Morphological and Mechanical Changes in Juxtarenal Aortic Segment and Aneurysm Before and After Open Surgical Repair of Abdominal Aortic Aneurysms

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Submitted 27 November 2007; accepted 28 April 2010
Available online 14 June 2010

KEYWORDS
Aortic aneurysms;
Ultrastructure;
Mechanical properties;
Surgical repair of the aneurysm;
Ultrasound;
Distensibility

Abstract Objective: The aim of study was to assess how the ultrastructure of the wall of aortic aneurysms, sac and neck influences aortic wall distensibility and proximal dilatation 2 years after open repair.
Methods: Biopsies for electron microscopy were taken from aneurysmal sac and neck of 30 patients. Patients were assessed by computed tomography (CT) and ultrasound for aneurysm diameter and distensibility (M-mode ultrasonography).
Results: Postoperative CT of the aortic stump distinguished two groups. Group I (n = 11) with little enlargement, median 1 mm (1–3 mm) and group II (n = 19) with significant aortic enlargement, median 5.2 mm (4–12 mm). In group II, changes in elastic fibres in the aneurysm neck were comparable to, but as extreme as in the aneurysm sac. For group I, the distensibility of the aneurysm sac was significantly lower than in the neck or at the renal arteries. For group II, the distensibility in both the neck and sac was significantly lower than at the juxtarenal segment (p = 0.01). The biopsies of group II patients showed the extensive degeneration of normal architecture, which was associated with altered wall distensibility in both the aneurysm neck and sac.
Conclusions: Disorganisation and destruction of normal aortic architecture at the ultrastructural level are associated with decreasing aortic distensibility. Low aortic neck distensibility is associated with proximal aortic dilatation at 2 years postoperatively.

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A normal aortic wall consists of three well-defined layers: endothelium, media and adventitia. Each layer has a well-defined morphological structure. In health, the endothelial lining consists of one-cell-thick layer of flat endothelial cells connected by tight junctions positioned on thin and regular basement membrane, which is attached to the lamina elastica interna. The media of normal aorta is composed of regularly distributed smooth muscle cells (SMCs). Between SMCs, there is a regular network of elastic fibres and collagen bundles. Within this network, collagen type III connects the network to elastic fibres, and collagen type VIII connects with collagen type VI in basement membranes. Any abnormality of regular connections of collagen to the elastic fibres leads to hyper-stretching of the latter.

The pathogenesis of abdominal aortic aneurysms (AAA) remains unclear. The aim of the present study was to evaluate the deterioration of mechanical properties of the aorta, both above and in the aneurysm sac, and AAA assess whether changes in distensibility were correlated with morphological changes. Few publications have focussed on the assessment of morphology of the AAA neck and its mechanical properties. Such studies may shed light on the reasons for late complications at the proximal aortic anastomosis (after open repair) or proximal aortic stent-graft fixation.

Morphological changes leading towards formation enlargement and rupture of AAA have been studied widely. The durability of both open and endovascular procedures depends on changes in the aortic neck. Long-term follow-up after open AAA repair shows dilatation of both the proximal aorta and iliac arteries after prosthetic graft implantation, this often being the reason for redo procedures. Successful aortic stent-graft placement may be followed later by the development of type I endoleak, perhaps due to continuing dilatation proximal to the original aneurysm. It may affect the neck of the aneurysm which, on preoperative imaging, was identified as normal. Open or endovascular graft fixation within a weak aortic wall may lead to several important complications. This emphasises the need for studies of aneurysm neck both before and after aortic surgery.

Few previous studies have assessed both morphology and mechanical properties. This is the first study to assess the histology of the aortic wall at different levels of the aneurysm and proximal ‘healthy’ aorta for comparison with the mechanical properties of the same aortic segments.

Material and methods

The study population consisted of 30 male patients (from 52 to 83 years, median 67 years) with American Society of Anesthesiologists (ASA) II or III preoperative risk assessment. Ethical committee approval for presented research was given by the Institutional Review Board at Poznan University of Medical Sciences, reference 633/04 valid from 08 April 2004. Patients with diabetes or auto-immune connective tissue disorders were excluded. In all patients, biopsies were taken from AAA main wall (anteriorty) and AAA neck (5 mm above the aneurysm) during open surgery. Biopsies were not taken if the aneurysm neck was less than 2 cm in length, to avoid inappropriate risk during the routine operation. No neck diameter exceeded 30 mm, which eliminated patients with pre-existing juxtarenal aneurysms.

All patients were assessed by preoperative ultrasound for aneurysm diameter length and aortic wall distensibility. Mechanical properties of the abdominal aorta wall were assessed in M-mode with a sector 3.5 MHz probe, Sonoline Siemens. Vessels could be assessed if the distance from abdominal wall to the aorta was not more than 70 mm, so that both anterior and posterior aortic walls can be visualised. The appropriate aortic segment was selected in two-dimensional (2-D) mode. We examined the aorta 5 mm below renal arteries and 5 mm above the aortic aneurysm (aneurysm neck) and at the point of maximum aneurysm diameter. Each point was examined thrice and the median data sample was used for the final analysis. Median systolic blood pressure was established from three brachial artery readings, preceded by 15-min rest. Using M-mode, we assessed the diameter of the selected segment in both systole and diastole. Distensibility was calculated as capacity of the artery to augment its diameter in relation to increasing intra-arterial pressure:

\[
\text{Distensibility}(D) = \frac{\Delta d}{\Delta p} \left(\text{mm}^2/\text{mm Hg}\right)
\]

where \(\Delta d\) — systolic—diastolic difference in aortic wall cross section, \(\Delta p\) — difference between systolic and diastolic arterial blood pressure computed tomography (CT) scanning was used in all patients for the assessment of exact anatomical details and open surgery planning. Every patient after surgery was assessed by clinical examination and abdominal ultrasound every 6 months within a 24-month follow-up period. At the final ultrasound, the exact diameter of the aortic neck was measured, as well distensibility of residual aneurysm neck.

All tissue samples were fixed and processed routinely in epoxy resin blocks (Peon 812) for transmission electron microscopy (TEM). After polymerisation resin blocks were cut, first into semi-thin sections, which, after olive blue stained sections, were evaluated under light microscope. Then ultra-thin sections were prepared, which were studied under TEM. TEM assessment was based on a protocol designed for the purpose of the study scoring system used by three independent pathologists with documented experience in TEM imaging interpretation. Each pathologist scored all available patients’ samples and mean score value obtained from each sample was used for statistical analysis.

Scores were attributed with regard to the following criteria:

1. Healthy aortic wall structure found in healthy donors was graded as 0.
2. Minimal disorganisation in the aortic wall with presumably regular endothelial lining with vacuolated cells within aortic media normal parallel arrangement of elastic fibres, collagen fibres and smooth muscle was graded as 1.
3. Samples where elastic fibres were partially disrupted and haphazardly organised collagen bundles were...
surrounded by glycosaminoglycans and inflammatory infiltration was graded as 2.

4. Samples with broad area without endothelial lining containing amorphous or fibrillar deposits and some fibrocyte-like cells were graded as 3 (this was mainly contributing to the aneurysm sac).

Statistical analysis of ultrasound distensibility data and the degree of structural changes was performed with use of the Mann--Whitney test. Correlation between structural changes and distensibility in vivo was established with the use of Spearman’s correlation.

Results

Measurement of aortic stump enlargement at 24-months follow-up and analysis of neck morphology allowed us to distinguish two groups of patients. Group I contained 11 patients with little enlargement of the aorta either below the renal artery or above the proximal anastomosis: median 1 mm (range 1–3 mm). Group II contained 19 patients with significant aortic enlargement with median of 5.2 mm (range 4–12 mm). In group II, at 24 months, two para-anastomotic aneurysms of 40 and 42 mm were diagnosed. CT scanning excluded the presence of pseudo-aneurysm. The initial diameter of the neck in those patients was 29 and 30 mm, respectively. Overall dilatation of the aortic neck with median growth of diameter of 4 mm (range 1–12 mm) was observed within 24 months’ follow-up.

For group I, the preoperative distensibility of the aorta 5 mm below the renal arteries, 5 mm above the aneurysm, and at the point of maximum diameter point was 11.29 ± 2.64 mm²/mm Hg, 9.58 ± 2.31 mm²/mm Hg, 4.98 ± 1.42 mm²/mm Hg, respectively. For group II, the distensibility of the aorta 5 mm below the renal arteries, 5 mm above the aneurysm and at the point of maximum diameter was 10.44 ± 3.72 mm²/mm Hg, 6.73 ± 2.94 mm²/mm Hg and 5.36 ± 1.47 mm²/mm Hg, respectively. The difference between aneurysmal neck and sac was not statistically significant but both were statistically lower than the distensibility at the juxtarenal aortic segment (p = 0.02; p = 0.01, respectively).

Ultrastructural changes in group I

In the 11 biopsies taken from the AAA neck in group I, we found regular endothelial lining with vacuolated cytoplasm but without basement membrane (Fig. 1) A and B; within the aortic media, there was normal parallel arrangement of elastic fibres, collagen fibres and SMCs were present (Fig. 1(D)). These cells were separated by regularly arranged collagen bundles, which were oriented parallel and obliquely to the SMC longitudinal axis (Fig. 1(C)). Between the collagen bundles, collagen fibres were regularly entrapped. In the peripheral part of media, there were well-preserved microvessels, often filled by red blood cells. Many of the elastic fibres were broken (Fig. 1(E)). The adventitia was composed by regular fibroblasts surrounded by collagen fibres, proteoglycans and glycosaminoglycans (Fig. 1(F)).

In media, next to regular collagen fibres and parallel arrangement of elastic fibres, we found wide extracellular spaces with only few collagen bundles, which additionally were accompanied by some remnants of cellular cytoplasm projections. The median (range) scores at each level of the aorta are shown in Table 1.

Ultrastructural changes in group II

In the 19 biopsies from group II AAA sac, we found no continuous endothelial lining and endothelial cells showed vacuolar changes and lipid accumulation in the cytoplasm (Fig. 2(A, B)). Around such areas, elastic fibres as well as proteoglycans were absent, in comparison with areas of more advanced changes found in larger size aneurysm (Fig. 2(C)). SMC present in adjacent areas were lying on irregularly formed and focally thickened basement membranes. In the basal SMC cytoplasm, only small groups of microvibrils were visible (Fig. 2(D)). Around such cells, there were only single collagen fibrils accompanied by proteoglycans and glycosaminoglycans (Fig. 2(D)). The majority of samples from the AAA neck showed partially disrupted elastic fibres, with haphazardly organised collagen bundles surrounded by glycosaminoglycans and infiltrating inflammatory cells.

In samples taken from AAA main sac wall in all patients (either group I or II), we found broad areas without endothelial lining containing amorphous or fibrillar deposits and some fibrocyte-like cells. Elastic fibres arranged in the outer one-third to one-fifth of the media were partially disrupted and surrounded by necrotic cells. The most important abnormalities of vascular wall structure were the focal presence of fragmented elastic fibres, sometimes totally absent (Fig. 2(E)). In some areas in the media, accumulations of haphazardly arranged collagen fibres and glycosaminoglycans were found. In other areas, acellular zone with loose amorphous material of necrotic remnants appeared. These zones contained no SMCs. In the intima-media complex of the AAA wall, monocytes/macrophages, lymphoid cells and, quite often, erythrocytes were present. Some macrophages contained electron-dense material, most probably lipids, phagolysosomes, some with similar ultrastructure to ceroid. Inflammatory cells were placed between collagen bundles, reticulin fibres and glycosaminoglycans. In such areas, fibrillar structures were completely irregularly arranged without any interweaved organisation (Fig. 2(F)). Fibrillar structures did not fill the entire extracellular space. We also found that vasa vaso rum in the tunica externa and capillaries in media were scanty and abnormal (Fig. 2(F)). They had non-continuous endothelial lining as well as irregular or even missing basement membranes. The median (range) scores at each level of the aorta are shown in Table 1.

Positive correlation was found for both groups between aortic neck distensibility and the degree of ultrastructural changes in the neck and AAA sac (r = 0.7 p = 0.001).

Discussion

After many years of studies on the pathogenesis of the aneurysm formation, the sequence of the changes leading to aneurysmal dilatation is still not known. There is no satisfactory explanation for the prevalence of infrarenal...
Aneurysm. Factors influencing length of the aneurysm neck and causes of its possible dilatation are unknown.\textsuperscript{7} In our studies, structural degradation of aortic matrix was found in all layers, especially in the media. These degenerative changes are related to the chronic inflammatory process (Figs. 2A–F). Among inflammatory cells, lymphocytes and macrophages have important roles in the degradation of extracellular matrix and weakening of aortic wall, through secretion and activation of matrix metalloproteinases (MMPs).

Pathological changes in aortic wall structures involved not only the main trunk of AAA, but also the neck, especially in the inner part of the media, proximal to the aneurysmal sac. However, in contrast to the neck of the aneurysm, the elastin concentration in aneurysmal sac was significantly reduced. Pathological changes of collagens and elastic fibres found in the neck and main trunk of the aneurysm were qualitatively but not quantitatively comparable to changes in the aneurysm sac in most cases. This suggests that aneurysmal processes may affect the whole infrarenal aorta, and hence influence any dilatation of the aorta, which may be observed after open or endovascular surgery.\textsuperscript{8}

Para-anastomotic aneurysm formation is an uncommon complication of both open and endovascular repair of AAA.\textsuperscript{9} The incidence of aneurysms in the region of arterial anastomosis (para-anastomotic aneurysm) is described in literature as 0.7–4%, and it depends on the time after the

<table>
<thead>
<tr>
<th>Aortic neck enlargement at 24 months (median, range)</th>
<th>Group 1 1 (1–3) mm</th>
<th>Group II 2 (4–12 mm)</th>
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<tr>
<td>Distensibility</td>
<td>Ultrastructure score (median)</td>
<td>Distensibility Ultrastructure score (median)</td>
</tr>
<tr>
<td>Pre-operative renal level</td>
<td>11.29 ± 2.64 mm(^2)/mm Hg, 1</td>
<td>10.44 ± 3.72 mm(^2)/mm Hg, 2</td>
</tr>
<tr>
<td>Pre-operative neck</td>
<td>9.58 ± 2.31 mm(^2)/mm Hg, 1</td>
<td>6.73 ± 2.94 mm(^2)/mm Hg, 2</td>
</tr>
<tr>
<td>Pre-operative sac</td>
<td>4.98 ± 1.42 mm(^2)/mm Hg, 3</td>
<td>5.36 ± 1.47 mm(^2)/mm Hg, 3</td>
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primary intervention. True aneurysms in the region of anastomosis are rare after reconstructive surgery. Their incidence in different studies is reported as 0.3–0.4%. However, it is frequently forgotten that supra-anastomotic aortic neck diameter enlargement should be monitored too as well as changes in its distensibility. After 10 years of follow-up, the incidence of para-anastomotic aneurysms is 8%, rising to as high as 27% 15 years after aneurysm repair. In our study, we found in 2 years observation only two cases, which could be classified as para-anastomotic aneurysm, which is comparable to other authors. However, if we take into consideration changes in aortic neck diameters, in all patients in abdominal ultrasound a median enlargement of 4 mm within a 24-month follow-up period could be observed. The length of supra-anastomotic aortic neck might influence the rate of aortic neck enlargement. This finding is comparable with authors assuming the implantation of the prosthetic graft in the proximity of the renal artery. Aortic neck diameter changes also have been reported in the follow-up of patients after endovascular aneurysm repair (EVAR).

All measurement of arterial wall distensibility performed in the present study are based on the hypothesis that observed vessels were strongly distended in the longitudinal aspect and attached to the surrounding tissue; hence, any longitudinal movement of aortic wall is impossible. The only reaction of the blood vessel to the blood pressure is the change of the diameter. That means that all measured distensibility indicators are functions of the arterial blood pressure. According to Borow’s observation, we presumed that blood pressure in brachial arteries is comparable to those found in the aorta. The principal factors influencing assessment of aortic mechanical properties are elastic and collagen fibres with SMCs. Continuous changes in aortic neck elasticity should be related to quantitative and qualitative changes in arterial wall structure, mainly in relationship to collagen and elastin fibres, as well as in the reorganisation of cellular population in different layers, decrease in elastin concentration within the wall produces gradual enlargement and elongation of arteries with aneurysm or ectasia. Such changes have been correlated with reduction of elasticity and distensibility in arterial walls. Imbalance in collagen production and degradation and its synthesis and transport is associated with impaired arterial distensibility and an increasing tendency to arterial wall rupture.

The positive correlation of preoperative aortic neck distensibility with both ultrastructural degeneration and risk of future neck dilatation, found in our study, could enable future selection of those patients at highest risk of para-anastomotic aneurysm by routine examination of distensibility of juxta-anastomotic aortic neck. Low aortic
neck distensibility shown preoperatively should warn the surgeon that the patient is at risk of para-anastomotic aneurysm and can lead to a high juxtarenal anastomosis, even when the neck anatomically is appropriate for a more distal anastomosis. Aortic neck dilatation is exacerbated by the presence of arterial hypertension and haemodynamic stress imposed on the supra-anastomotic aortic segment. Early postoperative changes in aortic wall distensibility should warn the physician on proper control of concomitant risk factors including hypertension. High infrarenal anastomosis may decrease the probability of para-anastomotic aneurysm formation, due to the more organised morphology of aortic wall at the level of the renal arteries and its improved distensibility. As pathological changes in the neck of the aneurysm are associated with impaired aortic wall distensibility, further non-invasive ultrasound assessment of aortic neck after open surgery should be conducted, because further surgical intervention, either open or endovascular, may be needed to exclude aneurysmal disease.

Conclusions

Reduced arterial wall distensibility at the level of aortic aneurysm neck was associated with increasing disorganisation of arterial wall morphology as shown by electron microscopy. Routine assessment of pre-operative aortic neck distensibility may reveal a group of patients who would benefit from earlier and/or more proximal intervention as well as proper risk factor management. Preoperative assessment of aneurysmal neck distensibility could be useful when planning both open and endovascular procedures.

Conflict of Interest/Funding

None.

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