mainly driven by experience in our lab, but it fits well in current literature. It could very well be that more components in the AAA wall have predictive properties that remain to be discovered. OPN appears predictive, but we're still far from clinical application. It needs to be validated in other cohorts first, and much of its biology remains to be clarified. Nevertheless, it is an interesting target and given the pro-inflammatory properties of OPN, and the importance of inflammation in AAA development and progression, it could also be that OPN has detrimental effects on the AAA itself as well. When this is further revealed, OPN might be an interesting therapeutic target.

Another protein that might actually have a direct negative impact on arterial wall degradation is osteoprotegerin (OPG). In **Chapter 5** we found that aortic concentration of OPG was

associated with a range of markers of AAA severity. OPG concentration was positively associated with AAA diameter. Furthermore, patients with high OPG levels also had higher matrix metalloproteinase (MMP) 2 and 9, and cathepsin A, B, and S. It also correlated with the numbers of lymphocytes and plasma cells, a specific staining revealed that OPG was present in the cytoplasm of these cell types. In an attempt to prove the mechanistic link between OPG and the protease levels, an in vitro study was conducted. We found that OPG appeared to stimulate the production of cathepsin S by VSMCs and monocytic cells, suggesting a possible role of OPG in promoting proteolysis.

The described results merit further research. As shown, longitudinal biobank studies facilitate the identification of predictive biomarkers. The idea that one single factor predicts adverse outcome is unrealistic. Probably a combination of factors that is predictive for adverse events might enable patient stratification that will allow individualized tailor made medicine and subsequently guide the choice for therapeutic interventions. When patients are identified as high risk for postoperative events, it is likely best for the patient to delay intervention as long as possible dependent on rupture risk. Analyzing predictive value of local AAA wall characteristics is currently limited to patients undergoing open vascular surgery. To make the step towards primary prevention and thereby increasing the amount of patients eligible for this kind of risk stratification, the found biomarkers need to be translated to markers available for measuring in patients who will not undergo vascular surgery, such as patients with small-diameter AAA. For instance, markers measurable in the peripheral circulation or detectable using imaging techniques would be appropriate candidates. This would also have the advantage of being able to track changes in biomarker levels over time and to better understand their role in pathophysiology. Biomarkers in AAA form potential targets for medical treatment, can be used for detecting (small) aneurysms and, most importantly, can be a measure for AAA progression and outcome and may therefore be a surrogate marker for treatment efficacy.

Pharmacological interventions

Currently no pharmacotherapeutic substance has been proven to directly influence human AAA. The reduction of aneurysm expansion could delay the need for AAA repair, which would postpone perioperative complications. Extrapolation of currently available growth rate data indicate that a modest (41%) reduction of growth can postpone treatment for a 3.5 cm AAA for 5 additional years.[19] Various approaches have been proposed to prevent aneurysm expansion during the period of aneurysm surveillance including hemodynamic control, as well as inhibition of inflammation and protease activity.[20]

Statins are frequently prescribed for the prevention of cardiovascular events. Initially they were thought to diminish inflammation, however, in the Athero-express it was shown that statin use was not associated with a consistent decrease in inflammation.[21] Small observational studies suggest that statins inhibit AAA expansion.[22, 23] Small studies indicated a attenuating effect of statins on AAA wall proteases.[24, 25] In **Chapter 6**, we wanted to confirm these findings, analyze differences between the different statins and correct for confounding variables. We found no decrease in protease levels or wall inflammation. Instead we found a slight increase in protease levels in patients taking pravastatin, suggesting that there might be pleiotropic differences within the group of statins. Recent literature added to the controversy about the

effect of statins on AAAs. A recently published large growth rate follow-up study could not confirm the earlier described findings of statins attenuating AAA growth.[26] This study included 652 patients with small AAA and after adjustment for risk factors, statins were not found to influence AAA growth. A randomized study of simvastatin (UK heart protection study) included 6,748 patients with peripheral arterial disease. Patients randomized to statins had a reduction in all major cardiovascular end points, but there was no reported reduction in the frequency of AAA repair or AAA-related mortality.[27] In addition, a follow-up study of 4,345 individuals for the detection of AAA failed to show a protective effect of statins on AAA development but showed instead an increased risk for AAA (OR 3.77[1.45-9.81]).[1]
Further studies are required to clarify the potential role of statin therapy in prevention of aneurysm expansion. In the meantime, with limited detrimental effects of statins described, their use in this group of patients with many cardiovascular risk factors is often indicated, in accordance with clinical guidelines.

Heterogeneity in aneurysm expressions

A higher number of small AAA are being detected because of the introduction of screening programs and the increased awareness of the disease. Therefore, it is becoming increasingly relevant to better understand the natural history of AAA, more specifically AAA expansion. A sub study of a large trial reported that the growth rate is higher in patients with larger aneurysms and in patients using tobacco products, whereas it appeared to be lower in patients with diabetes or with low ankle-brachial indexes. Interestingly, growth did not appear to be a continuous process as there were periods of rapid growth as well as episodes without any increase in diameter.[28] Additionally, growth rate analyses of small AAAs in a screening study of 1,231 patients showed that initial AAA diameter followed a unimodal distribution that in 5 years evolved into a bimodal distribution: half of the small AAAs remained quiescent with only little growth, whereas the other half expanded substantially, leading to either surgical repair or rupture.[19] Furthermore on a pathophysiological level, recent evidence is emerging that the development of AAA cannot completely be explained by 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.[29-31] In a recent study in 6,446 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.[32] In **Chapter 7** we assessed whether we could distinguish variants of AAA associated with clinical parameters and eventually outcome. After analyzing general wall inflammation on histology, we found a large variation in amount of inflammation. About half of all AAA had only few inflammatory cells, whereas the other half formed a broader spectrum of moderate to high amounts of inflammation. We found that AAAs with a relatively low amount of cellular inflammation were associated with more cardiovascular risk factors (diabetes, hypertension and hypercholesterolemia), more cardiovascular events during follow-up, and more frequently show an intimal cholesterol-rich lipid core. This observation suggests a closer relation between generalized atherosclerotic disease and AAA formation in patients with a low amount of inflammation in their AAA than in patients with a high amount of AAA inflammation. As could be expected, the levels of various cytokines and proteases were higher

in the patients with a higher amount of cellular inflammation. This observation suggests that AAA pathophysiology is multi-factorial, where in some AAA a closer relation with atherosclerosis appears to exist, in others inflammatory processes might have a more prominent role. Further research is needed to analyze the relation with AAA progression and prognosis, to analyze whether it should influence AAA treatment.

When we take the findings of **Chapter 4** into account, high OPN levels were associated with more postoperative events and OPN staining was present in the intimal atherosclerotic plaque, more specifically around the cholesterol core. This appears to be consistent with the increased presence of cholesterol cores in AAAs with low amounts of inflammation. We have not analyzed yet whether OPN and low inflammation both add up or interact in the prediction of postoperative adverse events, something we plan to do at a later point in time.

A limitation of this type of tissue research is that we're only able to analyze one moment in time. Specifically, during a late stage of the disease when repair was indicated. This raises the question whether a patient's AAA could move from the group with low inflammation to the group with high inflammation or vice versa. This question cannot be answered directly with the data from this study, however, indirect evidence suggests that group migration is not inevitably present. Over time AAA diameter increases, and average diameters didn't differ between groups. Moreover, there were differences in risk factors between groups. There could be certain variations between growth patterns[28], but that does not necessarily affect the ventral AAA wall (see below, Chapter 8). Regardless of possible migrations, the patient numbers appeared large enough to identify different variants of AAA.

For further investigating the difference in AAA progression and eventually rupture of the low- and high inflammation variants of AAA, an interesting next step could be to visualize the metabolically active inflammatory infiltrate in the aneurysm wall using fludeoxyglucose F 18 via positron emission tomography/computed tomography (PET/CT). Metabolic activity on PET/CT correlates well with histologic inflammation in the aneurysm wall, and a large variance in FDG uptake has been reported.[33, 34] It would be of great interest to prospectively monitor patients with smaller aneurysms with PET/CT, to translate present findings to a broader group of patients (not limited to patients undergoing open AAA repair).

The ventral wall is the most frequently studied part of the AAA wall in pathophysiological research. An AAA is a large structure, and it is not known whether its constituents are homogeneously distributed. Therefore, it is unknown if the ventral wall represents the magnitude of inflammatory processes within the AAA. In **Chapter 8** we collected AAA wall specimens of the entire circumference and found that lateral sides exhibit more active inflammation with more microvessels, more inflammatory cells on histology, and higher protease (MMP 9) and cytokine levels (IL 8) than the ventral and dorsal walls. This suggests that a specimen of the ventral AAA wall does not necessarily represent the entire AAA wall. Data from small studies using human specimens need to be interpreted carefully, because we have already shown in a limited number of patients that marked regional differences exist. OPN (**Chapter 4**) was also measured and had higher lateral expressions, when compared to the ventral wall, which was consistent with other measured inflammatory markers. This indicates that the predictive value might be different in the lateral AAA wall. However, the current study shows in a larger cohort that the easily accessible ventral wall can already be

predictive of adverse events. It also underlines that large patient numbers are necessary for AAA tissue research to compensate for regional variance.
The circumferential discrepancies inside the AAA further raises the question whether the ventral wall alone should be the focus of drug trials that aim to attenuate AAA wall inflammation and/or protease activity in order to retard growth. The effects of drugs on the lateral sides of the AAA may be more relevant than the currently collected ventral wall. Either the tissue should be collected in different regions (including lateral) or a more prominent role for imaging of inflammation or proteases as a primary outcome measure should be considered. For future research, it would be intriguing to analyze heterogeneity in inflammation in the entire AAA, for instance via imaging. Also changes over time would be illustrative, to analyze the association of the amount of inflammation with described stepwise growth patterns.[28] It could illustrate the suspicion that AAA expansion is more pronounced laterally, which we suggested based on the increased vulnerability in those regions.

AAA and popliteal artery aneurysms (PAA) often coincide and are frequently regarded as an expression of the same disease at a different location.[35] In **Chapter 9** we compared both aneurysms on tissue level. Despite original differences in histological composition between the aorta and the popliteal artery (elastic vs. muscular), aneurysm formation resulted in a similar degraded media in our study. Another study reported a marked overlap in inflammation between AAA and PAA.[36] Localization of the inflammatory infiltrate remained unreported. We show that the focus is different: where in AAA the majority of the inflammatory infiltrate lies in the adventitia, in PAA the infiltrate is situated in the intima. This could explain the difference in symptomatology between the two aneurysm types as the intimal infiltrate in PAA is adjacent to the intraluminal thrombus and could potentially influence frequently present processes such as thromboembolism and thrombosis.
With the focus of this thesis being the improvement of patient outcome, we also wanted to analyze discrepancies in AAA repair after our focus on discrepancies in AAA expressions.

Amelioriate patient outcome - improving outcome after repair

Besides patient and AAA characteristics, many factors influence outcome after repair. Elective AAA repair is undertaken in order to prevent future rupture with its accompanying high mortality rate.[37, 38] Most elective AAA repairs in the US are now performed using EVAR.[7] There are different ways of performing this type of procedure and level of experience and type of specialist executing this procedure can also differ. Both can have an impact on post procedural outcome.

Access type
Access to the common femoral artery can be obtained in multiple ways, and is necessary to perform an EVAR. We wanted to investigate if the least invasive method (percutaneous access, pEVAR) provides a better outcome than the current standard in many centers (femoral cutdown access, cEVAR). In **Chapter 10** we used the clinical database of the American College of Surgeons National Surgery Quality Improvement Program (ACS NSQIP) to analyze differences

between pEVAR and cEVAR in a more national setting (269 hospitals), as opposed to the reported single center studies. The single center studies reported lower access-related complications, such as wound infections.[39-41] Utilization of pEVAR increased from 36.5% in 2005 to 44.9% in 2009. We found that pEVAR was not associated with shorter operative time or shorter hospitalization, and no difference in wound infections was observed. pEVAR was associated with an increase in DVT. This study suggests that the reported benefits in single center studies are not being realized nationally.

In **Chapter 11** we used clinical records from our own institution (tertiary medical center) to answer the same research question. pEVAR increased from 0% in 2005 to 92.3% in 2010. Ultrasound guided percutaneous access has been shown to improve the accuracy and to lower the complication rate of percutaneous access [42, 43], and was used in our hospital from 2007 onwards. We found that pEVAR could be performed routinely with a high success rate and fewer wound complications even in arteries considered high risk for percutaneous failure. Percutaneous access decreased operative time and length of stay.

Interestingly, these studies seem to contradict each other. It is important to realize the type of data they are based on. The NSQIP database allows analyses in a more national setting, which means that patient numbers are bigger and the results are more generalizable. It is a clinical database, with data entered by trained clinical nurses.[44] This database comprises all types of surgical procedures, and therefore does not provide full details for every procedure. Factors such as use of ultrasound guidance, sheath size and anatomic information are lacking. Despite that, present variables are defined in great detail (such as concurrent procedures, different types of wound infection, 30 day outcome parameters, etc.). This level of detail combined with accuracy and large numbers make the NSQIP a valuable research tool. Use of electronic clinical records allows the recording of every detail of the hospitalization. Disadvantage is the retrospective nature and the sample size. The results can be indicative for a certain hospital setting, but are not generalizable.

An important caveat for comparing these studies lies in the proportion pEVAR used. In our hospital it involved 56% of total cases and in NSQIP 42%, furthermore the number of physicians performing these procedures varies from a few to numerous, with various levels of expertise. Our hospital is a tertiary urban teaching hospital, where NSQIP involves all kinds of hospitals in different settings. **Chapter 11** showed that pEVAR can offer a substantial advantage over cEVAR in terms of outcome, also when focusing only on variables present in both databases. However, these results cannot be generalized to a national level as **Chapter 10** demonstrated that the advantages of pEVAR are minimal or absent in these participating centers. The exact determinants of success after pEVAR need to be analyzed in a prospective manner so that these can be accounted for and implemented to improve the results on a national level.

Physician experience and volume

Surgeon specialty was reported to influence outcome after open AAA repair[45, 46], but for EVAR this remains unknown. In addition, the likelihood of receiving EVAR with its better postoperative outcome profile likely differs per specialist. **Chapter 12** described that vascular surgeons had a substantial increase in market share of AAA repair from 2001 to 2009, mainly caused by the increase of EVAR of which vascular surgeons perform the vast majority. Mortality was higher with lower surgeon volumes, especially for the open repairs. In the open group, the proportion

high volume decreased substantially in the studied period suggesting that it will be increasingly hard to deliver good quality care without centralizing treatment. For EVAR, the opposite trend was observed. More physicians have a medium or large annual volume, thereby significantly improving outcome.
After multivariable correction, being treated by a cardiac surgeon was beneficial for open repair but slightly detrimental for EVAR in terms of mortality. In addition, patients treated by cardiac surgeons were less likely to receive EVAR, where hospital volume but mainly high surgeon EVAR volume had a dramatically stimulating effect on the likelihood of undergoing this procedure. High surgeon volume was analyzed before and proved inversely related with outcome after AAA repair, however, the cut off for hospital volume was chosen in such a way that it was impossible to have a high volume surgeon at a low volume hospital.[47] We show that those surgeons do better than their medium and low volume counterparts within the low volume hospitals. Suggesting that these low volume centers should attract high volume surgeons (who would operate in different centers) to improve their quality of care, as an alternative to centralizing all AAA repair. It was previously reported that high volume hospitals have substantially lower mortality rates for both intact and ruptured open AAA repair.[48] When analyzed per type of repair, it was shown that with open repair a steady inverse relation between hospital volume and mortality exists. For EVAR, however, after a relatively low volume threshold mortality only increases slightly.[49] We confirmed these findings, also demonstrating in multivariable analyses that hospital volume is less relevant in predicting mortality after EVAR when compared to surgeon volume and patient characteristics. For open repair, being treated in a high volume hospital was beneficial in addition to surgeon volume. These results indicate that with a further decline in proportion of AAA treated with open repair, and the amount of high volume surgeons going down as well as the high volume hospitals, together with the inverse relation of these volumes with outcome (mortality), open AAA repair should be centralized to provide good quality of care. For EVAR opposite trends are noted, and when annual volume is above the minimal rate outcome differs only slightly, suggesting that centralization would only have limited befits for the patient.

Clinical decision making
Timing of repair is essential for optimizing patient outcome. In **Chapter 13** we discussed the current different aspects and determinants for making decisions on a patient's treatment. Factors such as rupture risk, patient's life expectancy, operative risk and natural history (AAA growth). For the majority of patients it is safe to delay AAA repair until the diameter exceeds 5.5 cm, grows rapidly or becomes tender. Meanwhile patients should be followed closely and risk factors for rupture need to be managed. A selected group of patients with a higher risk of ruptured should be offered repair at a lower threshold: women, irregular shaped AAA, tenderness, and young and healthy patients. Patients with short life expectancies should only be repaired when the risk of rupture is very high.

With advances in understanding discrepancies in AAA expressions and repair, we aim to contribute to the health of the patient with an AAA. Current and future advances in knowledge should lead to an individual approach with a tailored cut off for intervention and management of co-existing diseases. Ultimately, if AAAs are better understood, patients who need treatment

because of a higher rupture risk can be identified better. Also, the AAA repair of patients who are at high-risk for postoperative mortality and morbidity should be postponed as long as possible to delay the burden of repair, ideally to be repaired just before AAA rupture.

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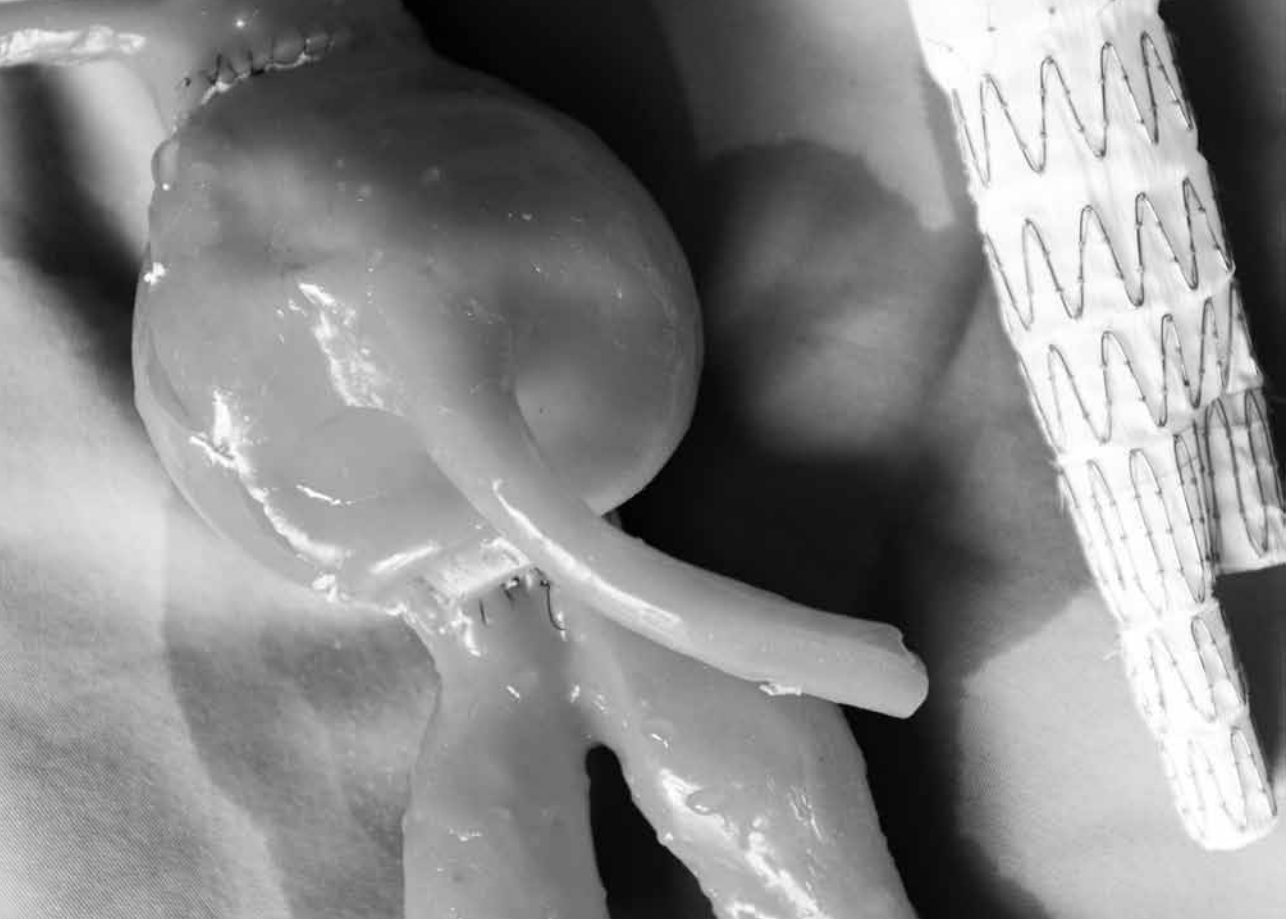

Summary in Dutch
Nederlandse samenvatting voor niet-ingewijden

15

"Omstreeks 3.30 uur 's morgens werd ik gewekt door een ondragelijke pijn in mijn buik. Ik kan de intensiteit enkel omschrijven als inhumaan, verschrikkelijke beelden oproepend van horror films waarbij het slachtoffer wordt geperforeerd door een industriële boor."[1] Dit citaat komt van een arts die de symptomen beschrijft die hij had toen zijn aneurysma van de Aorta abdominalis (AAA) scheurde. Hij had geluk dat hij op dat moment in het ziekenhuis was, met een vaatchirurg ter plaatse. Anders had hij het waarschijnlijk niet na kunnen vertellen.

Frequentie en risicofactoren voor AAA ontwikkeling

Een AAA is een aneurysma van de buikslagader (buikaorta) en betreft een locale verwijding van de slagader. Het bestaan ervan werd voor het eerst beschreven door de Belgische anatomist Vesalius in de 16e eeuw.[2] Normaal is de aorta in de buik 1,5 tot 2 cm, afhankelijk van geslacht en statuur. Vanaf 3 cm in diameter wordt er van een AAA gesproken.[3] Dit ziektebeeld komt steeds vaker voor in de Westerse samenleving[4, 5], in de leeftijdscategorie boven de 55 jaar hebben 4,1% van de Nederlandse mannen en 0,7% van de Nederlandse vrouwen zo'n aneurysma. Patiënten vergelijken het vaak met het hebben van een tijdbom in hun buik (figuur 1). Zo'n vaatverwijding geeft namelijk geen symptomen maar gaat gepaard met een dunnere en instabielere wand, waarbij het risico bestaat dat het AAA scheurt en leidt tot massief bloedverlies in de buik. Het gevolg is dat de helft van de patiënten die dit voorhebben overlijden voordat ze in het ziekenhuis belanden en van de overigen nog eens de helft tijdens of na de operatie om de scheur te herstellen. Tijdens het afgelopen decennium zijn er 10733 mannen en 4884 vrouwen overleden in Nederland met AAA als doodsoorzaak.[6] Ter vergelijking zijn AAA's in de VS doodsoorzaak nr. 15 bij mensen boven de 55 jaar.[7, 8] Het probleem met deze cijfers is dat er maar weinig autopsies plaatsvinden en vaak iets anders (onterecht) als doodsoorzaak wordt aangemerkt, waardoor er een onderschatting is van de daadwerkelijke cijfers.
De verwachting is dat het aantal gedetecteerde AAA's verder stijgt door enerzijds een ouder wordende en langer levende bevolking, anderzijds wordt er in de lekenpers meer aandacht

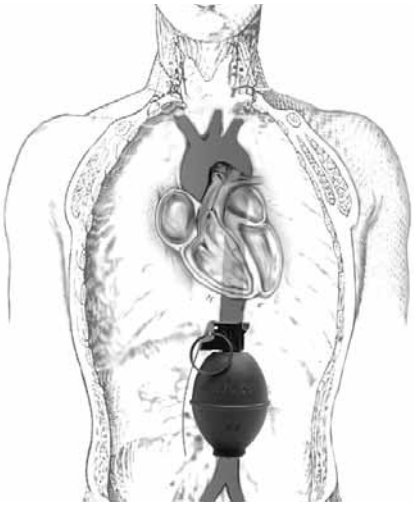

Figure 1 | Interpretatie van een AAA door de patiënt.

aan besteed.[9, 10] Maar de belangrijkste reden is de introductie van screening programma's. In de VS worden op steeds meer locaties mannen die ooit hebben gerookt tussen de 65 en 75 jaar gescreend met behulp van een eenmalige echografie van de buik.[11] In Nederland wordt ook over de mogelijkheden voor screening gediscussieerd.[12-14]
Er zijn diverse risico factoren voor het onstaan van AAA's bekend, waarbij de meest bepalende risicofactor roken is. Verder zijn met een groter risico geassocieerd: het mannelijk geslacht, een hogere leeftijd, aanwezigheid van slagaderverkalking, gestoorde bloedvetten en een eerste graad familielid met een AAA.[15-17] Verrassend genoeg blijkt het hebben van diabetes beschermend voor het ontstaan en de groei van aneurysma's.[18, 19]

Kans op ruptuur en (preventieve) behandeling
Zoals hoger vermeld, heeft het scheuren van een AAA een hoge mortaliteit (80% wanneer de patiënt zich buiten het ziekenhuis bevindt).[20] De belangrijkste determinant voor ruptuur risico is de grootte van het AAA: diameters tot 5 cm hebben een jaarlijks risico van <1%, terwijl AAA's groter dan 7 cm een jaarlijkse scheur kans van 30-33%.[21-23] Andere factoren die het optreden van een ruptuur bespoedigen zijn bijvoorbeeld snelle groei van het AAA, asymmetrische vorm, vrouwelijk geslacht, roken, hoge bloeddruk en chronisch obstructieve longziekten.[24-32]
Albert Einstein onderging een van de eerste behandelingen voor zijn AAA in 1948 op 69 jarige leeftijd. De chirurg kon het aneurysma niet verwijderen, maar verpakte het in cellofaan folie in een poging verdere groei en ruptuur te voorkomen. Deze folie veroorzaakt een ontstekingsreactie en verlittekening waardoor gehoopt werd dat de AAA wand steviger zou worden. Desondanks scheurde Einstein's AAA 7 jaar later wat hem fataal werd.[33]
Diverse nieuwere behandelingen volgden. Momenteel worden er 2 typen operaties uitgevoerd: open herstel en een variant via de liesslagader waarbij de buik gesloten blijft. Bij een open operatie wordt het AAA buiten de circulatie gebracht door een flexibele kunststof buisprothese (een bypass) in te hechten op de plaats van het aneurysma.[34] Bij de gesloten variant wordt via de lies in het AAA een buis geplaatst (een soort stent) waardoor het aneurysma buiten de circulatie gebracht wordt, dit heet endovasculair aneurysma herstel (EVAR).[35] Deze operaties kunnen zowel preventief als in urgente setting bij een ruptuur worden toegepast, maar zijn zeker niet zonder risico. Momenteel heeft preventief open AAA herstel een mortaliteit van 4,8% en EVAR 1,2%. Vervolgens treden vaak longontstekingen op (17,4% en 9,3%, respectievelijk), hartinfarcten (9,4% en 7,0%) en acuut nierfalen (10,9% en 5,9%). In de meeste analyses zijn de sterkste voorspellers voor mortaliteit hogere leeftijd, nierziekten en hartfalen.[36] Dit heeft tot gevolg dat de keuze tot interventie altijd een afweging is van het risico op ruptuur van het AAA en het operatierisico.

Doel van dit proefschrift
AAA's komen steeds vaker voor en vormen een belangrijk gezondheidsprobleem, zowel door de ziekte zelf als door de (preventieve) interventies. In dit proefschrift focussen we op strategieën om de uitkomst van patiënten met een AAA te verbeteren: het zoeken van *discrepanties in AAA expressies en herstel*. Twee benaderingswijzen werden verkend: het analyseren van varianten tussen verschillende AAA's en variatie binnen hetzelfde aneurysma, vervolgens analyseerden we hoe we de complicaties na de hersteloperaties kunnen verminderen.

Variatie en varianten in het AAA

EVAR besloeg 78% van alle behandelingen van intacte AAA's in de VS in 2008.[37] Aangezien weefsel van de AAA wand alleen tijdens open operaties kan worden verzameld, heeft dit tot gevolg dat het lastig is om grote aantallen te halen. Grote aantallen zijn nodig om meer subtiele verschillen te vinden en om te kunnen corrigeren voor mogelijk beïnvloedende (risico-)factoren. In Utrecht hebben we een unieke setting voor het uitvoeren van dit soort onderzoek aangezien er al ervaring was met de opslag en analyse van vaatweefsel in een biobank, vervolgens is al in 2003 eveneens begonnen met het verzamelen van AAA weefsel. Dit leidde tot het ontstaan van de Aneurysma-express biobank, die met informatie en materiaal van meer dan 300 patiënten de grootste biobank is momenteel.

Voorspellende waarde vaatwand

Het screenen naar patiënten met een verhoogd risico op complicaties gebeurt momenteel op basis van traditionele risicofactoren. Dit heeft tot gevolg dat er niet op individueel niveau kan worden gekeken naar het risico op optredende hart- en vaatziekten zoals een hartinfarct. In **hoofdstuk 2** beschrijven we hoe een locale plaque (een vernauwing door slagaderverkalking) voorspellende informatie bevat voor het optreden van hart- en vaatziekten op andere plekken in het lichaam. Waarbij die ene plaque als het ware een soort geconcentreerde expressie blijkt voor een systemische ziekte. Een slagader bestaat uit 3 lagen. Een plaque bestaat enkel uit de binnenste laag, dus lijkt het aannemelijk dat een AAA (dat alle drie de lagen van de slagader beslaat) nog meer voorspellende informatie bevat. Om dit te onderzoeken zijn grote biobanken met weefsel nodig en in **hoofdstuk 3** beschrijven we de opzet van de Aneurysma-express studie. In deze biobank wordt AAA vaatwand weefsel, bloed, klinische gegevens en follow-up na de operatie verzameld van patiënten die een open herstel van hun AAA ondergaan in het Universitair Medisch Centrum Utrecht in Utrecht of in het Sint Antoniusziekenhuis in Nieuwegein.

Een belangrijke focus voor het bepalen van voorspellende waarde is de ontsteking in de vaatwand, aangezien dat een belangrijke component is van AAA's.[38] Osteopontine (OPN) is een interessant eiwit in deze context aangezien het ontsteking bevordert, hoge waarden in bloed geassocieerd zijn met aanwezigheid van AAA's maar vooral aangezien het voorspellend bleek in de hoger beschreven plaques.[39, 40] In **hoofdstuk 4** beschrijven we dat het hebben van hoge OPN waarden in de vaatwand sterk voorspellend is voor het optreden van hart- en vaatziekten als complicaties na AAA herstel, onafhankelijk van bestaande risicofactoren. Door het identificeren van patiënten met een hoger risico, zouden deze patiënten zeer nauwlettend gevolgd en agressiever behandeld kunnen worden. In theorie zouden patiënten waarbij voor de operatie bekend is dat ze een verhoogd risico hebben op complicaties, pas in een later stadium worden geopereerd. OPN lijkt potentie te hebben, maar praktische toepassing laat nog even op zich wachten. Eerst moeten deze bevindingen ook door andere onderzoeksgroepen in andere patiënten worden bevestigd en de precieze werking van het eiwit moet nog opgehelderd worden. Desalniettemin is het een interessant target, ook aangezien het een actieve rol kan hebben in de groei van zo'n AAA. Een ander eiwit dat de vaatwand afbraak kan bevorderen is osteoprotegerin (OPG). In **hoofdstuk 5** analyseerden we de associatie van OPG met verschillende parameters in de AAA wand. Het resultaat leerde ons dat hoe hoger de OPG

concentratie, des te meer wandafbraak er was en des te groter was de diameter van het AAA. We toonde aan dat OPG de productie van afbraak enzymen in bepaalde ontstekingscellen kan stimuleren. Dit suggereert dat OPG een belangrijke rol heeft in de progressie van de ziekte en dit helpt ons de ziekte beter te begrijpen. Daarnaast kan dit mogelijk een target zijn om AAA groei te remmen met behulp van medicijnen.

Medicamenteuze behandelingen

Momenteel zijn er geen medicijnen bekend die menselijke AAA's rechtstreeks kunnen beïnvloeden. Het remmen van de groei van het AAA zou het mogelijk maken om het moment van het uitvoeren van een operatie verder uit te stellen, waardoor het optreden van mogelijke complicaties daarvan eveneens wordt verlaat. Het is gesuggereerd dat een bescheiden reductie van groei (met 41%) de behandeling van een 3,5 cm groot AAA kan uitstellen met 5 extra jaren.[41] In **hoofdstuk 6** onderzochten we het effect van statines op het AAA. Statines zijn medicamenten die zeer frequent worden voorgeschreven aan patiënten met een hoger risico op hart- en vaatziekten als preventief middel. Kleinere voorgaande studies suggereerden dat deze medicamenten de groei en de afbraak van de AAA wand vertragen.[42-45] Wij konden deze resultaten niet bevestigen, ook niet na correctie voor de mogelijke invloed van risicofactoren op deze processen. De ontstekings- en afbraakparameters van AAA's van patiënten die een statine slikten verschilden niet van die van patiënten die geen statine namen. Ook studies die naar groei en uitkomst keken en later gepubliceerd werden, konden de initieel beschreven gunstige effecten niet bevestigen.[46, 47] Aangezien er relatief weinig bijwerkingen optreden na het gebruik van statines en ze gunstige effecten hebben voor de preventie van hart- en vaatziekten, moeten ze worden voorgeschreven in lijn met internationale richtlijnen. Een rechtstreeks effect op het AAA blijft echter onbewezen.

Variatie in aneurysma expressies

Meer en meer kleine AAA's worden ontdekt door de introductie van screening programma's en de grotere bekendheid van de ziekte. Daarom wordt het nog relevanter om het proces dat leidt tot AAA expansie beter te begrijpen. Het is beschreven dat roken bijvoorbeeld de groei bespoedigd, terwijl aanwezigheid van diabetes groei remt. Daarnaast bleek de progressie geen continu proces, maar stapsgewijs: periodes van groei werden afgewisseld met stabielere periodes.[48] Sterker nog, een follow-up studie van kleine AAA's liet zien dat na 5 jaar de helft van de AAA's zo groot is dat het hersteld moest worden of scheurde, terwijl de andere helft nauwelijks groei vertoonde.[41] In **hoofdstuk 7** analyseerden we of we op weefselniveau verschillende typen AAA konden ontdekken die deze verschillende groeipatronen zouden kunnen verklaren. Het bleek dat er veel variatie is in de hoeveelheid ontsteking in de vaatwand. Ongeveer de helft had heel weinig ontsteking en de andere helft had een spectrum van matig tot veel ontsteking in de AAA wand. De patiënten met een AAA dat weinig ontsteking bevatte, hadden meer risicofactoren voor hart- en vaatziekten, meer tekenen van slagaderverkalkingen in de wand en meer complicaties na de operatie. Dit suggereert dat deze patiënten een AAA hebben dat een nauwer verwantschap heeft met slagaderverkalking in de rest van het lichaam. Deze observatie suggereert dat het ontstaan van AAA's multifactorieel is, waarbij verschillende invloeden een andere uitwerking kunnen hebben op het AAA. Verder onderzoek is nodig om de relatie tussen onze bevindingen en

de beschreven groeipatronen te analyseren en vervolgens of het de behandeling van het AAA zou moeten beïnvloeden.
Het huidige wetenschappelijk onderzoek met humaan AAA weefsel is gebaseerd op collectie van een stukje van de voorkant van het AAA. Zo'n aneurysma is een grote structuur en kan zelfs 17 cm groot worden in uitzonderlijke gevallen. Het is onduidelijk of de vaatwand op verschillende plekken binnen het AAA verschilt. Daarom hebben we in **hoofdstuk 8** weefsel verzameld van de volledige omtrek van het aneurysma. De resultaten toonden dat de zijkanten van het AAA fragieler blijken met meer aanwezige afbraak enzymen en meer ontsteking. Dit zou kunnen verklaren waarom AAA's vaker aan de zijkant scheuren. Daarenboven is deze bevinding relevant voor onderzoek naar het effect van medicatie op de stabiliteit van de AAA wand. Het stabiliseren van de zijkanten lijkt relevanter dan dat van de voorkant.
In theorie kan de wand elke slagader aneurysmatisch worden. De frequentie dat zoiets gebeurt, verschilt per type slagader. Het komt het meeste voor bij de buikaorta, maar daarna het frequentst bij de kniekuil slagader. Aangezien deze aneurysma's op verschillende locaties regelmatig gezamenlijk voorkomen hebben we beiden op weefsel niveau met elkaar vergeleken in **hoofdstuk 9**. Ondanks dat het oorspronkelijk twee verschillende typen slagaders waren op verschillende locaties, bleek de wand vergelijkbaar afgebroken te zijn. Het grote verschil bleek echter de exacte locatie van de ontsteking in vaatwand: waar in het AAA de ontsteking vooral in de buitenste laag zit van de slagaderwand, is het in de kniekuil aneurysma's vooral in de binnenste laag gelokaliseerd. Dit zou kunnen kloppen met het verschil in symptomen: AAA's zijn meestal zonder symptomen maar kunnen scheuren, kniekuil aneurysma's scheuren bijna nooit maar vormen vaak opstoppingen van bloed waardoor tenen of het hele onderbeen afgesloten worden van de bloedcirculatie.

Verbeteren van het resultaat na de hersteloperatie

Behalve patiënt- en AAA karakteristieken zijn er vele andere factoren van invloed op de uitkomst na AAA herstel. Preventieve operaties worden uitgevoerd om toekomstig scheuren te voorkomen. Zoals hoger vermeld worden de meeste operaties via de liesslagader uitgevoerd, met een EVAR. Deze procedure is op verschillende manieren uit te voeren en zowel de ervaring als het type specialist hebben invloed op het resultaat en de frequentie van optredende complicaties.

Type toegangsweg
De slagader in de lies kan op verschillende manieren worden benaderd en dit is noodzakelijk voor het kunnen uitvoeren van een EVAR. Wij wilden uitzoeken of de minst invasieve manier om dit te doen, pEVAR (direct aanprikken van de slagader), beter is voor de patiënt dan de traditionele manier cEVAR (snede maken, slagader vrijleggen en aanprikken). Eerdere kleinere studies suggereerden een lager aantal locale complicaties (zoals wondinfecties) na toepassing van pEVAR in plaats van cEVAR.[49-51] In **hoofdstuk 10** gebruikten we een meer nationale klinische database om dit te onderzoeken in 269 ziekenhuizen in de VS. We zagen dat de proportie pEVAR toenam van 36,5% in 2005 naar 44,9% in 2009. Onze resultaten toonden echter geen kortere operatietijden of kortere opnames na pEVAR als we het vergeleken met

cEVAR. Ook was er geen verschil in locale complicaties, want indiceert dat de gerapporteerde voordelen van pEVAR in individuele ziekenhuizen niet worden geëvenaard op nationaal niveau. Vervolgens analyseerden we de gegevens van ons eigen ziekenhuis in Boston in **hoofdstuk 11**. De proportie pEVAR nam toe van 0% in 2005 tot 92,3% in 2010. Het gebruik van echografie tijdens pEVAR zou nauwkeurigheid moeten verbeteren en de kans op complicaties verminderen[52] en werd in ons ziekenhuis vanaf 2007 toegepast. Analyse van deze data liet zien dat pEVAR routinematig kan worden toepast met een groot succespercentage en weinig complicaties. pEVAR zorgde voor kortere operaties en een korter verblijf in het ziekenhuis in vergelijking met cEVAR.
Een belangrijke kanttekening voor het vergelijken van deze studies is de proportie pEVAR toegepast: 42% in de nationale studie en 56% in ons ziekenhuis. Vervolgens varieert het aantal specialisten dat de procedure uitvoert van enkele in ons ziekenhuis tot velen in de andere studie, met allen variërende expertise. Ons ziekenhuis is een universitair opleidingsziekenhuis, terwijl de nationale database alle soorten ziekenhuizen omvat. Hoofdstuk 11 liet zien dat pEVAR een substantieel voordeel kan beiden over cEVAR wat betreft de uitkomst na de operatie, ook wanneer er enkel gefocust wordt op variabelen die in beide databases aanwezig zijn. Echter, hoofdstuk 10 laat zien dat deze resultaten niet te generaliseren zijn naar nationaal niveau, aangezien de voordelen van pEVAR minimaal of afwezig zijn in deze ziekenhuizen. De bepalende factoren voor een succesvolle pEVAR moeten worden uitgezocht, zodat hiermee rekening kan worden gehouden en de resultaten op nationaal niveau kunnen worden verbeterd.

Specialist ervaring en volume
Het type specialist dat een open AAA operatie uitvoert heeft invloed op de complicaties na de operatie.[53, 54] Voor EVAR is dit niet bekend. **Hoofdstuk 12** beschrijft dit op een nationaal niveau in de VS van 2001 tot en met 2009. Het aandeel geopereerde AAA's dat door een vaatchirurg werd uitgevoerd nam substantieel toe tijdens deze periode, voornamelijk veroorzaakt door een toename van EVAR waarvan het merendeel door de vaatchirurgen werd gedaan. Mortaliteit was hoger bij specialisten die minder opereerden, dit gold vooral voor open AAA herstel. In de open groep daalde de proportie specialisten met hoge behandelvolumes substantieel. Dit suggereert dat het moeilijker wordt om goede zorg te bieden zonder deze behandeling te centraliseren. Voor EVAR werd een omgekeerde trend gezien: meer specialisten halen hogere behandelvolumes en daardoor een beter resultaat.

Therapeutische besluitvorming
De timing van operatief herstel is essentieel om de patiënt zo goed mogelijk te behandelen en de kwaliteit van behandeling en van leven te waarborgen. In **hoofdstuk 13** bespreken we momenteel bekende verschillende aspecten en factoren die de afweging voor de behandeling van een patiënt met een AAA bepalen. Factoren zoals risico op scheuren, levensverwachting van de patiënt, operatief risico en natuurlijk verloop van het AAA (groei). Voor het merendeel van de patiënten is het veilig om operatief herstel van het AAA uit te stellen tot de diameter groter wordt dan 5,5 cm, snel groeit of pijnlijk wordt. Ondertussen moeten de patiënten nauwgezet worden gevolgd en risicofactoren voor het optreden van een scheur moeten worden behandeld. Een selecte groep patiënten met een hogere ruptuurkans komen in aanmerking voor een herstel bij een kleinere diameter: vrouwen, asymmetrische AAA,

pijnlijke AAA en jonge gezonde patiënten. Patiënten met een korte levensverwachtingen moeten alleen geopereerd worden als het ruptuurrisico erg groot is.

Met een beter begrip van *discrepanties in AAA expressies en herstel* hopen we bij te dragen aan de gezondheid van de patiënt met een AAA. Huidige en toekomstige toenamen van kennis van de ziekte zouden moeten leiden tot een specifieke aanpak met een individueel aangepaste behandelingsstrategie. Uiteindelijk zouden we patiënten die behandeling nodig hebben vanwege een hoog ruptuurrisico beter kunnen identificeren. Daarnaast zouden we het preventieve herstel van een AAA in patiënten met een hoog risico op complicaties na de operatie, zo lang mogelijk moeten uitstellen: idealiter tot net voor het scheuren van het AAA.

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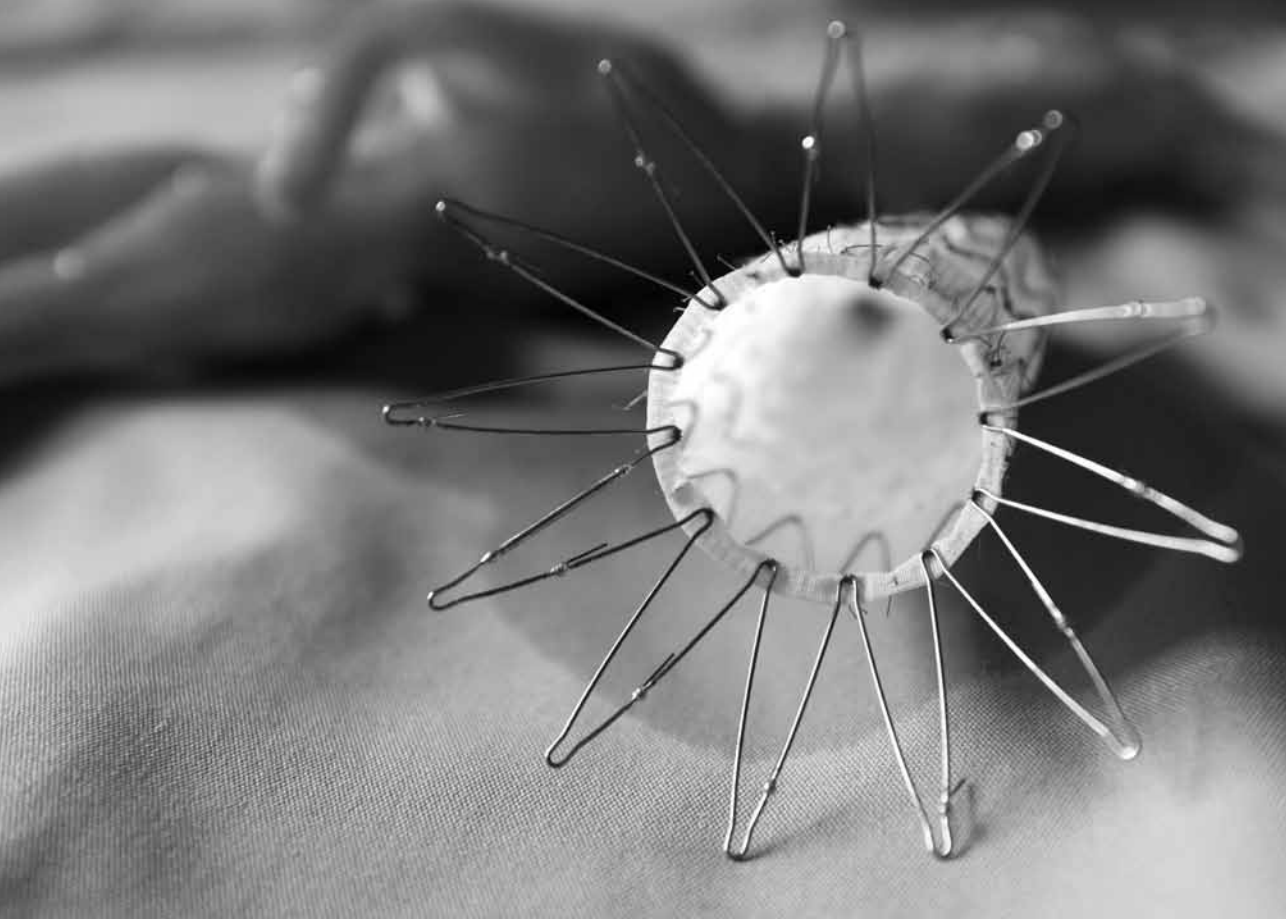

Appendix

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Publications

R Hurks, MJ Eisinger, I Goovaerts, J Hendriks, L van Gaal, C Vrints, P van Schil, J Weyler, P Lauwers. Early endothelial dysfunction in young diabetics. Eur J Vasc Endovasc Surg 2009; 37: 611-5

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R Hurks, T Sachs, RP Bensley, P Trivedi, FB Pomposelli, EL Chaikof, FL Moll, ML Schermerhorn. Limited benefit after percutaneous versus femoral cutdown access for endovascular aneurysm repair. Submitted

R Hurks, RP Bensley, MD Howell, GS DaSilva, EL Chaikof, FL Moll, ML Schermerhorn. Vascular surgeons repair an increasing majority of AAA, where volume load changes over time and determines outcome. Submitted

R Hurks, RP Bensley, DS Pinto, EL Chaikof, FL Moll, ML Schermerhorn. Traditional risk factors do not predict myocardial infarction after abdominal aortic aneurysm repair. Submitted

R Hurks*, D Koole*, AH Schoneveld, A Vink, DPV de Kleijn, JA van Herwaarden, JPPM de Vries, FL Moll, G Pasterkamp. Osteopontin in the abdominal aortic wall predicts adverse cardiovascular events. Submitted

R Hurks*, P Liang*, RP Bensley, FB Pomposelli, AD Hamdan, MC Wyers, EL Chaikof, ML Schermerhorn. The rise and fall of renal artery angioplasty and stenting in the United States, 1988-2009. Submitted

D Koole, **R Hurks**, AH Schoneveld, A Vink, J Golledge, CS Moran, DPV de Kleijn, JA van Herwaarden, JPPM de Vries, G Pasterkamp, FL Moll. Osteoprotegerin is associated with aneurysm diameter and proteolysis in abdominal aortic aneurysm disease. Submitted

RP Bensley, **R Hurks**, Z Huang, FB Pomposelli, AD Hamdan, MC Wyers, D Campbell, EL Chaikof, ML Schermerhorn. Ultrasound guided percutaneous endovascular aortic aneurysm repair can be performed routinely with high success and minimal complications. Submitted

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RP Bensley, **R Hurks**, RC Lo, FB Pomposelli, AD Hamdan, MC Wyers, EL Chaikof, ML Schermerhorn. Isolated iliac artery aneurysms: management and outcomes in the endovascular era. Submitted

RP Bensley, ML Schermerhorn, **R Hurks**, T Sachs, RC Lo, AJ O'Malley, P Cotterill, BE Landon. Late onset incisional hernia and adhesion related complications after laparotomy and laparoscopy. Submitted

ML Schermerhorn, RP Bensley, KA Giles, T Sachs, **R Hurks**, AJ O'Malley, P Cotterill, BE Landon. Changes in short term mortality from abdominal aortic aneurysm repair and rupture 1995-2008. Submitted

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Curriculum Vitae

Rob Hurks was born on January 7, 1982 in Eindhoven, the Netherlands. After graduating from secondary school (S.G. Augustinianum, Eindhoven, the Netherlands) in 2000 he studied Medicine at the University of Antwerp, Belgium. During this 7-year program he was an active member of student society Aesculapia, took additional courses and worked as a nurse during the summer leaves. As a student he started conducting research on the department of Vascular Surgery (Dr PR Lauwers, Prof Dr PE van Schil), which resulted in his first publication. During the last year of his studies he followed the pre-specialization program for Surgery, and in 2007 he graduated with great distinction. Afterwards he started a PhD-training program on the departments of Vascular Surgery (Prof Dr FL Moll) and Experimental Cardiology Laboratory (Prof Dr G Pasterkamp) of the UMC Utrecht, Utrecht, the Netherlands. After 3 years of basic and translational research the next step was conducting clinical research. In 2010, he continued his PhD-training on the department of Vascular Surgery (Dr ML Schermerhorn) of the Beth Israel Deaconess Medical Center and Harvard Medical School, both in Boston Massachusetts, USA. The work in this thesis was presented on numerous (inter) national meetings. Following his work as a research fellow, he started working at the department of Surgery (Dr PMNYH Go) in the St Antonius Hospital Nieuwegein, the Netherlands, in anticipation of residency training.