ABDOMINAL AORTIC ANEURYSMS

LUIGI PASCARELLA, M.D.

VASCULAR SURGERY CONFERENCE

FRIDAY FEB., 19 2010
ABDOMINAL AORTIC ANEURYSMS

• ETIMOLOGY
• DEFINITION
• EPIDEMIOLOGY
• RISK FACTORS
• ETIOPATHOGENESIS
• GENETICS
• CANDIDATE GENES
• ATHEROSCLEROSIS AND INFLAMMATION
• OXYDATIVE STRESS
• ANIMAL MODELS
• POTENTIAL PHARMOCOLOGIC TARGETS
Abdominal Aortic Aneurysm

ETYMOLOGY

- Abdomen: Unknown; Maybe from the Latin “Abdere” that means to hide; Others believe from Adeps, Adipis: Fat

- Aorta: Greek Origin ➔ Αορτη ➔ What is hung up, What is carried. Term first used by Aristotle to describe “the great artery from which the heart hungs”.

- Aneurysm: Greek Origin ➔ Ανευρισμα ➔ (Ανα: across; ευρις: broad) Dilation.
Definition

Abdominal aortic aneurysm: dilation of the abdominal aorta.

No clear general agreement on how to define an abdominal aortic aneurysm (AAA) in the scientific community.
How to define an AAA?

• In 1965 Steinberg and Stein established normal diameters for AAA: a diameter in excess of 30 mm well above the average (+2SD) for both genders (Steinberg CR at al., Am. J. Roentgenol. 1965).

• In 1975 McGregor defined an AAA as the maximum infrarenal artic diameter being 30 mm or more (McGregor JC et al. Scott. Med. J. 1975).

• Several authors have reported that the actual aortic diameter depends on age, gender and body size
How to define an AAA?

Age and Body Size

• Age: In the ADAM study in which 120,000 subjects aged 50-79 years were screened for AAA, the age was associated with aortic diameter but the effect was small with a 0.1 cm difference in diameter between the youngest and the oldest patients. (Lederle FA et al. Arch. Intern Med. 2000)

“Considering the small influence of age and that most of the patients with clinically relevant AAA are >60 years, it is questionable whether an AAA definition needs to be corrected for age”


Several reports noticed very little variability in aortic diameter by variation in body size. (Wanhainen et al. JVS, 2008)
How to define an AAA? Gender

• Gender difference in aortic diameter was reported to be 0.3 cm in 70 years old subjects (Sonesson B et al. EJVS, 1994).

• In the ADAM study female gender was associated with a 0.14 cm reduction in aortic diameter (Lederle FA et al. Arch. Intern Med. 2000).
Definition

- ISCVS/SVS Ad Hoc Committee defined an AAA as the maximum infrarenal aortic diameter at least 1.5 times larger than the expected normal infrarenal aortic diameter (Johnston et al., J.V.S. 1991).
Epidemiology

• AAA causes 1 % of all deaths in developed countries and 2% of all deaths in elderly men.
• M:F 9:1
• Prevalence : 6-10%
• Estimated prevalence in men is between 1.3 and 8.9%; in women is 1-2.2 %.
• Prevalence increases with age.
• Prevalence in men increases by 6% per decade.

Epidemiology

• Annual Incidence 40.6 to 49.3/100000 men and 6.8 to 12 /100000 women
• Clinical relevant aneurysms (at least 4 cm in diameter) are found in about 1% of men 55 to 64 years of age and the prevalence increases by 2 to 4% thereafter.
• AAA are more frequently diagnosed in male patients over 55 years of age.
• Rupture seldom occurs before 65 years of age.
• In women AAA is diagnosed about 10 years later than in men.
Risk Factors

- Smoking
- Hypertension
- COPD
- Family History of aneurysms
- Family history of atherosclerotic disease
- Male Gender
- Age
- Hylipidemia
- Peripheral obstructive vascular disease
- Hereditary connective tissue disorders: Marfan’s and Ehlers-Danlos
Smoking and AAA

• Tobacco Smoking is the strongest independent risk factor.
• 90 % of patients with AAA have previous or current history of tobacco abuse. (Vardulaki KA, Br. J. Surg. 2000)
• Incidence of AAA in individuals that have smoked for more than 40 years is increased by factor 6 and by factor 7 among those who have smoked more that 20 cigarettes per day. (Wilmink TBM at al., J.V.S. 1999)
Risk factors for rupture

• Aneurysm size and expansion rate
• Cardiovascular risk factors: Smoking, Hypertension, Family history of AAA
• Presence of Blood within the thrombus
• Recent onset of abdominal pain
• Female Gender
• Inflammatory aneurysm
• Possible serum markers predicting rupture:
  High levels of MMP9
  Reduced serum level of α1-antitrypsin

Risk Factors for rupture

- UK Small Aneurysm Trial:
  Aneurysms<4 cm: 0.3% annual risk of rupture.
  Aneurysms 4.0-4.9 cm: 1.5% annual risk of rupture.
  Aneurysm 5.0-5.9 cm: 6.5% annual risk of rupture.
- Rapid rate of expansion in AAA > 5.0 cm
- Smoking increases growth rate by 5 to 20% (increasing the degradation of aortic tissue)
- ABI and Aneurysm expansion seem to be inversely associated (Reduction by 0.2 mm/year for each fall of 0.2 in the ABI) (Brady et al. Circulation 2004).
- Diabetes and Aneurysm expansion have been shown to be inversely related (Growth Reduction of over 30% compared with non diabetic). (Brady et al. Circulation 2004).
- MMP9: mRNA for MMP9 was increased in larger (5.0 to 6.9 cm) AAA (Longo GM et al. J. Clin. Invest., 2002).
### Summary of AAA Screening Guidelines

<table>
<thead>
<tr>
<th>Group</th>
<th>Year</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>USPSTF</td>
<td>2005</td>
<td>Recommends for screening in men aged 65–75 years who have ever smoked No recommendation in men aged 65–75 years who have never smoked</td>
<td>Recommends against screening in all women</td>
</tr>
<tr>
<td>ACC/AHA</td>
<td>2006</td>
<td>Recommends for screening in men aged ≥65 years who have ever smoked Recommends for screening in men aged ≥60 with family history of AAA</td>
<td>No recommendation</td>
</tr>
<tr>
<td>SVMB/SVS/AAVS</td>
<td>2004</td>
<td>Recommends for screening in all men aged 60–85 years</td>
<td>Recommends for screening in women aged 60–85 years with a family history of AAA</td>
</tr>
</tbody>
</table>

Screening

• Physical examination: In a meta-analysis of 15 studies of abdominal palpation for detection of AAA the sensitivity rates ranged from 29% to 76%, with increasing accuracy for larger aneurysms and a positive predictive value near 43%. Although abdominal palpation may detect AAA, however cannot rule it out with certainty.

• US: Simple, safe. It has a sensitivity of 95% and a specificity of 100% to detect AAA greater than 3 cm.

Ethiopathogenesis

• Formation of a AAA is a complex and multifactorial process
• Synergy of Environmental and Genetic Factors primes the onset of atherosclerosis and an inflammatory reaction which contribute to the formation of the aneurysm.
Biomechanical factors in Aneurysm formation and rupture

AAA formation is accompanied by an increase in wall stress and decrease in tensile strength.

The relationship between Aneurysm size and risk of rupture is referred to the biophysical principle expressed by the law of Laplace where the wall stress is proportional to the aortic diameter and the blood pressure.

\[ \sigma = \frac{P \times r}{t} \]

Choke at al., EJVS 2005
Wall Stress

Finite element analysis is a recognized discipline in engineering sciences. It is a numerical technique for finding approximate solutions of partial differential and integral equations.

In vascular biology it has been used to predict stress distribution in hypothetical aneurysm model with great accuracy.

FEA analysis of an asymmetric and fusiform AAA model by Elger et al. found that the maximum stress was a function of the shape, geometry rather than the diameter.

Choke at al., EJVS 2005
Mower et al., using FEA analysis, found that the stress was the greatest on the inner surface of the vascular wall.

3D computer tomography reconstruction of abdominal wall geometry showed that wall stress is complexly distributed.

Peak wall stress seemed to be localized to the posterior surface where the majority of rupture occurs.
Genetic background in AAA

- First degree relatives with AAA have odds ratio of 9.7 (95% CI 4-23).
- Odds ratio for siblings of AAA patients is 4.1 (95% CI 1.5-11.2) and for brothers 4.2 (95% CI 1.4-12.8).
- Using infrarenal aortic diameter based on ultrasound measurements, the siblings of AAA patients have an odds ratio between 2.6 (95% 1.0 and 7.1) and 3.3 (95% CI 1.8-37.5).

Because these studies are Cross-Sectional, it is uncertain whether any other relative will develop an aneurysm in the future.

C.J. Van-Vlijmen-van Keulen at al. EJVS 2002
Genetic background-Inheritance

• Hereditary pattern of inheritance of 50 families. Only one gene responsible for inheritance. This is autosomal dominant with a strong bias toward male to female ratio (8:1) (Tilson MD et al., Am. J. Surg., 1984).

• First degree relatives on 91 probands. Thirteen families had at least one affected first degree relative. In these families the most likely genetic model was a recessive gene at an autosomal diallelic major locus. (Majumder PP et al., Am. J. Hum. Genet., 1991)

• van Vlijmen–van Keulen C.J. in several studies reported inheritance related to an autosomal dominant pattern with reduced/variable penetrance (C.J. Van-Vlijmen-van Keulen at al. EJVS 2002).
Candidate Genes

• **Collagen I** mutations are associated with Osteogenesis Imperfecta and rare forms of Ehlers Danlos.

• **Collagen III** mutations may sporadically cause AAA. In patients with family history of AAA lower amounts of collagen III in the aortic media were found in 6-18% of patients. Type III procollagen mutations and aminoacids substitutions were found to be responsible for familial AAA (Gly619Arg, Gly136Arg, and Leu002Phe). These mutations influence the triple helical domain of type III procollagen and make the protein unstable.

  - An analysis of 50 patients indicated a causal relation with mutation of the type III procollagen gene only in 2% of aortic aneurysms.

  - Immunohistochemical staining with antibodies for the aminoterminal propetide of collagen III (PIIINP) that represent new collegen synthesis was mainly present in the media of AAA. Increased type III in AAA may result in impaired fibril formation causing aortic dilation.

  - Mutation of COL3A1 gene, associated to the EDS IV, is present in small subset of family with AAA.

C.J. Van-Vlijmen-van Keulen at al. EJVS 2002
Candidate Genes

• No clear evidence that Elastin gene mutation may be implicated in the development of AAA. In the Williams-Beuren syndrome (elfin facies) is associated with mutations of the elastin gene and with supravalvular aortic stenosis and not with AAA. However a transgenic mouse with a deletion 19-31 exon in the tropoelastin gene, similar to the mutation of the Williams-Beuren syndrome, developed aortic rupture at 7 months of age.

• Mutations of the Fibrillin 1 gene are responsible for the Marfan’s syndrome. The mutation is an aminoacid substitution in the Gly1127Ser Gene that cause weakening of the elastic tissue. It has been suggested that variation of the fibrillin 1 gene modulates the elastic properties of ageing aortas, influencing the arterial pulse pressure. The current hypothesis is that a combination of hypertension and variation of the fibrillin 1 gene may be influence the development of aneurysms.
Candidate Genes

- 5A allele of **MMP3** was found to be a risk factor for AAA among Finnish patients. Although **TIMP 1** activity has been found decreased in AAA, however no mutation of TIMP1 and TIMP 2 genes have been found in aneurysm patients.

- 4 G homozygous variant Allele **Inhibitor of the activator of the Plasmin** (PAI-1) was found in patients with familial AAA.

- Common polymorphism of **Apo E** may be related to aneurysm expansion. Apo-E and u-PA deficient mice seem to be protected for aneurysm formation, probably because less plasmin is activate to clive the proMMPs.

- **Cathepsin D,H and L**, involved in the turnover of structural proteins, have higher activities in the aneurysmal wall and in the aneurysmal thrombus.

C.J. Van-Vlijmen-van Keulen et al. EJVS 2002
Candidate Genes

• **Cystatin C**, extracellular inhibitor of the Cystein proteases, deficiency is associated with increased aneurysm size and expansion rate.

• **Circulating cytokins** (TNFα, IL1-β and IL6) have been suggested to influence integrity of the aortic wall, priming in increased activity, aortic wall dilation. The 174 G/Cp polymorphism of the IL6 gene may predict cardiovascular mortality in patients with small AAA.

• **HLA alleles** have been suggested as possible risk factors for AAA development. The Antigen **HLA-DQ3** appear to protect from AAA formation while **HLA-DQ15** promote the disease.

Several other genes have been studied (Haptoglobin, Cholesterol ester transfer protein, Fibulin 2, α1-antitrypsin, Proteglycans and Hyperhomocystenemia genes) have been studies but no definitive and clear evidence of their involvement in the AAA formation has been proven.
Linkage analysis

• Linkage of a disease of a disease phenotype, with a DNA marker of chromosomal location, suggests that the 2 are located in close proximity on the DNA. Meiotic recombination frequencies of less than 50 % indicate that the disease phenotype and the marker are linked.

C.J. Van-Vlijmen-van Keulen at al. EJVS 2002
Linkage analysis

Linkage analysis was performed on families with patients with familial Thoracic Aortic Aneurysms (TAA) and dissection. A major locus was mapped on the Chromosome 5q13-14 with in 9/15 families. In this region the Betaine – homocysteine methyl transferase gene may be a possible gene. The encoded enzyme catalyses the conversion of betaine and homocysteine to dimethylglycine and methionine. Hyperhomocysteinemia may also involved in the development of TAA and not only of arterial occlusive disease.

Guo et al. Circulation 2001
Caldwell S., EJVS 1998
Mohan IV A.DJ, EJVS 1997
Linkage analysis

Genome Wide scan in 3 large dutch families with 4 or 5 affected siblings found a locus on the chromosome 19 q13.3.

Fine mapping of the locus on the chromosome found some putative genes:
1. Fibroblast Growth Factor 21
2. Hyaluronan Synthase 1 (HAS1)

Sequencing of the FGF21 in one of the three families did not identify any mutations.

van Vlijmen-van Keulen CJ EJVS 2005
Chlamydia Pneumoniae

• Chlamydial antigens in human aortas compared to controls.
• In a rabbit model of AAA, topical treatment with Chlamydial antigens primed formation of AAA that was partially inhibited by treatment with Azithromycin.
• Roxithromycin inhibits AAA expansion rate but this seems to be unrelated to the presence of Chlamydial antigens.
• No strong evidence that Chlamydial infection could lead to development of AAA
Atherosclerosis and Inflammation

• AAA are primarily associated with atherosclerosis.
• Only 9-16% of patients with atherosclerotic abdominal aortas develop AAA.
• Mechanical and Chemical Endothelial Insult (HTN, Smoking Metabolites, Oxidized lipids).
• Expression of Adhesion Molecules on Leukocytes and mostly on endothelial cells (VCAM1).
• Chemoattraction of Inflammatory Cells (Macrophages, Monocytes, Leukocytes). Elastin degradation peptides seem to attract Monocytes through interaction with a 67KDa elastin binding receptor on the phagocytes.
Atherosclerosis and Inflammation

Adhesion of Inflammatory Cells to the Endothelium, Diapedesis and Propagation into the subintima and Media.
Infiltration of T Cells, B Cells, Plasma Cells.
Deposition of IGg
Apoptosis of VSMCs with deposition of Calcium in the apoptotic bodies.
Vascular Wall Remodeling, Medial Degeneration

Medial Degeneration

Medial Degeneration:
• Disarray and Loss of Elastic fibers
• Disarray and Loss of VSMCs
• Smoke may increase elastin degeneration promoting aneurysm expansion.

He et al., J Cardiovasc. Surg., 2006
Bergoeing MP, JVS 2007
Medial Degeneration

Curci JA, Vascular 2009
Extracellular Matrix

• Excess of MMP1, MMP2, MMP3 and MMP9 compared with normal aortic tissue. (Thompson et al. Ann NY Acad Sci 1996)
• Increased amount of MMP12 in AAA, localized by the residual elastin fibers fragments.
• Increased amount of Stromelysin have also been detected by immunoblotting and and mRNA analysis. Stromelysin may play a role in activating pro-MMPs (Curci JA, Vascular 2009).
Extracellular Matrix

- VSMCs from AAA had a significant increase in TIMP-1 synthesis in response to stimulation to IL-1β. No such a response has been seen in VSMCs from normal aortas (Galis ZS et al., Ann NY Acad Si 1995).
- VSMCs from AAA were found to produce detectable amounts of Plasminogen Activators Urkinase (uPA) and Tissue Plasminogen Activator (tPA). Plasminogen Activators have been demonstrated to activate MMPs. Evidence exists to suggest that increases in expression of fibrinolytic proteins are important to the progression of small aortic aneurysms. (Curci JA, Vascular 2009).
Extracellular Matrix in Abdominal Aortic Aneurysms.

Curci JA, Vascular 2009
Cytokines in AAA

• Increased expression of TNFα, IL6, IL1β, IL8, Macrophage Migration Inhibitory Factor
• Elevated levels of IFNγ seems to correlate with a more rapid expansion.

Curci JA, Vascular 2009
Oxidative Stress in AAA

• Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been demonstrated to cause tissue and cell damage in several conditions.

• In human studies ROS and RNS were increased in the aneurysmal wall compared with normal aorta and adjacent non aneurysmal aortic wall. (Miller FJ Jr et al., Arterioscler. Thromb. Vasc. Biol., 2002)

• Infiltrated inflammatory cells are the main source of ROS through the upregulation of NADPH oxidase.

• Overexpressed ROS and NO increased the expression of MMPs through the activation of the Nuclear-kappaB and induced apoptosis in the aneurysmal wall. (Zhang J et al. J.V.S. 2003)
Insights From animal Models of AAA

Genetically predisposed animal models
Medial Injury animal models
Hemodynamically Induced animal models
Genetically Predisposed Animal Models

- **Blotchy Mouse**: Chromosomal Mutation that affects copper metabolism and causes abnormal cross-linking of collagen and elastin fibers with weakening of connective tissue: Emphysema, aortic arch and abdominal aorta aneurysms. Used to investigate the effect of anti-hypertensive therapy on the development of AAA.

- **Knockout mouse**, lacking Apolipoprotein E gene, develops atherosclerotic plaques in the aortic wall when fed a high cholesterol diet. Aortic lesions include fragmentation of the elastic lamellae in the media and perforation of the media with development of pseudomicroaneurysms and true aneurysms.

Genetically Predisposed Models

- Transplantation of aortic segments from Guinea pigs to rats produces a rejection process that include elastin degradation and aneurysm formation. Plasminogen Activator Inhibitor seems to inhibit the aneurysm formation and expansion.
- Importance of atherosclerosis and aneurysms has been supported by observation in Cynomolgus monkeys fed with high lipid, atherogenic diet. A proportion of these monkeys developed aneurysms after the cessation of the atherogenic diet. This may suggest that the aneurysm formation may be cause by the regression of the atherogenic plaque.

Model of Medial and Adventitia degeneration

- Anidjar and Dobrin in 1989 described a rat model of AAA via the intra-arterial infusion of porcine elastase, causing medial degeneration.

Models of Medial Degeneration

- Aortic Dissection and formation of AA was observed via infusion of Angiotensin II.
- Aneurysms formed in the abdominal aorta of apoE<sup>−/−</sup> mice infused with Ang II. The aortic segments shown are from approximately the last intercostal branch to the ileal bifurcation. The aorta on the right is an example from an apoE<sup>−/−</sup> mouse infused with AngII (1,000 ng/min/kg) for 28 days. The aorta on the left is an example from an age- and gender-matched apoE<sup>−/−</sup> mouse infused with vehicle for the same interval.

Daugherty A et al. J. Clinic Investigation, 2000
Models of Media and Adventitia degeneration

- Medial degeneration was produced by the intrarterial injection of plasmin and by the infusion of peptides of elastin degradation.
- Doxycycline has shown to inhibit the aneurysm formation in this model.
- Inhibition of inflammatory cell infiltration via the monoclonal antibody anti-CD-18 reduces aneurysm formation.
- Adventitial application of Calcium Chloride may cause aortic dilation and aortic wall thickening in the rabbit. If this is combined with a high cholesterol diet and thioglycollate (activator of macrophage), aneurysms form in the abdominal aorta. Infiltration of inflammatory cells is noticed in this model.
- None of these small animal models reproduces the complex intraluminal thrombosis that is in human AAA.
Hemodynamically induced animal models

• Model of AAA via partial ligation of the aorta between the renal arteries.
• Poststenotic dilation has been used to study the protease activity in thoracic aortic aneurysms in Cynomolgus monkeys. When a stenosis was produced, collagenase activity increased over a three months period.

Pharmacological Treatment of abdominal aortic aneurysms

Miyake T. et al, Cardiovascular Research, 2009
Potential Targets for AAA treatment

• Inhibition of proteolytic activity
• Inhibition of Inflammatory response
• Suppression of oxidative stress
• Upregulation of synthesis of extracellular matrix

Miyake T. et al, Cardiovascular Research, 2009
Statins

• Hydroxymethylglutaryl-Coenzyme A inhibitors have been noted to have an inflammatory effect, anti-oxidative effect and reduction of MMP secretion.
• In a mouse model of AAA, Statins suppressed the AAA progression. This was accompanied by reduced MMP9 activity and increased TIMP1 activity. Infiltration of inflammatory cells was not inhibited.
• In an elastase induced rodent model of AAA, atorvastatin suppressed macrophage recruitment into the vascular wall, reduced expression of ICAM1, MCP1, MMP12 but not MMP9.
• In an ex vivo human aortic culture system, the application of cervistatin reduced the tissue levels of MMP9 in a concentration dependent manner.

Miyake T. et al, Cardiovascular Research, 2009
Angiotensin –converting enzyme inhibitors and Ang II receptor blockers

• Three different ACE Inhibitors (Captopril, Lisinopril, Enalapril) but not an ARB (Losartan) suppressed the development of elastase-induced AAA in rats.
• Losartan was noted to inhibit the Ang II-induced formation of AAA.
• In an APOE defective mouse Losartan increased the severity and incidence of AAA.
• Valsartan has been demonstrated to prevent the formation of AAA in a rat model through inhibition of NFκB activation, MMP expression, and infiltration of Macrophages.

Miyake T. et al, Cardiovascular Research, 2009
Antibiotics: Chlamydia Pnemoniae and MMP Inhibition

• The administration once per day of 300 mg of Roxithromycin reduced the growth of small AAA compared with placebo. This effect was observed only during the first year. (Lindholt JS, Br. J. Surg., 1999)

• In a prospective double blinded, randomized, placebo controlled study, 32 patients with AAA received either Doxycycline or placebo. The aneurysm expansion rate in the Doxy group was much lower than in the placebo. (Mosorin M. et al., J.V.S. 2000).

• Rapamycin is a immunosuppressive medication, used as a second line agent in Transplanted patients. In a rat model of AAA, Rapamycin demonstrated to reduce the expansion rate by 40% and was associated with reduced MMP9 expression and NFκB expression. (Lawrence DM et al., J.V.S. 2004).
Anti-Inflammatory Agents

• COX2 Inhibitor, Celecoxib, decreased the incidence and severity of AAA formation in Apoe deficient mice.

• After 28 days of infusion of Angiotensin II, AAA incidence in the wild-type mice was 54%, whereas AAAs were not detected in the COX2 deficient animal.
Novel Agents

• Inhibitors of Mast Cell degranulation: Disodium Chromoglicate
• Inhibition of c JUn N Terminal Kinase
• Oligodeoxybuceotide therapy