

The Pathohistology of Abdominal Aortic Aneurysm

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1. Introduction

Atherosclerosis is a chronic condition characterised by the formation of lipid-rich plaques within the walls of medium and large arteries (Ross, 1993; Lusis, 2000) and underlies many forms of vascular disease, including abdominal aortic aneurysm (AAA). The development of all vascular disease phenotypes is dependent on multiple genetic and environmental determinants (Lusis, 2000) though the relative contribution of each of these risk factors may vary with different vascular disease phenotypes. This is certainly true for AAA, which appears to have particularly strong associations with male gender, tobacco smoking (Singh et al., 2001) and a family history of AAA (Verloes et al., 1995; Ogata et al., 2005). Interestingly, diabetes, a well-established occlusive atherosclerotic disease risk factor is not associated, and may even be protective for AAA (Shantikumar et al., 2010).

Abdominal aortic aneurysm is a common condition being responsible for 1.3% of all deaths in 65-85 year old white males (Sakalihasan et al., 2005). The reported prevalence rates vary depending on factors such as the threshold aortic diameter used to define an aneurysm and age strata (relevant when reviewing rates from screening studies). If the most widely accepted value of 30mm is applied, 1% of women and 4.2% of men between the ages of 50 to 79 years are affected in a predominantly white (north American) population (Lederle et al., 2000). In British males, over the age of 65 years, this rate has been reported to be as high as 7.6% (Scott et al., 1995), although there are indications that incidence may be falling internationally (Sandiford et al., 2011). An abdominal aortic aneurysm is typically defined as being localised in the infrarenal abdominal aorta and may either extend up to involve the renal ostia, or down to involve the aortic bifurcation and into common iliac arteries (Sakalihasan et al., 2005).

These epidemiologic and anatomical features are important when considering the pathobiology of AAA. While the initial pathobiology is atherosclerotic, at some stage it diverts to a distinctive dilating, rather than aortic occlusive, phenotype. A schema outlining the key pathogenic components was proposed by Thompson and colleagues in 1995 (Holmes et al., 1995), a modified interpretation of which is shown in Figure 1. This chapter will briefly describe each of the pathohistological features.

2. Histology of the abdominal aorta

2.1 Histological features in the aging arteries

The abdominal aorta is a transitional elastic artery. In the prenatal abdominal aorta the intima consists of an endothelial monolayer and a scant pad of sub-endothelial fibrous

connective tissue upon an intact internal elastic lamina (IEL). The only openings between the intima and underlying media are fenestrations in the IEL, which are spanned by elastic tissue trabeculae. The media is composed of approximately 28-30 concentric lamellar subunits (Wolinsky & Glagov, 1969), one lamellar unit consisting of an elastic lamina and the smooth muscle and extracellular matrix contents of the adjacent interlamellar zone (Fig. 2). The medial elastic laminae are approximately two thirds the thickness of the internal elastic lamina and are interconnected by a network of finer elastic fibres. Collagen and elastin fibres are uniformly dispersed around the circumferentially orientated smooth muscle cells (Keech, 1960; Karrer, 1961; Wolinsky & Glagov, 1964).

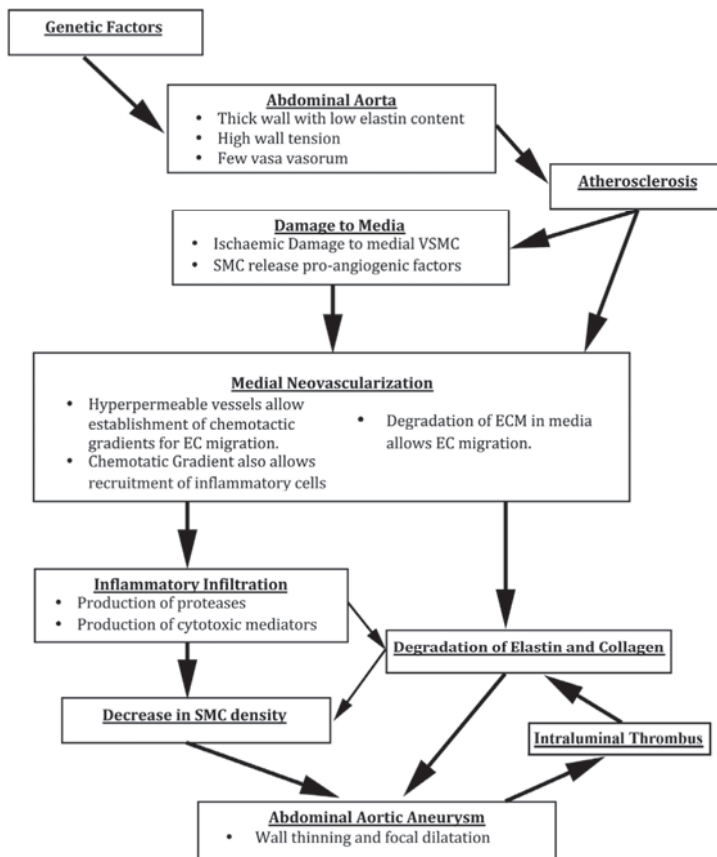


Fig. 1. The Pathogenesis of Abdominal Aortic Aneurysm. This schema is a modification of that proposed by Thompson and colleagues (Holmes et al., 1995). Originally to highlight the central role of medial neovascularisation it shows the progressive steps of aneurysm histopathology outlined in this chapter. Atherosclerosis superimposed on a structurally vulnerable infrarenal abdominal aorta leads to medial degeneration which in-turn stimulates a neovascularisation and inflammatory response from the adventitia. These processes result in a pernicious cycle leading to wall thinning, vessel expansion and eventually aneurysm rupture.

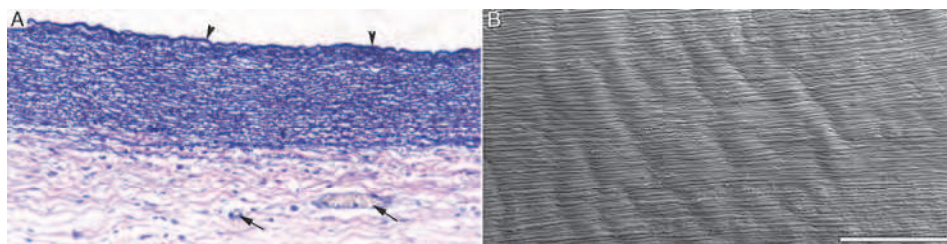


Fig. 2. Infrarenal abdominal aorta, 21 weeks-gestation. A. The intima consists of a monolayer of endothelial cells in close apposition to a thick continuous internal elastic lamina (arrowheads). The aortic media consists of vascular smooth muscle interspaced between approximately 30 elastic laminae (stained black). A network of vasa vasorum (arrows) is found throughout the adventitia but very rare penetrates even the most external layers of the media. Original magnification x50. Verhoeff's elastic stain and van Gieson's counterstain (elastin black, collagen red). B. *An en face* (endothelium removed) scanning electron preparation of the IEL from the same subject. The IEL forms an intact corrugated layer punctuated by small fenestrations (not visible at this magnification. Scale bar 500 μ m

Within the aortic adventitia a network of vasa vasorum is present that originates from adjacent intercostal, lumbar and mesenteric arteries (Heistad & Marcus, 1979). The vasa vasorum has been shown to only penetrate the media of vessels with greater than 29-30 lamellae (Wolinsky & Glagov, 1969) and consequently the media of the human infrarenal aorta is vastly avascular, despite the presence of the adventitial vasa vasorum (Fig. 3). However, if the luminal diffusion is insufficient to supply nutrients to the intima, vasa vasorum becomes an important alternative nutrient supply (Heistad & Marcus, 1979). Consequently, as the intima thickens, the vasa vasorum extends channels into the outer medial lamellae (Fig. 3B). Significantly however, unlike vasa vasorum in larger vessels such as the thoracic aorta, which are well integrated into the lamellar structure of the wall during its vasculogenesis, these new vessels appear to disrupt the medial structure during their invasion of the wall, as described below in section 2.4.

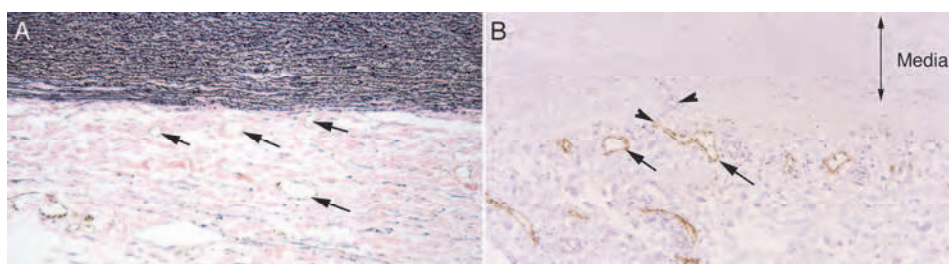


Fig. 3. Vasa vasorum at the medial-adventitial boundary. (A) numerous channels (arrows) within the infrarenal aortic adventitia of a 9-year old female (Verhoeff's elastic stain and van Gieson's counterstain). (B) anti-von-Willebrand factor immunostaining (DAB (3,3-diaminobenzidine) staining resulting in a brown reaction product) showing an extensive network of vessels in the adventitia (arrows), including some small vessels extending into the medial layer (arrowheads) in the infrarenal aorta of a 35-year old male. Original magnification (A) x33, (B) x25

Compared to the thoracic segment and other mammalian aortas of the same thickness, human abdominal aortic media contains less lamellar units, and therefore medial elastic laminae. Consequently, the vessel appears to be stiffer, due to a higher collagen-to-elastin ratio. The flow on effect of this is that the mean tension per lamellar unit is higher in the human abdominal aorta (Wolinsky & Glagov, 1969; Wolinsky, 1970). As will be described below, loss of load bearing elastin, along with the avascular nature of the abdominal aortic media, are key pathologic features of AAA. Therefore the vessels initial lower elastin content and lack of medial vasa vasorum may make it structurally vulnerable to degenerative alteration and the subsequent development of aneurysmal disease later in life (Dobrin, 1989; He & Roach, 1994).

2.2 Early life elastic lamina degeneration, the nidus of atherosclerosis?

After birth one of the first changes in aortic structure consists of disruption of the IEL (Fig. 4). These spontaneous, transversely orientated, elastic lamina defects have been reported within the arteries of newborn and adolescent humans, particularly those vessels known to be prone to the subsequent development of atherosclerosis, such as the aorta (Meyer & Lind, 1972), coronary (Levene, 1956; Moon, 1957; Sims, 1985; Ikari et al., 1999) and carotid (Meyer et al., 1980) arteries. Critically, Meyer and colleagues demonstrated that elastic lamina defects occur in very young humans, at sites prone to the eventual development of atherosclerosis (Meyer & Lind, 1972; Meyer et al., 1980).

The intima also undergoes early cellular alteration, with the formation of a smooth muscle cell layer between the endothelium and IEL. The origins of these smooth muscle cells has been considered controversial, with some authors suggesting that they are an expansion of a resident intimal cell population (Stary, 2000), while others have argued that they originate from the media and migrate into the intima (Schwartz, 1997; Willis et al., 2004). The most significant contributor to intimal smooth muscle thickening is likely to be a medial origin, as evidenced by the fact that the intima is thicker above regions of IEL disruption in children (Fig. 4). This is further supported by the ApoE deficient mouse model of atherosclerosis, which clearly shows that early intimal smooth muscle cell accumulation is due to medial smooth muscle cell migration through defects in the IEL (Jones et al., 2005). The thickening intima typically forms two distinct layers, a deep musculoelastic layer and a less compact superficial subendothelial layer (Figs 5 & 6C).

The literature also reports the internal elastic lamina beneath the thickened intima and subsequent atherosclerotic plaques as becoming 'frayed', 'reduplicated' or undergoing laminated elastosis. All of these terms refer to intimal elastic tissue deposition and as such should be considered as a distinct, though often co-localised, processes to the disruption of the original IEL. The key cells in producing intimal elastic tissue are smooth muscle cells (Kojimahara, 1988) with a possible contribution by the endothelial monolayer (Jones & Stehbens, 1995) and macrophages (Krettek et al., 2003).

Intimal elastic tissues appear to have an increased affinity for lipoproteins compared with the 'original' elastic laminae (Adams & Tuqan, 1961; Urry, 1975; Meyer et al., 1980). The lipophilic features of intimal elastic tissue (Fig. 5) appears to be a key early contributor to intimal lipid retention (Williams & Tabas, 1995).

Calcification has been shown to be localised to the elastin adjacent to the break edges of spontaneous IEL defects in the arteries of children (Meyer & Lind, 1972; Meyer et al., 1980). While calcium containing matrix vesicles have been shown to preferentially accumulate in

microfractures within elastic laminae adjacent to abrupt, haemodynamically induced, elastic tissue defects in experimental animals (Jones & Stehbens, 1995). As such failure of the elastic laminae may facilitate accumulation of granulo-vesicular debris, including calcospherites, and thereby contribute to vascular connective tissue calcification.

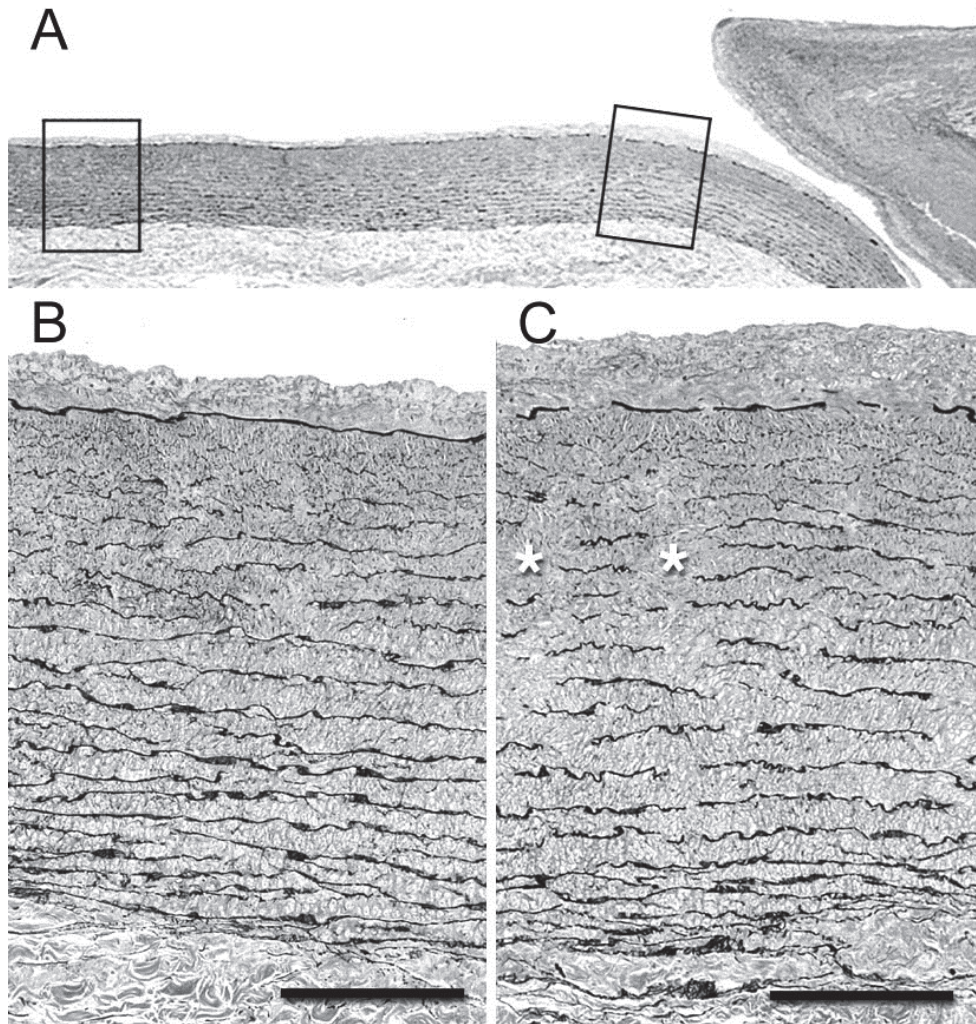


Fig. 4. Longitudinal sections of the anterior infrarenal abdominal aortic wall from a 9 year old female. (A) The two regions indicated represent a proximal segment of the infrarenal aorta (B) and the lateral angle of the inferior mesenteric artery ostium (C). (B) In this young subject IEL defects were not observed in abdominal aortic segments away from branch ostia. (C) In contrast the segment within the lateral branch angle has a fragmented IEL, a more thickened intima, and disrupted medial lamellae (asterisks). Verhoeff's elastic stain. Scale bars 200 μ m.

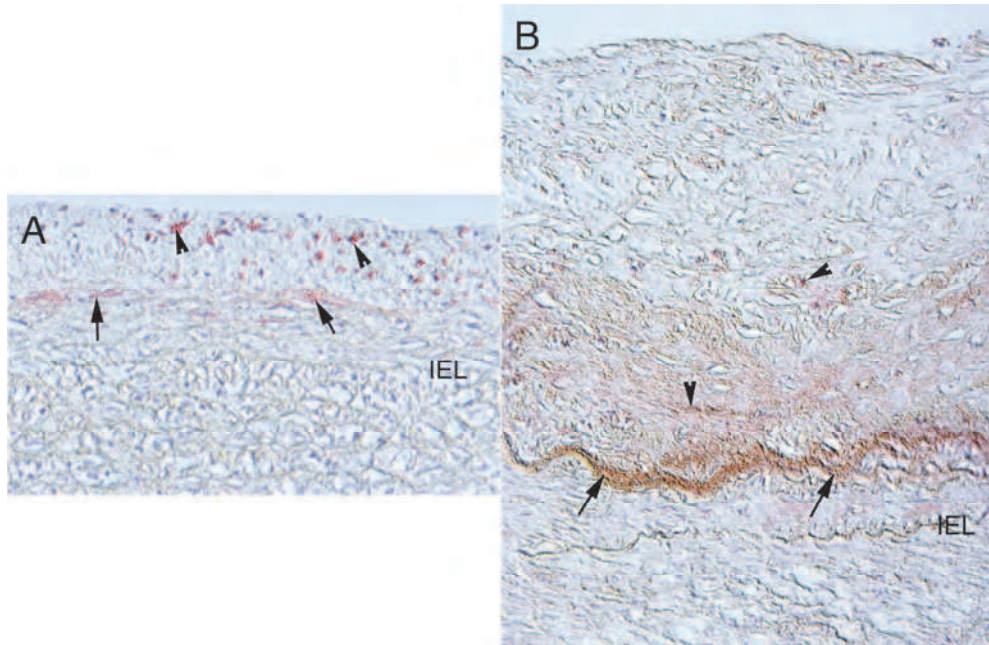


Fig. 5. Intimal lipid disposition. Oil-red-O staining of the distal abdominal aorta in a (A) 37-year old male and an (B) 84-year old female showing diffuse lipid deposits within (subendothelial) macrophages (arrowheads) and bound to extracellular matrix, particularly intimal pseudo-elastic laminae (arrows). The intima is demarcated by the internal elastic lamina (IEL). As the intima thickens with age (B) note the localisation of lipid in the deep (fibroelastic layer) intima as a precursor to the formation of a lipid core. Original magnification both x33. Photographed using differential interference contrast (DIC) microscopy.

The localisation of atherogenic lipids and granulo-vesicular debris within elastic laminae break edges and the intimal elastic tissue may act as chemotactic targets for migrating smooth muscle cells and macrophages effectively 'lighting up' these intimal / medial gateways.

When assessing the histopathology of early atherosclerotic lesions, care should therefore be taken to differentiate between intimal elastic tissue and the original IEL. Such a distinction is clearly significant given the possible differences in cell permeability and affinity for lipophilic debris of these two forms of elastic tissue. Certain stains, such as phosphotungstic acid haematoxylin (PTAH), differentially stain these two forms of elastic connective tissue (Gillman et al., 1955), though common elastic tissue stains, such as Verhoeff's elastic stain, do not.

Of relevance to the study of abdominal aortic aneurysm histopathology the distal abdominal aorta and common iliac arteries appear to be particularly vulnerable to both IEL degeneration and intimal elastosis (Figs. 4-6).

Even at its earliest stages of development, atherosclerosis displays preferential localisation about the segmental branch ostia of the posterior abdominal aortic wall (Miller et al., 1993; Stehbens, 1995). While systemic factors, such as serum LDL levels, smoking, hypertension and diabetes (Grundy, 1995; Schaefer et al., 1995; Strong & Group, 1995; Jousilahti et al., 1999), have been implicated in the progression of atherosclerosis they fail to fully account for the

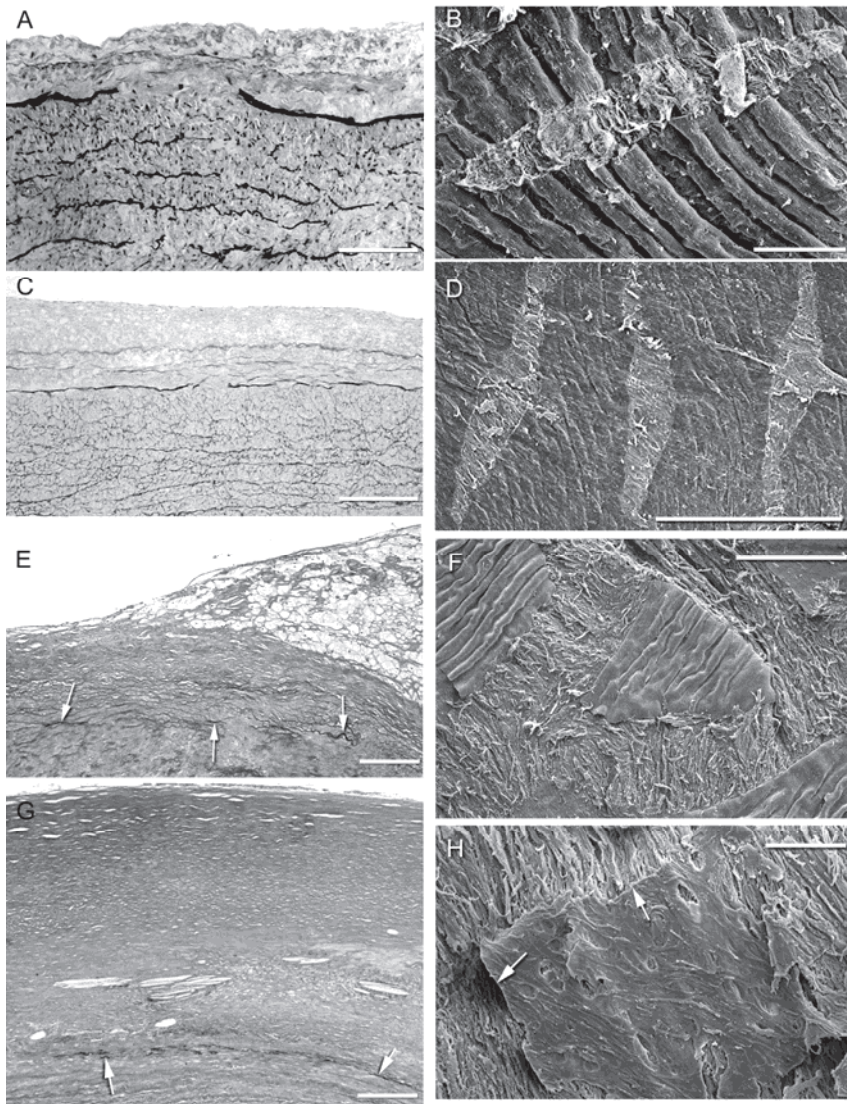


Fig. 6. Histological and en face appearance of abdominal aortic IEL defects. Abdominal aortic specimens from subjects aged (A&B) 9-years, (C&D) 35-years (E&F) 61-years with atherosclerosis, (G&H) 63-year old with abdominal aortic aneurysm. The primary lesions were transversely orientated (B&D), and become interconnected longitudinally in older subjects (F&H). Remnants of the IEL are indicated (E& G arrows). The asterisk in D indicates adherent intimal tissue not fully removed during sample preparation. Note the formation of elastic tissue, within the intimal fibroelastic layer, above the disrupted IEL in early intimal thickenings. In the AAA specimen the small IEL fragment has abrupt break edges (arrows). Scale bars (A&B) 100µm, (C, E-F) 200µm, (D) 500µm, (G) 400µm (H) 50µm.

localised nature of the disease. Haemodynamic stresses, including pulse reflection and summation, may predispose various anatomical sites to the development of vascular disease (Stehbens, 1995) as may local intrinsic wall abnormalities. Early atherosclerotic alterations appear to involve the development of intimal thickenings, which are partly derived from medial smooth muscle cells (Fig. 6). Normally the intact internal elastic lamina (Meyer et al., 1980; Ikari et al., 1999) restricts intimal medial cell migration. In latter stages of atherosclerosis the structural and molecular modification of elastic tissues is believed to reduce arterial compliance leading to increased pulse pressure and pressure wave velocities in aging individuals (Urry, 1975; Robert et al., 1995).

This raises the possibility that early-life structural and functional modification of the aortic wall may directly contribute to the progression of atherosclerosis (Stehbens, 1997) rather than simply being normal physiological remodeling. As shown in figure 6, it is somewhat remarkably that the elastic lamina defect edges remain so abrupt, even in elderly aneurysmal aortae, given the considerable elastolytic activity reported in atherosclerotic tissues, and in particularly AAA (Thompson & Parks, 1996). This is undoubtedly a testament to the stability and low turnover rate of elastin, which is estimated at approximately 70 years in humans (Shapiro et al., 1991).

Nevertheless, given the proteolytic activity that occurs within developing atherosclerotic lesions (Knox et al., 1997) and aneurysms (Vine & Powell, 1991; Gargiulo et al., 1993; McMillan et al., 1995; Thompson et al., 1995), the lack of change to the elastic lamina defect edges, apparently over many decades, is noteworthy in that it may provide a stable cumulative index of connective tissue degeneration, without the confounding effect of connective tissue remodelling (as is the case with the fibrillar collagens).

Though elastolytic activity undoubtedly occurs, the histo-morphological features indicate that the elastin within condensed laminae is remarkably resistant to enzymatic degradation. This is not to say that proteolysis is not a significant pathophysiological process in the loss of elastin, for example proteolytic activity may play an indirect role by degrading structural fibrillar collagens. The effect of this would be to shift mechanical stress from these high tensile strength connective tissue components to the relatively weaker elastic tissues, resulting in their accelerated structural fatigue.

2.3 Early atherosclerotic lesions

The earliest lesion generally considered to be atherosclerotic consists focal accumulations of intracellular lipoproteins in the intima and formation of fatty streaks (Fig. 5A). Fatty streaks may be present in the aorta from early childhood and may even start to develop during the foetal life, especially in foetuses of mothers with hypercholesterolaemia (Napoli et al., 1997). The major cellular constituents of fatty streaks are monocyte-derived macrophages recruited into wall via endothelial cell mediated diapedesis. Within the intima, these macrophages may engulf the blood-derived low density lipoprotein (LDL) and become lipid-filled foam cells. By the age of puberty (12-15 years), almost all children will develop lesions containing macrophages and foam cells in the intima of the aorta. This initial accumulation of lipoprotein and formation of fatty streaks does not protrude into the lumen and are thus asymptomatic (Stary, 2000).

The initial accumulation of lipids within the intima is then followed by progressive accumulation of both intra- and extra-cellular lipids. The origin of these lipoproteins can be classified as either blood-derived or that released from the death of resident macrophage/foam cells (Guyton, 2001). Intimal smooth muscle cells also contain lipid droplets (Stary, 2000), but, not having the oxidised LDL scavenger receptor, never develop

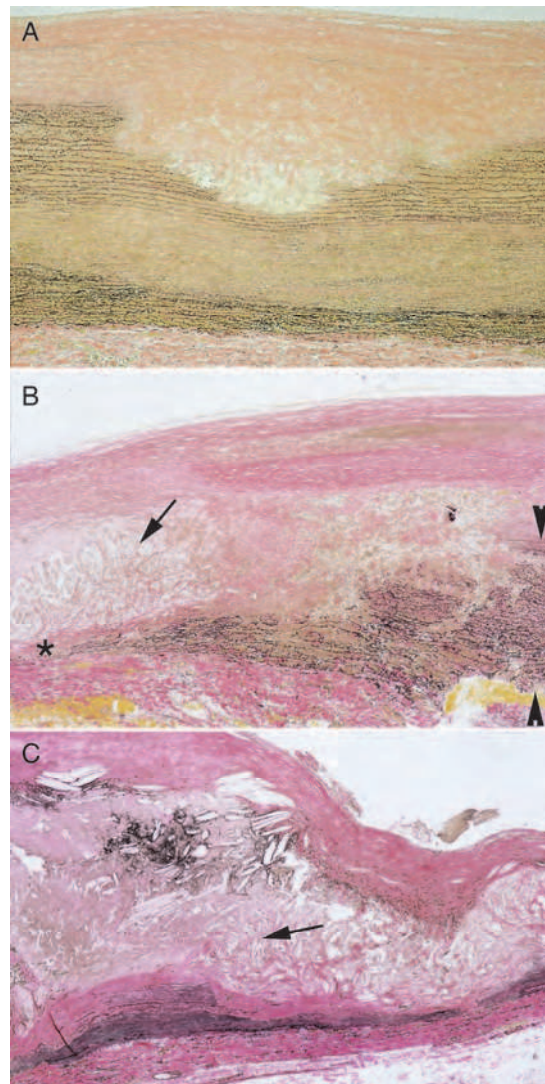


Fig. 7. Infra-renal aortic medial degeneration. A. Mild atherosclerosis (male 84 years). The atherosclerotic intimal lipid core has eroded the medial elastic laminae. (B) Proximal neck of an AAA from a 83-year old female. The region beneath the lipid core (*) has undergone almost complete medial degeneration, while the media on the right hand side still has numerous, at least partially intact, elastic laminae and layers of smooth muscle (between arrow heads). (C) Mid-sac region of an AAA, showing extensive, but heterogenous, medial atrophy (male 69-years). Note accumulation of cholesterol crystals (arrows) and extracellular calcification (black stained region overlying the lipid core in C) in the more advanced lesions. Verhoeff's elastic stain and van Gieson's counterstain (elastin black, collagen red), magnification A x13, B&C x10.

the foam cell appearance observed in tissue macrophages. The process of intimal lipid sequestration is pernicious and eventually results in the formation of a so-called lipid-core within the deep intima. As the size of this region increases it becomes filled with cholesterol ester crystals (Tangirala et al., 1994) and calcium deposits (Fig. 7). Beginning as regions of vesicular debris, termed matrix vesicles (Tanimura et al., 1983; Bobryshev et al., 2008), these small (approximately 20nm in diameter) calcium phosphate rich membrane bound vesicles appear to act as a nidus for subsequent vascular calcification (Fig. 7C). The lipid core is covered by a 'fibrous cap' composed of proteoglycan-rich extracellular matrix, smooth muscle and inflammatory cells (Stary, 2000).

As the disease progresses the atherosclerotic intima becomes more fibrotic, due to the production of extracellular matrix by smooth muscle cells (Davies & Hagen, 1994; Kaiura et al., 2000).

The increased intimal thickness results in decreased luminal diffusion to the deep intima and inner media, producing a hypoxic zone (Pugh & Ratcliffe, 2003). This is generally considered to occur when the intima reaches 500µm in thickness (Moreno et al., 2006).

2.4 Medial atrophy

There are two histopathological features associated with the formation of a hypoxic intimal-medial zone. Firstly the lipid-laden foam cell rich lipid core appears to erode the underlying media (Fig. 7). While, in children and young adults, the initial failure of the internal and medial elastic laminae appears to be driven by biomechanical fatigue, there is little doubt that a strong proteolytic component drives the medial erosion observed in more advanced plaques. The matrix metalloproteinases (MMP), in particular MMP-9 (Fig. 8), have been strongly implicated in the pathogenesis of both atherosclerosis and AAA progression (Thompson & Parks, 1996). It is this outward remodeling of the media, and eventually the adventitia, that preserves luminal patency during the early-mid phases of atherogenesis (Ge et al., 1993; Hermiller et al., 1993).

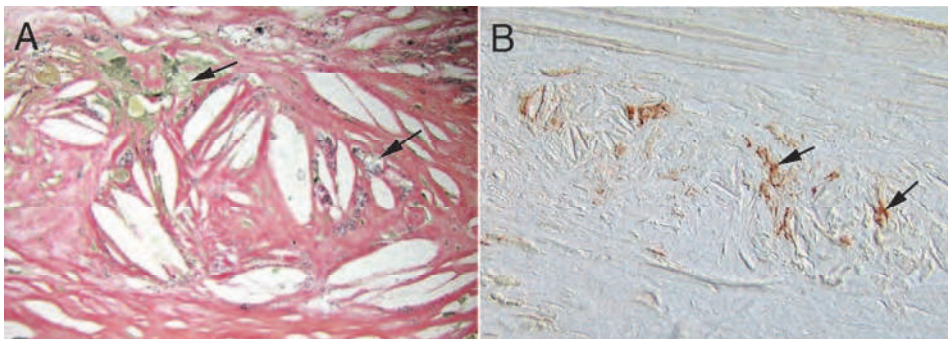


Fig. 8. Proteolytic activity within the developing aneurysm wall (female 70-years). (A) Macrophages (arrows) surrounded by cholesterol ester crystals clefts (Verhoeff's elastic tissue stain). (B) In a matching section, these same macrophages (arrows) are matrix metalloproteinase-9 positive (DAB (brown) reaction product, with DIC microscopy). Magnification A x132, B x66.

Secondly, as a result of the deep intima hypoxia, the adventitial vasa vasorum network begins to penetrate the media (Moreno et al., 2006). In addition, the process of elastic tissue degradation

produces small elastin-derived peptides, which have also been shown to stimulate angiogenesis (Nackman et al., 1997; Robinet et al., 2005). The neovascularisation process disrupts the lamellar structure of the media, with heavily vascularised regions of the media having significantly reduced elastic tissue and smooth muscle cell content (Fig. 9). Paralleling the process of medial neovascularisation, the adventitia typically undergoes progressive thickening due to the expansion and proliferation of the vasa vasorum network (Table 1).

Vessel density (channels/0.1mm ²)	Control n=11	AAA n=28	p-value
Media	0.1 (0-1.5)	1.6 (0.4-2.9)	<0.002
Adventitia	3.2 (1.3-5.2)	6.1 (2.8-9.3)	<0.003

Table 1. Vasa vasorum (von Willebrand factor positive) channel density in the media and adventitia of the infrarenal aortic wall. Data presented as median and interquartile range. Aneurysmal aortae had significantly increased medial and adventitial vasa vasorum densities compared with controls. Unlike wall cellularity measures (Fig 11), there was no difference in vessel density between different sized aneurysms. (Data courtesy of Dr Jun Li).

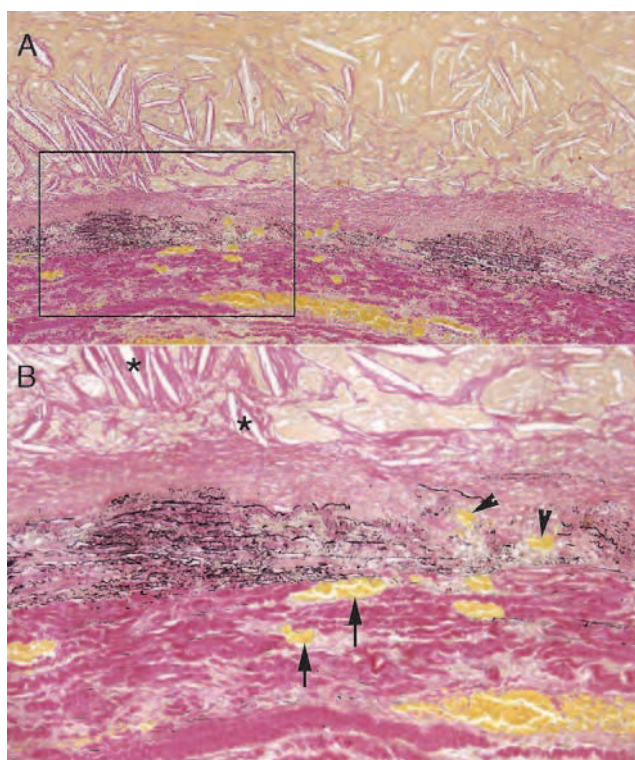


Fig. 9. Medial neovascularization. Histology of an abdominal aortic aneurysm (60-year male), showing a cholesterol crystal (*) rich intima with atrophy of the tunica media. Note the neovascularization of the media (arrowheads) originating from the adventitial vasa vasorum (arrows). Verhoeff's elastic tissue stain- van Gieson's counterstain (elastic tissue black, collagen red, Red blood cells orange). Original magnification A x13, B x66

The combined effects of intimal atherosclerosis and medial neovascularisation result in progressive medial atrophy (Crawford & Levene, 1953; Isner et al., 1986). This is associated with a loss of medial smooth muscle cells due to migration into the intima (Fig. 10) and increased cell death due to apoptosis (Lopez-Candales et al., 1997; Henderson et al., 1999; Boyle et al., 2001). This process can be demonstrated by quantifying the cellularity of the media within infrarenal aortae of varying diameters (Fig. 11). Large AAAs have approximately half the number of (smooth muscle) cells compared with normal sized aortae.

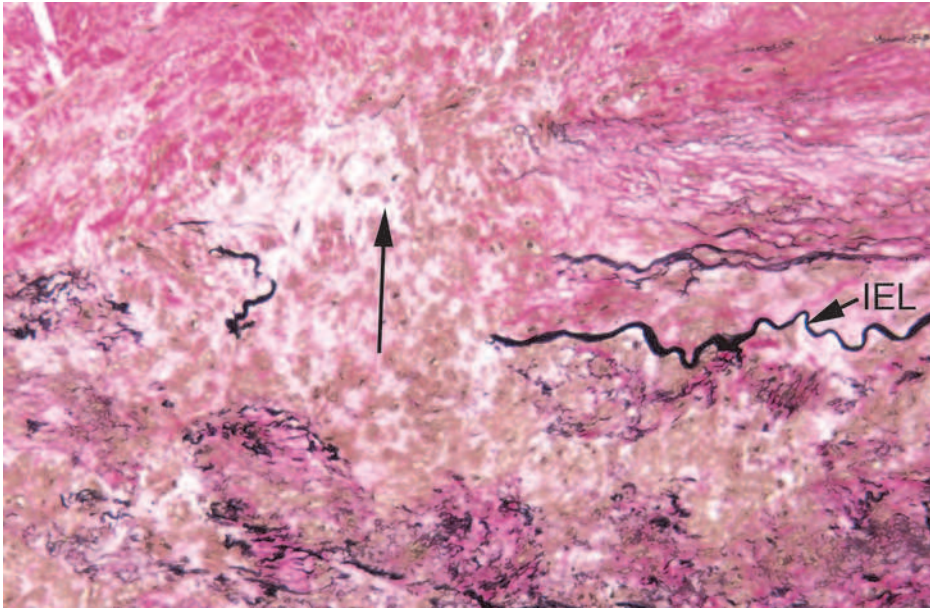


Fig. 10. Medial smooth muscle cell migration into the intima. Histology of the intimal-medial boundary in an 85-year old male. The closely packed medial smooth muscle cells (stained brown) are shown migrating (long arrow) into the intima through a disrupted internal elastic lamina (IEL). Within the intima they are loosely arranged and surrounded by abundant extracellular matrix (stained pink/red). Verhoeff's elastic tissue stain- van Gieson's counterstain. Original magnification x66.

2.5 Medial and peri-aortic inflammation

As described above, the formation of an atherosclerotic intima stimulates a neovascular response from the adventitia towards the intima. The presence of these new channels allows for direct recruitment of inflammatory cells, such as macrophages, plasma cells and T-lymphocytes, into the arterial wall. These cells secrete cytotoxic mediators, such as perforin, which induce smooth muscle apoptosis (Henderson et al., 1999; Lindeman et al., 2008) and further weakens the aortic wall. Moreover, these cells also release chemoattractants thereby stimulating further inflammatory recruitment and sustaining the inflammatory tissue reaction. In this way, medial and adventitial neovascularization establishes a chronic inflammatory state within the outer aortic wall (Figs 12 &13), resulting in continued matrix

degradation and remodelling, propagating outward expansion of the aorta and consequent aneurysm formation.

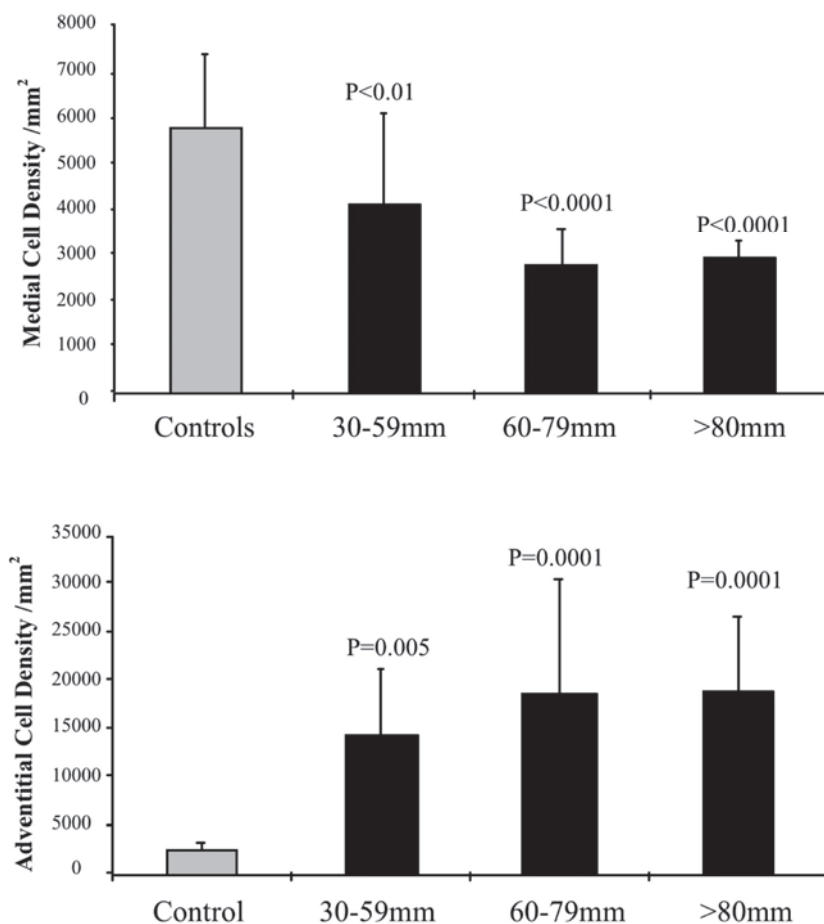


Fig. 11. Cell densities (number of nuclei) in different layers of the infrarenal aortic wall. Non-aneurysmal controls (n=11) were compared with AAAs of varying sizes (30-59mm n=7, 60-79mm n=12 and >80mm n=9). (Top) Within the degenerated media of aneurysms there is a progressive reduction in cell density to approximately half that of controls. (Bottom) In the adventitia there is a marked increase in cell density, vastly in the form of inflammatory cell infiltrate. (Data courtesy of Dr Jun Li, Vascular Research Group, Dunedin School of Medicine).

In a small but significant subset (3-10%) of AAA patients this process can become so extensive that the adventitia undergoes significant macroscopic thickening, developing peri-aortic (Fig 14) and retroperitoneal fibrosis and even adhesions of adjacent abdominal organs. These are typically referred to as inflammatory aneurysms (Tang et al., 2005), but it is generally accepted that, rather than representing a separate pathobiological entity, the

inflammatory AAA is an extreme manifestation of the same medial and adventitia inflammation which characterizes all abdominal aneurysms (Koch et al., 1990). While there is a spectrum of inflammatory cell infiltration, ranging from minimal (Fig. 9), moderate (Fig. 12) to extensive (Figs. 13 & 14), the adventitia cellularity tends to be increased in all aneurysms compared with non-aneurysm vessels (Fig 11). This is due a combination of both cells forming neovascular channels and variable degrees of associated inflammatory infiltrate.

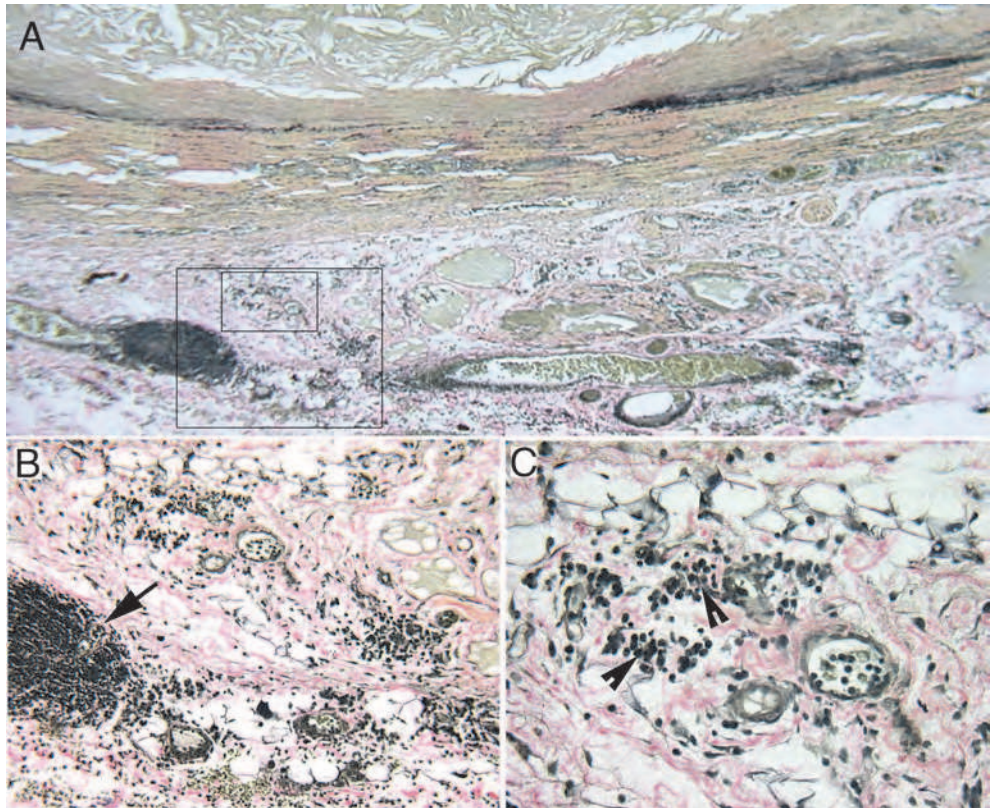


Fig. 12. Microscopic adventitial inflammation. A. Low magnification image of the aneurysm wall from an 82-year old female. (A) Magnified regions of the adventitia are indicated by boxes. Lymphocyte infiltrate forms either large aggregates surrounding blood vessels (arrow in B) or smaller diffuse collections within the extracellular matrix (arrowheads in C). Verhoeff's elastic tissue stain- van Gieson's counterstain. Magnification A x25, B x66, C x132

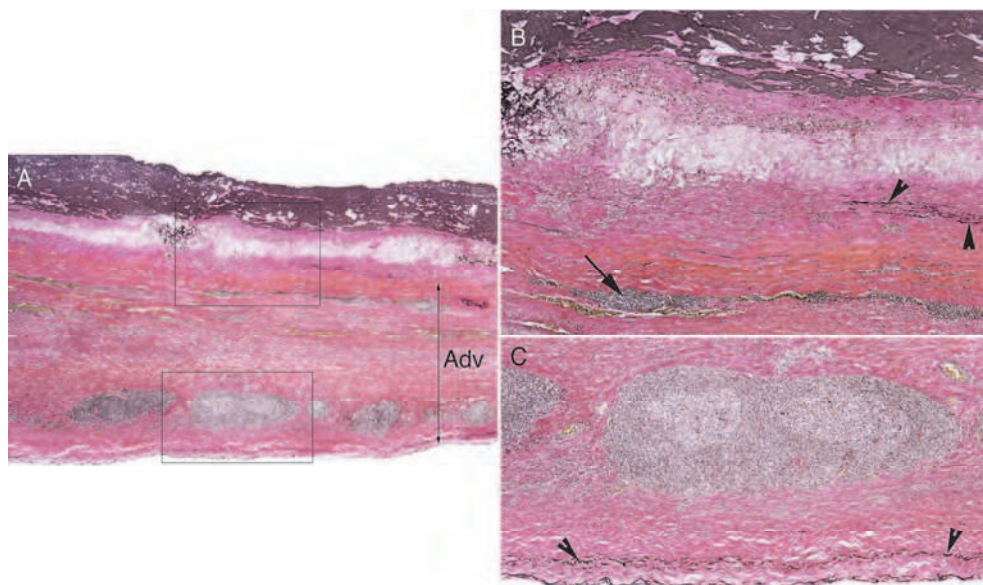


Fig. 13. Moderate adventitial inflammation. (A) Low magnification image of the AAA wall from an 77-year old male. (B & C) Higher magnification images of the boxed regions in A. The adventitia (Adv) is the thickest layer of the aneurysm wall with increased fibrosis and the formation of numerous lymphoid aggregates towards the outer adventitia (C). Regions of more diffuse inflammatory infiltrate are present within the inner adventitia and beneath the atherosclerotic core (arrows in B). Note the remnants of medial and adventitia elastic tissue (arrowheads in B and C respectively). Verhoeff's elastic tissue stain- van Gieson's counterstain. Magnification A x4, B x13, C x13

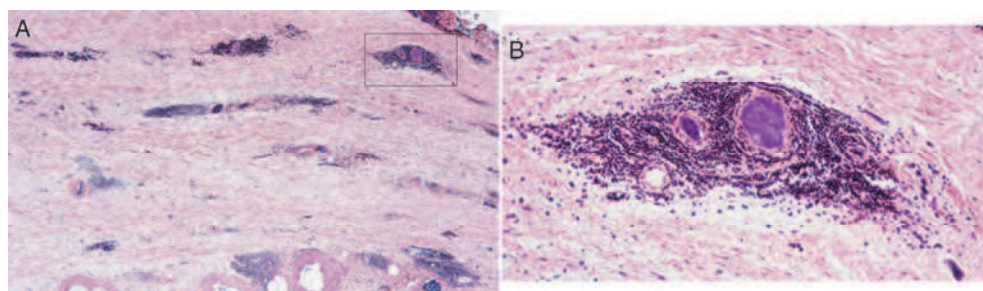


Fig. 14. Adventitia of an inflammatory AAA (female 77-years). (A) low magnification image of the thick adventitial layer. Part of the atrophic media is visible in the top right corner and large vasa vasorum are present towards the bottom. There is extensive fibrosis throughout the adventitia, largely consisting of fibrillar collagen bundles and lymphoid follicles. (B) higher magnification a small network of 3-4 vasa vasorum channels surrounded by a dense inflammatory infiltrate. PTAH Stain (nuclei blue, fibrin within the vasa vasorum blue, collagen red) Original magnification A x10, B x50.

2.6 Intraluminal thrombus

Blood flow studies suggest that the shape of an abdominal aortic aneurysm results in disordered flow, an increased wall tension and thrombus formation within the sac (Moore et al., 1992). Chronic intraluminal thrombus (ILT) forms as a multi-layered structure, consisting of a luminal layer of fresh red clot, a middle laminated thrombus and an actively fibrinolyzed abluminal layer (Fig 15), with variable incorporation into the aortic intima, including regions of intramural haemorrhage. The presence of ILT contributes to aneurysm progression in multiple ways (Michel et al., 2011). Firstly, when present, ILT significantly thickens the aortic wall (Fig. 16) and acts as a barrier for oxygen transport, resulting in further hypoxia-associated degeneration (Vorp et al., 1998), as described above. Secondly, ILT is a rich source of proteases and their activators, including MMPs and urokinase-type plasminogen activator (Houard et al., 2007).

The luminal zone is enriched with neutrophils, due to these cells strong affinity for the fibrin-fibronectin network. Neutrophil proteases are centrifugally propelled towards the abluminal zone and underlying aortic wall through a network of canaliculi (Adolph et al., 1997). The plasmin within the fibrinolytic abluminal zone results in MMP activation and facilitates the release of matrix sequestered growth factors such as transforming growth factor beta. An association between the presence of ILT and risk of AAA rupture (Fig 16B) has been suggested by many authors but the relationship remains controversial. Recent evidence suggests that a key factor may be the degree of ILT fissuring, leading to localised regions of increased wall stress (Polzer et al., 2011).

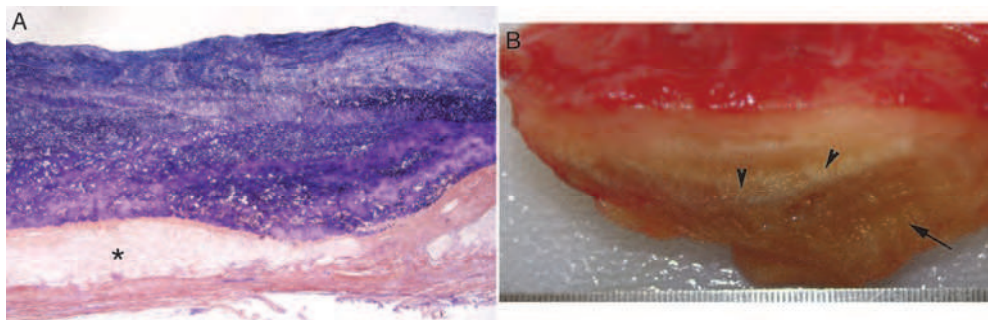


Fig. 15. Intraluminal thrombus. (A) Histology of a AAA (from an 71-year old male) stained with Phosphotungstic Acid Haematoxylin (PTAH). A thick layer of adherent thrombus (fibrin blue), is observed overlying an atheromatous intima (*), pronounced medial atrophy and adventitial collagen (red bands towards the bottom). Notice that the fibrinolytic abluminal zone stains a paler blue. Original magnification x4 (B) A gross thrombus specimen removed from the aneurysm sac of a 74-year old male. Notice the multiple layers consisting of fresh red thrombus on the luminal surface, an organized middle layer including lines of Zahn (arrowheads), and an abluminal fibrinolytic layer (arrow) adjacent to the aortic wall. Scale in millimeters.

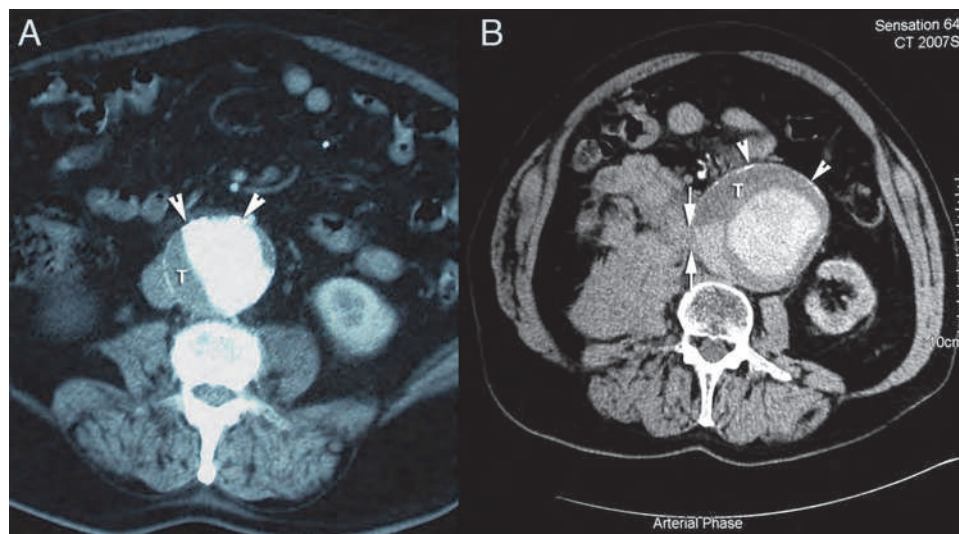


Fig. 16. Computed tomography of a (A) 6.5cm AAA in a 74-year old male and (B) ruptured 8.3cm AAA in a 79-year old male. The anterior aneurysm wall is calcified (arrowheads) in both cases. Notice the effect of adherent intraluminal thrombus (T) on total wall thickness. (B) the posterolateral (retroperitoneal) rupture site (arrows). This is a common site for AAA rupture.

3. Conclusion

The pathohistology of AAA involves the initial formation of intimal atherosclerosis followed by medial atrophy, neovascularisation, inflammatory cell infiltration and intraluminal thrombus formation. Elastic tissue fragility, both in terms of the initial intimal connective tissue degeneration and subsequent medial atrophy, along with the vascular inflammatory response to atherosclerosis appear to be key pathogenic features of AAA.

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5. References

Adams, C. W. M. & Tuqan, N. A. (1961). Elastic degeneration as source of lipids in the early lesion of atherosclerosis. *J Pathol Bacteriol* 82: 131-139.

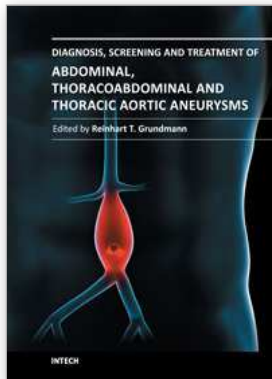
- Adolph, R., Vorp, D. A., Steed, D. L., Webster, M. W., Kameneva, M. V. & Watkins, S. C. (1997). Cellular content and permeability of intraluminal thrombus in abdominal aortic aneurysm. *J Vasc Surg* 25(5): 916-926.
- Bobryshev, Y. V., Killingsworth, M. C., Lord, R. S. & Grabs, A. J. (2008). Matrix vesicles in the fibrous cap of atherosclerotic plaque: possible contribution to plaque rupture. *J Cell Mol Med* 12(5B): 2073-2082.
- Boyle, J. J., Bowyer, D. E., Weissberg, P. L. & Bennett, M. R. (2001). Human blood-derived macrophages induce apoptosis in human plaque-derived vascular smooth muscle cells by Fas-ligand/Fas interactions. *Arterioscler Thromb Vasc Biol* 21(9): 1402-1407.
- Crawford, T. & Levene, C. I. (1953). Medial thinning in atheroma. *J Pathol Bacteriol* 66(1): 19-23.
- Davies, M. G. & Hagen, P.-O. (1994). Pathobiology of intimal hyperplasia. *Brit J Surg* 81: 1254-1269.
- Dobrin, P. B. (1989). Pathophysiology and pathogenesis of aortic aneurysms. Current concepts. *Surg Clin North Am* 69(4): 687-703.
- Gargiulo, M., Stella, A., Spina, M., Faggioli, G., Cenacchi, G., Degani, A., Guiducci, G., Tonelli, M., Bertoni, F. & D'Addato, M. (1993). Content and turnover of extracellular matrix protein in human "nonspecific" and inflammatory abdominal aortic aneurysms. *European Journal of Vascular Surgery* 7(5): 546-553.
- Ge, J., Erbel, R., Zamorano, J., Koch, L., Kearney, P., Gorge, G., Gerber, T. & Meyer, J. (1993). Coronary artery remodeling in atherosclerotic disease: an intravascular ultrasonic study in vivo. *Coron Artery Dis* 4(11): 981-986.
- Gillman, T., Penn, J., Bronks, D. & Roux, M. (1955). Abnormal elastic fibres. *Arch Pathol* 59: 733-749.
- Grundy, S. M. (1995). Role of low-density lipoproteins in atherogenesis and development of coronary heart disease. *Clin Chem* 41: 139-146.
- Guyton, J. R. (2001). Phospholipid hydrolytic enzymes in a 'cesspool' of arterial intimal lipoproteins: a mechanism for atherogenic lipid accumulation. *Arterioscler Thromb Vasc Biol* 21(6): 884-886.
- He, C. M. & Roach, M. R. (1994). The composition and mechanical properties of abdominal aortic aneurysms. *J Vasc Surg* 20(1): 6-13.
- Heistad, D. D. & Marcus, M. L. (1979). Role of vasa vasorum in nourishment of the aorta. *Blood Vessels* 16(5): 225-238.
- Henderson, E. L., Geng, Y. J., Sukhova, G. K., Whittemore, A. D., Knox, J. & Libby, P. (1999). Death of smooth muscle cells and expression of mediators of apoptosis by T lymphocytes in human abdominal aortic aneurysms. *Circulation* 99(1): 96-104.
- Hermiller, J. B., Tenaglia, A. N., Kisslo, K. B., Phillips, H. R., Bashore, T. M., Stack, R. S. & Davidson, C. J. (1993). In vivo validation of compensatory enlargement of atherosclerotic coronary arteries. *Am J Cardiol* 71(8): 665-668.
- Holmes, D. R., Liao, S., Parks, W. C. & Thompson, R. W. (1995). Medial neovascularization in abdominal aortic aneurysms: a histopathologic marker of aneurysmal degeneration with pathophysiologic implications. *J Vasc Surg* 21(5): 761-771; discussion 771-762.
- Houard, X., Rouzet, F., Touat, Z., Philippe, M., Dominguez, M., Fontaine, V., Sarda-Mantel, L., Meulemans, A., Le Guludec, D., Meilhac, O. & Michel, J. B. (2007). Topology of

- the fibrinolytic system within the mural thrombus of human abdominal aortic aneurysms. *J Pathol* 212(1): 20-28.
- Ikari, Y., McManus, B. M., Kenyon, J. & Schwartz, S. M. (1999). Neonatal intima formation in the human coronary artery. *Arterioscler Thromb Vasc Biol* 19(9): 2036-2040.
- Isner, J. M., Donaldson, R. F., Fortin, A. H., Tischler, A. & Clarke, R. H. (1986). Attenuation of the media of coronary arteries in advanced atherosclerosis. *Am J Cardiol* 58(10): 937-939.
- Jones, G. T., Jiang, F., McCormick, S. P. & Dusting, G. J. (2005). Elastic lamina defects are an early feature of aortic lesions in the apolipoprotein E knockout mouse. *J Vasc Res* 42(3): 237-246.
- Jones, G. T. & Stehbens, W. E. (1995). The ultrastructure of arteries proximal to chronic experimental carotid-jugular fistulae in rabbits. *Pathology* 27(1): 36-42.
- Jousilahti, P., Vartiainen, E., Tuomilehto, J. & Puska, P. (1999). Sex, age, cardiovascular risk factors, and coronary heart disease. A prospective follow-up study of 14786 middle-aged men and women in Finland. *Circulation* 99: 1165-1172.
- Kaiura, T. L., Itoh, H., Kubaska, S. M., 3rd, McCaffrey, T. A., Liu, B. & Kent, K. C. (2000). The effect of growth factors, cytokines, and extracellular matrix proteins on fibronectin production in human vascular smooth muscle cells. *J Vasc Surg* 31(3): 577-584.
- Karrer, H. E. (1961). An electron microscope study of the aorta in young and in aging mice. *J Ultrastruct Res* 5: 1-27.
- Keech, M. K. (1960). Electron microscope study of the normal rat aorta. *J Biophys Biochem Cytol* 7: 533-538.
- Knox, J. B., Sukhova, G. K., Whittemore, A. D. & Libby, P. (1997). Evidence for altered balance between matrix metalloproteinases and their inhibitors in human aortic diseases. *Circulation* 95(1): 205-212.
- Koch, A. E., Haines, G. K., Rizzo, R. J., Radosevich, J. A., Pope, R. M., Robinson, P. G. & Pearce, W. H. (1990). Human abdominal aortic aneurysms. Immunophenotypic analysis suggesting an immune-mediated response. *Am J Pathol* 137(5): 1199-1213.
- Kojimahara, M. (1988). Intimal laminated elastosis in the intrarenal arteries. An electron microscopic study. *Acta Pathol Jpn* 38(3): 315-323.
- Krettek, A., Sukhova, G. K. & Libby, P. (2003). Elastogenesis in human arterial disease: a role for macrophages in disordered elastin synthesis. *Arterioscler Thromb Vasc Biol* 23(4): 582-587.
- Lederle, F. A., Johnson, G. R., Wilson, S. E., Chute, E. P., Hye, R. J., Makaroun, M. S., Barone, G. W., Bandyk, D., Moneta, G. L. & Makhoul, R. G. (2000). The aneurysm detection and management study screening program: validation cohort and final results. Aneurysm Detection and Management Veterans Affairs Cooperative Study Investigators. *Arch Intern Med* 160(10): 1425-1430.
- Levene, C. I. (1956). The early lesions of atheroma in the coronary arteries. *J Pathol Bacteriol* 72: 79-83.
- Lindeman, J. H., Abdul-Hussien, H., Schaapherder, A. F., Van Bockel, J. H., Von der Thusen, J. H., Roelen, D. L. & Kleemann, R. (2008). Enhanced expression and activation of pro-inflammatory transcription factors distinguish aneurysmal from atherosclerotic aorta: IL-6- and IL-8-dominated inflammatory responses prevail in the human aneurysm. *Clin Sci (Lond)* 114(11): 687-697.

- Lopez-Candales, A., Holmes, D. R., Liao, S., Scott, M. J., Wickline, S. A. & Thompson, R. W. (1997). Decreased vascular smooth muscle cell density in medial degeneration of human abdominal aortic aneurysms. *Am J Pathol* 150(3): 993-1007.
- Lusis, A. J. (2000). Atherosclerosis. *Nature* 407(6801): 233-241.
- McMillan, W. D., Patterson, B. K., Keen, R. R., Shively, V. P., Cipollone, M. & Pearce, W. H. (1995). In situ localization and quantification of mRNA for 92-kD type IV collagenase and its inhibitor in aneurysmal, occlusive, and normal aorta. *Arteriosclerosis, Thrombosis & Vascular Biology* 15(8): 1139-1144.
- Meyer, W. W. & Lind, J. (1972). Calcifications of iliac arteries in newborns and infants. *Arch Dis Child* 47(253): 364-372.
- Meyer, W. W., Walsh, S. Z. & Lind, J. (1980). *Functional morphology of human arteries during fetal and post-natal development. Structure and function of the circulation*. C. J. Schwartz, N. T. Werthessen and S. Wolf. New York, Plenum Press. 1: 95-380.
- Michel, J. B., Martin-Ventura, J. L., Egido, J., Sakalihasan, N., Treska, V., Lindholt, J., Allaire, E., Thorsteinsdottir, U., Cockerill, G. & Swedenborg, J. (2011). Novel aspects of the pathogenesis of aneurysms of the abdominal aorta in humans. *Cardiovasc Res* 90(1): 18-27.
- Miller, E. J., Malcom, G. T., McMahan, C. A. & Strong, J. P. (1993). Atherosclerosis in young white males: arterial collagen and cholesterol. *Matrix* 13: 289-296.
- Moon, H. D. (1957). Coronary arteries in fetuses, infants, and juveniles. *Circulation* 16(2): 263-267.
- Moore, J. E., Jr., Ku, D. N., Zarins, C. K. & Glagov, S. (1992). Pulsatile flow visualization in the abdominal aorta under differing physiologic conditions: implications for increased susceptibility to atherosclerosis. *J Biomech Eng* 114(3): 391-397.
- Moreno, P. R., Purushothaman, K. R., Sirol, M., Levy, A. P. & Fuster, V. (2006). Neovascularization in human atherosclerosis. *Circulation* 113(18): 2245-2252.
- Nackman, G. B., Karkowski, F. J., Halpern, V. J., Gaetz, H. P. & Tilson, M. D. (1997). Elastin degradation products induce adventitial angiogenesis in the Anidjar/Dobrin rat aneurysm model. *Surgery* 122(1): 39-44.
- Napoli, C., D'Armiento, F. P., Mancini, F. P., Postiglione, A., Witztum, J. L., Palumbo, G. & Palinski, W. (1997). Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. *J Clin Invest* 100(11): 2680-2690.
- Ogata, T., MacKean, G. L., Cole, C. W., Arthur, C., Andreou, P., Tromp, G. & Kuivaniemi, H. (2005). The lifetime prevalence of abdominal aortic aneurysms among siblings of aneurysm patients is eightfold higher than among siblings of spouses: an analysis of 187 aneurysm families in Nova Scotia, Canada. *J Vasc Surg* 42(5): 891-897.
- Polzer, S., Gasser, T. C., Swedenborg, J. & Bursa, J. (2011). The Impact of Intraluminal Thrombus Failure on the Mechanical Stress in the Wall of Abdominal Aortic Aneurysms. *Eur J Vasc Endovasc Surg*.
- Pugh, C. W. & Ratcliffe, P. J. (2003). Regulation of angiogenesis by hypoxia: role of the HIF system. *Nat Med* 9(6): 677-684.
- Robert, L., Jacob, M. P. & Fulop, T. (1995). Elastin in blood vessels. *Ciba Foundation Symposium*(192): 286-299.

- Robinet, A., Fahem, A., Cauchard, J. H., Huet, E., Vincent, L., Lorimier, S., Antonicelli, F., Soria, C., Crepin, M., Hornebeck, W. & Bellon, G. (2005). Elastin-derived peptides enhance angiogenesis by promoting endothelial cell migration and tubulogenesis through upregulation of MT1-MMP. *J Cell Sci* 118(Pt 2): 343-356.
- Ross, R. (1993). The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 362(6423): 801-809.
- Sakalihasan, N., Limet, R. & Defawe, O. D. (2005). Abdominal aortic aneurysm. *Lancet* 365(9470): 1577-1589.
- Sandiford, P., Mosquera, D. & Bramley, D. (2011). Trends in incidence and mortality from abdominal aortic aneurysm in New Zealand. *Br J Surg*.
- Schaefer, E. J., Lichenstein, A. H., Lamon-Fava, S., McNamara, J. R. & Ordovas, J. M. (1995). Lipoproteins, nutrition, aging and atherosclerosis. *Am J Clin Nutr* 61(Suppl): 726S-740S.
- Schwartz, S. M. (1997). Smooth muscle migration in atherosclerosis and restenosis. *J Clin Invest* 100(11 Suppl): S87-89.
- Scott, R. A., Wilson, N. M., Ashton, H. A. & Kay, D. N. (1995). Influence of screening on the incidence of ruptured abdominal aortic aneurysm: 5-year results of a randomized controlled study. *Br J Surg* 82(8): 1066-1070.
- Shantikumar, S., Ajjan, R., Porter, K. E. & Scott, D. J. (2010). Diabetes and the abdominal aortic aneurysm. *Eur J Vasc Endovasc Surg* 39(2): 200-207.
- Shapiro, S. D., Endicott, S. K., Province, M. A., Pierce, J. A. & Campbell, E. J. (1991). Marked longevity of human lung parenchymal elastic fibers deduced from prevalence of D-aspartate and nuclear weapons-related radiocarbon. *J Clin Invest* 87(5): 1828-1834.
- Sims, F. H. (1985). Discontinuities in the internal elastic lamina: a comparison of coronary and internal mammary arteries. *Artery* 13(3): 127-143.
- Singh, K., Bonna, K. H., Jacobsen, B. K., Bjork, L. & Solberg, S. (2001). Prevalence of and risk factors for abdominal aortic aneurysms in a population-based study : The Tromso Study. *Am J Epidemiol* 154(3): 236-244.
- Stary, H. C. (2000). Lipid and macrophage accumulations in arteries of children and the development of atherosclerosis. *Am J Clin Nutr* 72(5 Suppl): 1297S-1306S.
- Stehbens, W. E. (1995). *Atherosclerosis and degenerative diseases of blood vessels. Vascular Pathology*. W. E. Stehbens and J. T. Lie. London, Chapman & Hall: 175-270.
- Stehbens, W. E. (1997). Mechanisms underlying arterial fragility and the complications of atherosclerosis. *Pathobiology* 65(1): 1-13.
- Strong, J. P. & Group, P. R. (1995). Natural history and risk factors of early human atherogenesis. *Clin Chem* 41: 134-138.
- Tang, T., Boyle, J. R., Dixon, A. K. & Varty, K. (2005). Inflammatory abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg* 29(4): 353-362.
- Tangirala, R. K., Jerome, W. G., Jones, N. L., Small, D. M., Johnson, W. J., Glick, J. M., Mahlberg, F. H. & Rothblat, G. H. (1994). Formation of cholesterol monohydrate crystals in macrophage-derived foam cells. *J Lipid Res* 35(1): 93-104.
- Tanimura, A., McGregor, D. H. & Anderson, H. C. (1983). Matrix vesicles in atherosclerotic calcification. *Proc Soc Exp Biol Med* 172(2): 173-177.
- Thompson, R. W., Holmes, D. R., Mertens, R. A., Liao, S., Botney, M. D., Mecham, R. P., Welgus, H. G. & Parks, W. C. (1995). Production and localisation of 92-kilodalton gelatinase in abdominal aortic aneurysms. *J Clin Invest* 96: 318-326.

- Thompson, R. W. & Parks, W. C. (1996). Role of matrix metalloproteinases in abdominal aortic aneurysms. *Annals of the New York Academy of Sciences* 800: 157-174.
- Urry, D. W. (1975). Molecular aspects of the elastic fiber as a site of vascular pathology. *Alabama Journal of Medical Sciences* 12(4): 361-368.
- Verloes, A., Sakalihan, N., Koulischer, L. & Limet, R. (1995). Aneurysms of the abdominal aorta: familial and genetic aspects in three hundred thirteen pedigrees. *J Vasc Surg* 21(4): 646-655.
- Vine, N. & Powell, J. T. (1991). Metalloproteinases in degenerative aortic disease. *Clinical Science* 81: 233-239.
- Vorp, D. A., Wang, D. H., Webster, M. W. & Federspiel, W. J. (1998). Effect of intraluminal thrombus thickness and bulge diameter on the oxygen diffusion in abdominal aortic aneurysm. *J Biomech Eng* 120(5): 579-583.
- Williams, K. J. & Tabas, I. (1995). The response-to-retention hypothesis of early atherogenesis. *Arterioscler Thromb Vasc Biol* 15(5): 551-561.
- Willis, A. I., Pierre-Paul, D., Sumpio, B. E. & Gahtan, V. (2004). Vascular smooth muscle cell migration: current research and clinical implications. *Vasc Endovascular Surg* 38(1): 11-23.
- Wolinsky, H. (1970). Comparison of medial growth of human thoracic and abdominal aortas. *Circulation Research* 27(4): 531-538.
- Wolinsky, H. & Glagov, S. (1964). Structural Basis for the Static Mechanical Properties of the Aortic Media. *Circulation Research* 14: 400-413.
- Wolinsky, H. & Glagov, S. (1969). Comparison of abdominal and thoracic aortic medial structure in mammals. Deviation of man from the usual pattern. *Circulation Research* 25(6): 677-686.



Diagnosis, Screening and Treatment of Abdominal, Thoracoabdominal and Thoracic Aortic Aneurysms

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This book considers mainly diagnosis, screening, surveillance and treatment of abdominal, thoracoabdominal and thoracic aortic aneurysms. It addresses vascular and cardiothoracic surgeons and interventional radiologists, but also anyone engaged in vascular medicine. The high mortality of ruptured aneurysms certainly favors the recommendation of prophylactic repair of asymptomatic aortic aneurysms (AA) and therewith a generous screening. However, the comorbidities of these patients and their age have to be kept in mind if the efficacy and cost effectiveness of screening and prophylactic surgery should not be overestimated. The treatment recommendations which will be outlined here, have to regard on the one hand the natural course of the disease, the risk of rupture, and the life expectancy of the patient, and on the other hand the morbidity and mortality of the prophylactic surgical intervention. The book describes perioperative mortality after endovascular and open repair of AA, long-term outcome after repair, and the cost-effectiveness of treatment.

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