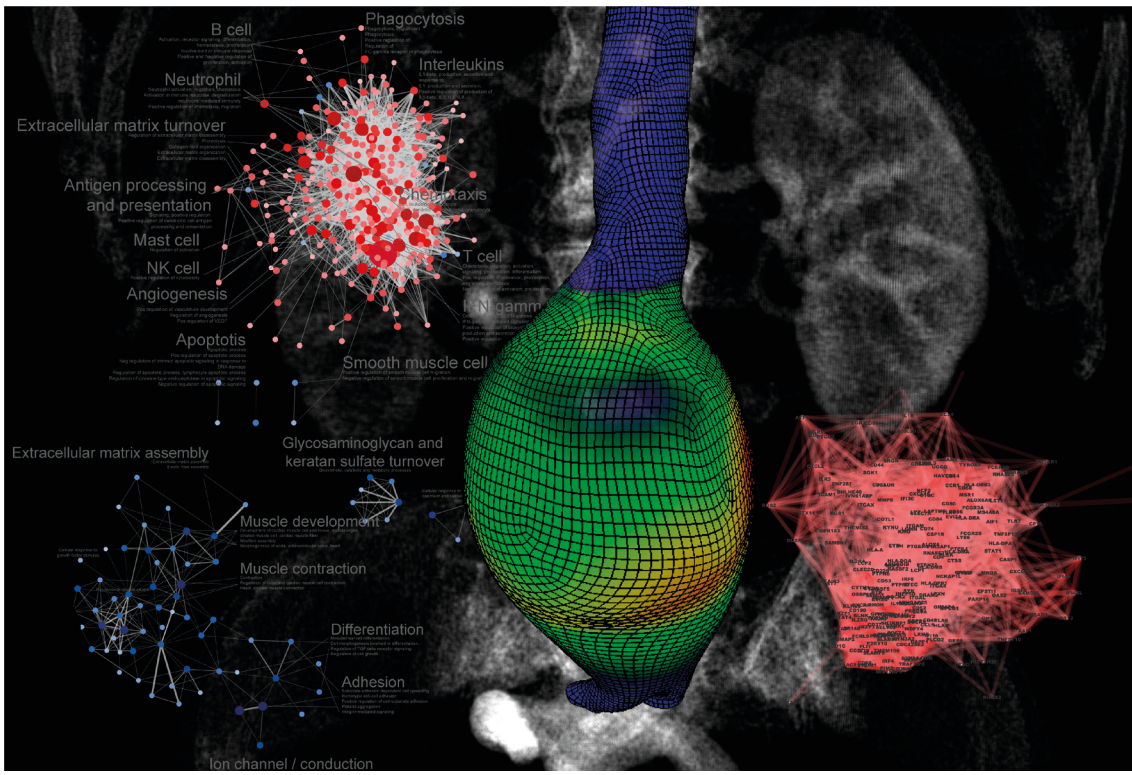
Thesis for doctoral degree (Ph.D.)
2020

Geometric, Biomechanical and Molecular Analyses of Abdominal Aortic Aneurysm



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Stockholm 2020

Published by Karolinska Institutet.
Printed by E-Print AB 2020

ISBN 978-91-7831-918-3

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Public defense on Friday 11th of September 2020, at 14:00.
Nanna Svartz, J3:12, Bioclinicum, Nya Karolinska Sjukhuset

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To my family and friends.

ABSTRACT

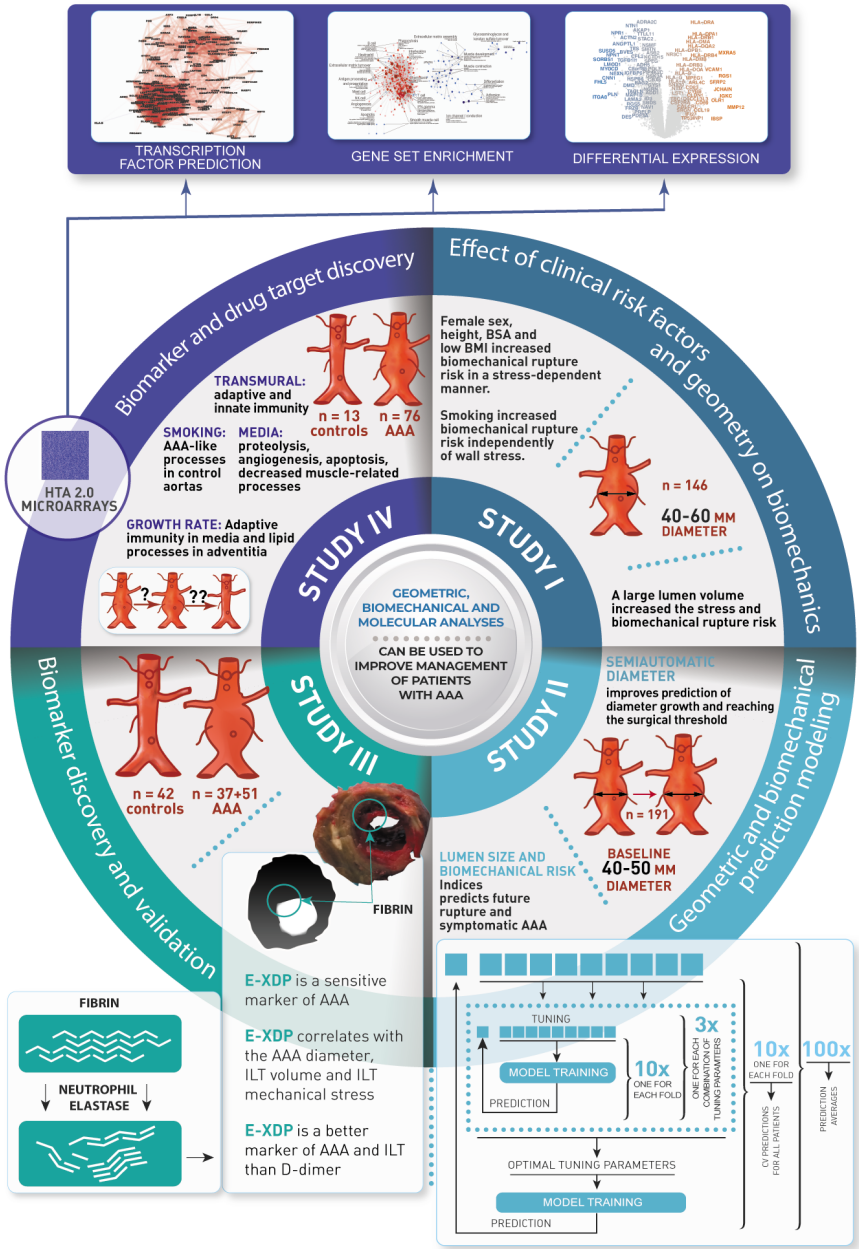
Background: Abdominal aortic aneurysm (AAA) is defined as a dilatation of the abdominal aorta of 30 mm in diameter or more. Main risk factors are smoking, age and male sex. Pathophysiological features include inflammation, smooth muscle cell loss and destruction of the extracellular matrix. The AAA is typically asymptomatic but can expand and eventually rupture, with a mortality of 70-80% as a result. Risk factors for rupture include a large diameter, female sex, active smoking, high blood pressure and low body mass index (BMI). There is no medical treatment to inhibit growth or rupture of AAA. The only measure to prevent rupture in a large AAA is aortic surgery. This intervention carries its own significant risk of morbidity and mortality, necessitating a risk stratification method. The diameter is currently used to decide when to operate on an AAA and it is repeatedly monitored until the threshold for surgery is reached. However, this measurement leaves room for improvement, as the individual aneurysm growth rate is difficult to predict and some large AAAs do not rupture while in other patients, small AAAs rupture during surveillance. Finite element analysis (FEA) is a method by which biomechanical rupture risk can be estimated based on patient characteristics and a computed tomography (CT)-derived 3D model of an AAA. Microarray analysis allows high-throughput analyses of tissue gene expression.

Aims: The overall aim of this thesis was to explore and develop new strategies to improve, refine and individualize management of patients with AAA, by applying geometric, biomechanical and molecular analyses.

Methods and Results: In study I, the CTs of 146 patients with AAAs of diameters between 40 and 60 mm were analyzed with three-dimensional (3D) segmentation and FEA. Simple and multiple regression analyses were performed. Female sex, patient height, lumen volume, body surface area (BSA) and low BMI were shown to be associated with the biomechanical rupture risk of AAA. Study II included 191 patients with AAAs of diameters between 40-50 mm. The AAAs were analyzed with 3D segmentation and FEA after which prediction algorithms were developed by use of machine learning strategies. More precise diameter measurements improved prediction of growth and four-year prognosis of small AAAs. Biomechanical indices and lumen diameter were predictive of future rupture or symptomatic AAA. Growth and rupture required different prediction models. In study III, 37 patients, 42 controls and a validation cohort of 51 patients were analyzed with respect to their circulating levels of neutrophil elastase-derived fibrin degradation products (E-XDP). The results showed that E-XDP was a sensitive marker for AAA, independently of examined comorbidities, and its concentration in peripheral blood correlated with the AAA diameter and the volume and mechanical stress of the intraluminal thrombus (ILT). It was further increased by the presence of coexisting aneurysms. Study IV included 246 tissue samples, divided into tunica media and adventitia, from 76 patients with AAA and 13 organ donor controls, analyzed by microarrays. There were large differences between the transcriptomes of AAA and control media and adventitia. Processes related to inflammation were transmural, whereas the upregulation of proteolysis, angiogenesis and apoptosis along with downregulation of smooth muscle- and differentiation-related gene sets were specific for the aneurysm media. Active smoking increased oxidative stress in all tissues and increased inflammation and lipid-related processes in AAA. The growth rate of the AAA diameter correlated with adaptive immunity in media and lipid processes in adventitia.

Conclusions: In this thesis, we show that known clinical risk factors and certain geometric properties are associated with biomechanical deterioration of AAAs. Furthermore, geometric and biomechanical analyses can enhance prediction of outcome. Importantly, there are differences between prediction of AAA growth and rupture. Finally, a biomarker was discovered and the transcriptome of AAA including effects of the ILT, smoking and rapid diameter growth rate, was mapped and we envision that the data may be used for future biomarker and drug target discovery.

VISUAL ABSTRACT



POPULÄRVETENSKAPLIG SAMMANFATTNING

Introduktion: Pulsåderbråck på buksegmentet av den stora kroppspulsådern kallas bukaortaaneurysm (BAA) och drabbar ca 1,5% av alla 65-åriga män och 0,5% av alla 70-åriga kvinnor i Sverige. Riskfaktorer för att utveckla BAA är bland andra rökning, manligt kön och hög ålder. Sjukdomen innebär att kroppspulsådern utvidgas i diameter på grund av nedbrytning av den normalt stabiliserande vävnaden i kärlväggen. I vanliga fall ger inte BAA några symptom. Med ökande diameter ökar dock risken för bristning, ett tillstånd med svår rygg-buksmärta och inre blödning. Ett brustet BAA orsakar en dödlighet på 70-80%. Riskfaktorer för bristning hos patienter med BAA är kvinnligt kön, rökning, högt blodtryck och lågt kroppsmasseindex (BMI). Det enda sättet att förhindra tillväxt och bristning av ett BAA är operation, det finns ingen medicinsk behandling. Operation av BAA är dock ett omfattande ingrepp med risk för död och komplikationer. Därför opereras inte alla patienter med BAA. Kirurgi övervägs när diametern är 55 mm hos män och 52 mm hos kvinnor. Mindre BAA övervakas regelbundet med ultraljud och/eller skiktröntgen tills de uppnår denna operationsindikation. Diametern är inte en perfekt indikator på risk för bristning. Det är svårt att förutsäga hur snabbt ett specifikt BAA kommer att tillväxa samt när, och om, det kommer att bli föremål för kirurgi. De senaste decennierna har datoriserade hållfasthetsberäkningar, baserade på 3D-modeller, utvecklats för att uppskatta biomekanisk belastning i ett BAA och det har också blivit möjligt att mäta uttrycket av alla gener i en vävnad samtidigt, med så kallad microarray-teknik.

Målsättningar: Den övergripande målsättningen var att utveckla nya geometriska, biomekaniska och molekylärbiologiska metoder som kan förbättra och individanpassa handläggningen av patienter med BAA.

Metod och Resultat: I den första studien inkluderades 146 patienter och hållfasthetsberäkningar av BAA utfördes baserade på skiktröntgenbilder. Ett starkt samband kunde observeras mellan kvinnligt kön, kroppslängd, rökning, lågt BMI och ökad biomekanisk belastning i BAA. I den andra studien inkluderades 191 patienter och analyser av geometri samt hållfasthet användes för att förutsäga prognos. Ökad precision i diametermätningar med hjälp av 3D-modellering förbättrade förutsägelser om vilka patienter som behövde operation inom fyra år samt hur snabbt diametern skulle tillväxa. Hållfasthetsberäkningar, men inte maximal diameter, kunde förutsäga vilka patienter som i framtiden skulle riskera att drabbas av bristning av BAA. I den tredje studien inkluderades 37+51 patienter och 42 kontroller och nivåerna av en nedbrytningsprodukt av den kroniska väggfasta tromb som bildas på insidan av de flesta BAA (kallad E-XDP) uppmättes. Studien visade att mätning av E-XDP i blod kunde avslöja om en person har BAA eller inte och att dess koncentration korrelerar med BAA-diametern och trombens storlek. Den sista och fjärde studien inkluderade 246 vävnadsprover från 76 patienter och 13 kontroller och uttrycket av alla gener i dessa prover jämfördes. Resultaten kunde avslöja tusentals gener som var annorlunda uttryckta i BAA jämfört med kontroller. När dessa gener analyserades tillsammans framträdde ett flertal processer som exempelvis inflammation, nedbrytning av stödjevävnad samt förlust av muskelceller i kärlväggen i BAA. Några av dessa processer påverkades också vid rökning, samt var högt uttryckta vid snabb diametertillväxt.

Slutsatser: Kliniska riskfaktorer för bristning är associerade med biomekanisk belastning av BAA och geometrisk-biomekanisk analys ökar precisionen i prognostiseringen av denna sjukdom. Mätning av E-XDP i blod kan avslöja BAA samt dess storlek. Det fullständiga genuttrycket i BAA kartlades och kan användas för framtida utveckling av blodprover och läkemedel. Geometriska, biomekaniska och molekylärbiologiska analyser kan användas för att förbättra handläggningen av patienter med AAA.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following publications and manuscripts:

I. Gender, smoking, body size, and aneurysm geometry influence the biomechanical rupture risk of abdominal aortic aneurysms as estimated by finite element analysis. **Lindquist Liljeqvist M**, Hultgren R, Siika A, Gasser TC, Roy J. *J Vasc Surg*. 2017;65:1014-1021.e4.

II. Geometric and Biomechanical Prediction Modeling of Growth, Treatment and Outcome of Small Abdominal Aortic Aneurysms Using Machine Learning. **Lindquist Liljeqvist M**, Bogdanovic M, Siika A, Gasser TC, Hultgren R, Roy J *Manuscript*

III. Neutrophil Elastase-Derived Fibrin Degradation Products Indicate Presence of Abdominal Aortic Aneurysms and Correlate with Intraluminal Thrombus Volume. **Lindquist Liljeqvist M**, Silveira A, Hultgren R, Frebelius S, Lengquist M, Engström J, Gasser TC, Eriksson P, Roy J. *Thromb Haemost.* 2018;118:329–339.

IV. Tunica-Specific Transcriptome of Abdominal Aortic Aneurysm and the Effect of Intraluminal Thrombus, Smoking and Diameter Growth Rate. **Lindquist Liljeqvist M**, Hultgren R, Bergman O, Villard C, Kronqvist M, Eriksson P, Roy J. *Manuscript, accepted in Arterioscler Thromb Vasc Biol. 2020*

Related publications not included in this thesis:

Dipeptidyl peptidase-4 is increased in the abdominal aortic aneurysm vessel wall and is associated with aneurysm disease processes. **Lindquist Liljeqvist M**, Eriksson L, Villard C, Lengquist M, Kronqvist M, Hultgren R, Roy J. *PLoS ONE*. 2020;15:e0227889.

A large proportion of patients with small ruptured abdominal aortic aneurysms are women and have chronic obstructive pulmonary disease. Siika A, **Lindquist Liljeqvist M**, Zommorodi S, Nilsson O, Andersson P, Gasser TC, Roy J, Hultgren R. *PLoS ONE*. 2019;14:e0216558.

Does Finite Element Analysis Enable Us to Predict Rupture? - The benefits and limitations of finite element analysis in patients with abdominal aortic aneurysms. Erhart P, **Lindquist Liljeqvist M**, Roy J, Gasser TC, Böckler D. *Endovascular Today.* March 2019 vol 18, no. 3

Aortic Lumen Area Is Increased in Ruptured Abdominal Aortic Aneurysms and Correlates to Biomechanical Rupture Risk. Siika A, **Lindquist Liljeqvist M**, Hultgren R, Gasser TC, Roy J. *J Endovasc Ther*. 2018;1526602818808292.

CD40L Deficiency Protects Against Aneurysm Formation.
Kusters PJH, Seijkens TTP, Beckers L, Lievens D, Winkels H, de Waard V, Duijvestijn A, **Lindquist Liljeqvist M**, Roy J, Daugherty A, Newby A, Gerdes N, Lutgens E. *Arterioscler Thromb Vasc Biol*. 2018;38:1076–1085.

Localized Hyperattenuations in the Intraluminal Thrombus May Predict Rupture of Abdominal Aortic Aneurysms.
Talvitie M, **Lindquist Liljeqvist M**, Siika A, Hultgren R, Roy J. *J Vasc Interv Radiol*. 2018;29:144–145.

Resolution of Inflammation Through the Lipoxin and ALX/FPR2 Receptor Pathway Protects Against Abdominal Aortic Aneurysms.
Petri MH, Thul S, Andonova T, **Lindquist-Liljeqvist M,** Jin H, Skenteris N-T, Arnardottir H, Maegdefessel L, Caidahl K, Perretti M, Roy J, Bäck M. *JACC Basic Transl Sci*. 2018;3:719–727.

Value of risk scores in the decision to palliate patients with ruptured abdominal aortic aneurysm.
Sweeting MJ, Ulug P, Roy J, Hultgren R, Indrakusuma R, Balm R, Thompson MM, Hinchliffe RJ, Thompson SG, Powell JT, Ruptured Aneurysm Collaborators: AJAX Trial investigators, ECAR Trial investigators, IMPROVE Trial investigators: management committee, **STAR Cohort investigators**. *Br J Surg*. 2018;105:1135–1144.

Biomechanical changes during abdominal aortic aneurysm growth.
Stevens RRF, Grytsan A, Biasetti J, Roy J, **Lindquist Liljeqvist M**, Gasser TC. *PLoS ONE*. 2017;12:e0187421.

Local Diameter, Wall Stress, and Thrombus Thickness Influence the Local Growth of Abdominal Aortic Aneurysms.
Martufi G, **Lindquist Liljeqvist M**, Sakalihasan N, Panuccio G, Hultgren R, Roy J, Gasser TC. *J Endovasc Ther*. 2016;

Volume growth of abdominal aortic aneurysms correlates with baseline volume and increasing finite element analysis-derived rupture risk.
Lindquist Liljeqvist M, Hultgren R, Gasser TC, Roy J. J Vasc Surg. 2016.

Prediction of Rupture Sites in Abdominal Aortic Aneurysms After Finite Element Analysis.
Erhart P, Roy J, de Vries J-PPM, **Liljeqvist ML**, Grond-Ginsbach C, Hyhlik-Dürr A, Böckler D. *J Endovasc Ther*. 2016;23:115–120.

CONTENTS

LIST OF ABBREVIATIONS

SI-units, software or company names and commonly accepted gene and protein abbreviations are not listed.

^{18}F-FDG: Fluorodeoxyglucose

^{18}F-NaF: 18F–sodium fluoride

3D: Three-dimensional

AAA: Abdominal aortic aneurysm

AUC: area under curve

BMI: Body mass index

BSA: Body surface area

CSE: Cigarette smoke extract

CT: Computed tomography

CTA: Computed tomography Angiography

E-XDP: Neutrophil elastase-derived fibrin degradation products

ECM: Extracellular matrix

ELISA: Enzyme-linked immunosorbent assay

eQTL: Expression quantitative trait loci

EVAR: Endovascular aneurysm repair

FDR: False detection rate

FEA: finite element analysis

GO:BP: Gene Ontology biological processes

GO:MF: Gene Ontology molecular functions

GWAS: Genome-wide association study

ILT: Intraluminal thrombus

IQR: Interquartile range

LASSO: Least absolute shrinkage and selection operator

LMIC: Low- and middle-income countries

lncRNA: long non-coding RNA

MAP: Mean arterial pressure

Mean WS: Mean wall stress

miRNA, miR: microRNA

MRI: Magnetic resonance imaging

OAR: Open aortic repair

OPLS-DA: Orthogonal projection to latent structures discrimination analysis

PAD: Peripheral arterial disease

PET: Positron emission tomography

PWRI: Peak wall rupture risk index

PWS: Peak wall stress

RCT: Randomized controlled trial

ROC: Receiver operating characteristic

RRED: Rupture risk equivalent diameter

SNP: Single-nucleotide polymorphism

StAAAB: Stockholm AAA Biobank

SVM: Support vector machine

t-SNE: t-distributed stochastic neighbor embedding

US: Ultrasound

USPIO: Ultrasmall superparamagnetic particles of iron oxide

vSMC: Vascular smooth muscle cell

1 INTRODUCTION

1.1 OVERVIEW OF ABDOMINAL AORTIC ANEURYSM

1.1.1 Definition and risk factors

An arterial aneurysm is a focal and irreversible dilatation of an artery to a diameter larger than 1.5 times the expected normal diameter (1). Aneurysms may be classified based on the affected arterial segment, its etiology, histopathologic features and clinical characteristics. A true aneurysm refers to the dilatation of the arterial vessel wall, whereas a false "pseudoaneurysm" refers to the contained rupture through the same. Aneurysms occur along the entire vascular system and etiologies range from congenital, caused by connective tissue diseases such as Marfan's syndrome or Ehlers-Danlos, traumatic, inflammatory, infectious and degenerative (1), the latter being the exceedingly most common. Further, aneurysms may be asymptomatic or cause symptoms and can in the worst-case scenario lead to rupture. Abdominal aortic aneurysm (AAA) is an aneurysm of the abdominal aorta which preferentially affects its infrarenal segment (Figure 1), typically diagnosed when the diameter exceeds 30 mm (2,3). The most common shape of an AAA is fusiform, ie diffuse circumferential dilatation, whereas some aneurysms are saccular with a focal, spherical and asymmetric dilatation. Saccular aneurysms appear more prone to rupture at smaller diameters compared with fusiform aneurysms (4). The present thesis mainly concerns the common degenerative and fusiform AAA. Most clinically relevant AAAs contain an intraluminal thrombus (ILT), which is adherent to the vessel wall, very rarely cause embolic/thrombotic events and does not require specific treatment (5).

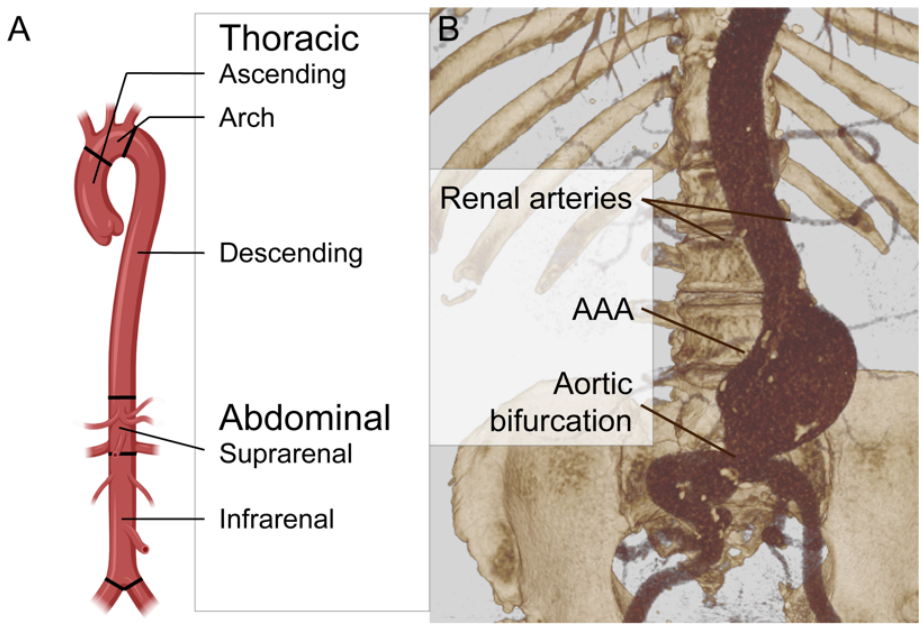


Figure 1: Infrarenal abdominal aortic aneurysm. A: Aortic segments. B: infrarenal AAA imaged by computed tomography angiography
Abbreviations: AAA: Abdominal aortic aneurysm

In contemporary Swedish studies, the screening-detected prevalence of AAA is 1.5% for men at age 65 (6) and 0.5% for women at age 70 (7). Nonmodifiable risk factors for AAA include old age, male sex and positive family history whereas African-American, Hispanic or Asian ethnicities appear to be protective (8,9). Smoking is the exceedingly most important modifiable risk factor and increases the risk of AAA in a highly dose-dependent manner. Moreover, individuals who quit smoking have a decreased risk of developing AAA compared with those who continue (8,10). Hypercholesterolemia, hypertension, cardiovascular disease and increased body mass index (BMI) are potentially modifiable risk factors. Another proposed risk factor is depression (11). Finally, diabetes mellitus, which is a strong risk factor for cardiovascular disease in general, appears to be negatively associated with AAA (8). Importantly, however, epidemiologically derived risk factors are associated but not necessarily causally related to the disease in question.

1.1.2 Rupture

The feared and often lethal complication of AAA is rupture, where a structural failure of the aneurysm wall occurs and a massive retro- and/or intraperitoneal hemorrhage follows. The patient who survives long enough to be admitted to a hospital classically presents with hypotension, abdominal and/or back pain and a pulsatile abdominal mass. These symptoms are not present in all patients and initial misdiagnosis, with for example myocardial infarction or ureteric colic, is common. (12). Rupture will occur in around 20% of patients with AAA that are denied preventive surgery, competing with other causes of death (13). The total mortality for patients with ruptured AAA is around 81% in women and 67% of men (14). If left untreated, the mortality of aortic rupture is 100% and more than half of the affected patients succumb before reaching hospital (15). For patients admitted to hospital, mortality remains high at 30-40% (16,17). Factors associated with mortality in patients operated for rupture include preoperative hemodynamic instability, cardiac arrest, impaired renal function, reduced consciousness, congestive heart failure, female sex and significant anemia as well as post-operative multi-organ, renal and/or respiratory failure, abdominal compartment syndrome and massive blood transfusion (3,14).

The risk of rupture correlates with the aneurysm diameter, and this measurement is considered the major rupture risk indicator in current practice (18–20). Further, although women have a lower risk than men of developing AAA, female patients with AAA have an almost four times higher risk of rupture compared to male patients with an AAA of the same diameter (21). Other risk factors for rupture in patients with AAA include current (as opposed to previous) smoking, low BMI and hypertension (21). High age has been identified as a risk factor for rupture in a large-scale ($n > 15\,000$) meta-analysis performed by Sweeting MJ et al, but in this study, full adjustment could not be performed for all potential confounders. However, in a smaller ($n > 2000$), previous study conducted by the same group, in which full adjustment could be made, age was not related to aneurysm rupture risk. Several risk factors, such as age, female sex, BMI, lung function and income, are however simultaneously related to operative outcome (22–26). Thus, balancing these risks clinically is challenging and a more patient- and aneurysm-specific rupture risk estimation would facilitate the operative decision.

1.1.3 Diagnosis and screening

AAAs may be diagnosed via targeted screening or incidentally when a computed tomography (CT), ultrasound (US), magnetic resonance imaging (MRI) or clinical examination of the abdomen is performed for other reasons. It is diagnosed by diameter measurement of the

abdominal aorta, where a maximal diameter of 30 mm is considered aneurysmal and 25-29 mm could be considered sub-aneurysmal, and a quarter of the latter category will develop a large AAA in 15 years (2,3,27). The preferred modality to measure diameter is by US (Figure 2A) as it is widely accessible and does not expose the patient to radiation. The measurement method used in Sweden is leading edge-to-leading edge (outer anterior wall to inner posterior wall) but 'inner-to-inner' and 'outer-to-outer' protocols are used in other countries, affecting the measured size of the aneurysm and thereby to some extent the prevalence of the disease (28). CT with intravenous contrast agent in arterial phase (CT angiography, CTA, Figure 2B) is the preferred modality to image in detail the anatomy of the aorta and its branches and is typically performed to detect any coexisting aneurysms and when the threshold for surgery is reached in order to plan the operation (2,3,29). With CT imaging, the recommended method to measure the diameter is from outer-to-outer wall perpendicularly to the estimated direction of the blood flow (2,3)

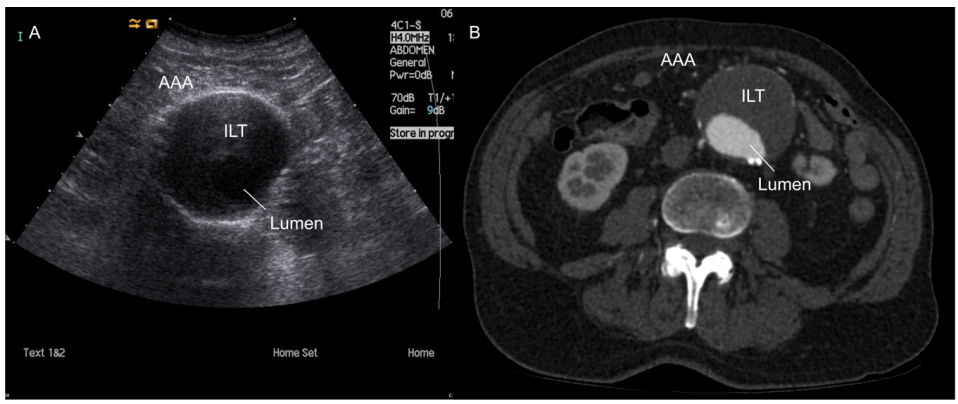


Figure 2: Cross-sections of abdominal aortic aneurysms. A: Ultrasound. B: Computed tomography angiography.
Abbreviations; AAA: abdominal aortic aneurysm, ILT: intraluminal thrombus.

Classic screening criteria apply to AAA due to the high mortality of ruptured AAA, an effective preventive treatment (open or endovascular surgery), an asymptomatic latent stage and an excellent mean of diagnosis (US) (6). Screening for AAA has been examined in four randomized controlled trials (RCTs) and been shown to decrease aneurysm-related and, at later time points, all-cause mortality (30,31). A population-wide screening program of 65-year-old men was gradually introduced in Sweden between 2006 and 2015. Despite a relatively low prevalence of AAA in Sweden, an evaluation in 2016 showed that the Swedish screening program reduced the AAA-specific mortality and was cost-effective, with a numbers-needed-to-screen to prevent 1 premature death of 667 and an incremental cost-efficiency ratio of €7770 per quality-adjusted life-year (6). Prevalence of AAA in women is three times lower than that in men (32). An RCT on screening for AAA in women observed similar, <0.2%, aneurysm-related mortality in both case and control group but the study was underpowered to evaluate differences at those rates (33). Female current smokers have a six times higher prevalence of AAA and the effect of selectively screening female smokers is not known. Relatives of patients with AAA have an increased risk of developing AAA themselves (34), and screening of individuals with a positive family history is recommended but has not yet been evaluated in an RCT (2,35).

1.1.4 Non-surgical management and timing of surgery

The only treatment to prevent the growth and rupture of an AAA is surgery (2,32,36). The rate of diameter growth is positively associated with smoking and negatively with diabetes mellitus (21). The cessation of smoking is therefore advised to slow the progression of the AAA towards rupture and to decrease operative risks (2,3). This could be considered the only available non-surgical intervention for AAA, which makes the study of the biological effects of smoking highly relevant. While the mortality of a AAA rupture is striking, an elective aortic operation is a high-risk procedure with mortality rates at around 2-3% in contemporary Sweden (17). This fact calls for some sort of risk stratification when surgery is considered. The risk of rupture increases with the diameter of the aneurysm and this measurement is used as indication for surgery (18–20)

Four RCTs demonstrated no benefit of operating patients with an AAA < 55 mm in diameter, as opposed to repeated diameter measurements ('surveillance', see below) until the diameter reaches 55 mm, Table 1 (37–41). The first two of these trials, the 'United Kingdom Small Aneurysm Trial' (UKSAT) and the 'Aneurysm Detection and Management Veterans Affairs Cooperative Study' (ADAM), compared open aortic repair (OAR) to surveillance of AAAs of between 4 and 5.5 cm in diameter (37,38). The two following studies 'Comparison of Surveillance Versus Aortic Endografting for Small Aneurysm Repair' (CAESAR) and the Positive Impact of Endovascular Options for treating Aneurysms Early (PIVOTAL) instead studied endovascular aneurysm repair (EVAR) in small AAAs compared with surveillance (39,40). These studies shared similar diameter thresholds for intervention, surveillance time intervals and included few women. Differences include age criteria and diameter measurement protocols. Due to the low proportions of women, the resulting evidence is not necessarily generalizable to a female AAA population, which has been shown to feature a markedly increased rupture risk compared with male counterparts (21,41). While it has been shown that the rupture rate for female patients with an AAA of 4.5 cm is similar to that of a male patient with a 5.5 cm AAA, female patients with AAA also generally show higher perioperative mortality than males, why a compromise of a 50 mm as surgical threshold for female patients has been proposed in recent guidelines (2,3). Before surgery is performed, cardiac, pulmonary, renal and nutritional statuses are evaluated and optimized. The patients should also be started on statins several weeks preoperatively (3).

Table 1: Summary of randomized controlled trials comparing surveillance to surgery in small AAAs.

Attribute	UKSAT	ADAM	PIVOTAL	CAESAR
Diameter, inclusion range	4-5.4 cm	4-5.4 cm	4-5 cm	4.1–5.4 cm
Ages, min-max	60-76 y.o.	50-79 y.o.	40-90 y.o.	50-79 y.o.
Female patients included	17.2%	0.8%	13.3%	4.2%
Diameter definition	US Antero-Posterior, Outer-to-Outer	CT Maximal cross-sectional diameter in any direction. If aneurysm was tortuous, it was measured perpendicularly to tortuosity	CT	CT Maximum external cross-sectional measurement in any direction but perpendicular to the vessel axis
Protocol for surveillance in non-surgery group	US. 4 to 5 cm; 6 months intervals, 5 to 5.5 cm; 3 months intervals	US. 6 months intervals	CT or US. 6 months intervals	US: 6 months intervals and CT: 1 year intervals

Threshold for surgery	Diameter ≥ 5.5, symptomatic AAA, growth rate ≥ 1cm/y or 0.7 cm / ½ y	Diameter ≥ 5.5, symptomatic AAA, growth rate ≥ 1cm/y	Diameter ≥ 5.5, symptomatic AAA, growth rate ≥ 0.5 cm/ ½ y	Diameter ≥ 5.5, symptomatic AAA, growth ≥ 1cm/y
Primary Outcome	All-cause mortality	All-cause mortality	Aneurysm-related mortality after 3 y	All-cause mortality
Result	No difference in primary outcome	No difference in primary outcome	No difference in primary outcome	No difference in primary outcome
Authors' interpretation	Survival not improved by surgery of small AAAs	Survival not improved by surgery of small AAAs	Early EVAR and surveillance are both safe alternatives for patients with small AAAs	Surveillance is safe for small AAAs

Abbreviations; AAA: Abdominal aortic aneurysm, UKSAT: United Kingdom Small Aneurysm Trial, ADAM: Aneurysm Detection and Management Veterans Affairs Cooperative Study, CAESAR: Comparison of Surveillance Versus Aortic Endografting for Small Aneurysm Repair, CT: computed tomography, EVAR: endovascular aneurysm repair, PIVOTAL: Positive Impact of Endovascular Options for treating Aneurysms Early, US: ultrasound, y.o.: years old

1.1.5 Open and endovascular surgery

Two principal surgical treatments exist; OAR or EVAR. Laparoscopic aneurysm repair has been described but is associated with an increased risk of serious adverse events and is not recommended (3,42). An OAR is performed either by transperitoneal or left retroperitoneal incision, both of which show similar outcomes in RCTs (43). The commonly used transperitoneal approach is versatile and allows access to all abdominal organs, whereas the retroperitoneal approach offers better exposure of the suprarenal aorta, facilitates repair of inflammatory AAA or that associated with a horseshoe kidney while allowing the surgeon to avoid a hostile abdomen (2,3). After exposure of the AAA, OAR proceeds by suturing a tube-shaped or bifurcated synthetic graft into the aorta, after which the aneurysm sac is wrapped around it to prevent viscera adhering and potentially forming fistulas against the reconstructed aorta.

The most common treatment modality of AAA in Sweden is EVAR (17). In EVAR, access to the circulation is obtained via the femoral arteries after which a stent graft is inserted into the aorta (Figure 3). Thus, blood flows through the stent graft and pressure is removed from the aneurysm vessel wall. So called endoleaks are important EVAR-specific complications where for different reasons, opposite to what is intended, blood flows between the stent graft and the vessel wall of the aneurysm sac (44). Endoleaks are divided into four classes; type I indicates leakage of blood at proximal or distal attachment sites, type II is retrograde flow from aortic branches, type III is leakage at defects in the stent graft wall or in a junction between stent grafts and type IV indicates leakage through the stent graft due to fabric porosity. Type I or III occur in 1-5% of patients and expose the aneurysm to pressurized circulation which necessitates prompt reintervention (2,3). Type II endoleaks occur in around 20% of patients and can be considered benign if not associated with persistent aneurysm growth (2,3). Other complications include endotension ("type V endoleak"; increasing aneurysm sac size despite no demonstrable leak on imaging), migration, kinking and limb occlusion. Due to these complications, patients treated with EVAR are typically required to undergo imaging surveillance, often by CTA, at regular intervals for the rest of their lives, which is not required after OAR.

A number of RCTs have compared outcomes of OAR and EVAR for treatment of asymptomatic and ruptured AAA (45–54). The trials EVAR-1 (49), OVER (50), DREAM (48) and ACE (47) compared OAR with EVAR for the treatment of asymptomatic AAAs. A recent meta-analysis of these trials showed that the mortality was lower with EVAR in the short term but within 3 years, the survival after EVAR and OAR was the same after which the aneurysm-related mortality was higher in the EVAR-group compared with OAR, although one of the included studies showed no difference (45,47–50). The current recommendation of the European Society for Vascular Surgery is to make individual patient-specific decisions of OAR vs EVAR but that EVAR should be considered the first-hand choice in patients with anatomically suitable AAAs, due to its early mortality benefit and the 9 years post-operative survival of the average AAA patient (3). In contrast, the United Kingdom's National Institute for Health and Care Excellence (NICE) did not consider EVAR cost-effective and based on formal analyses of above RCTs instead recommends OAR as the first-hand choice (55).

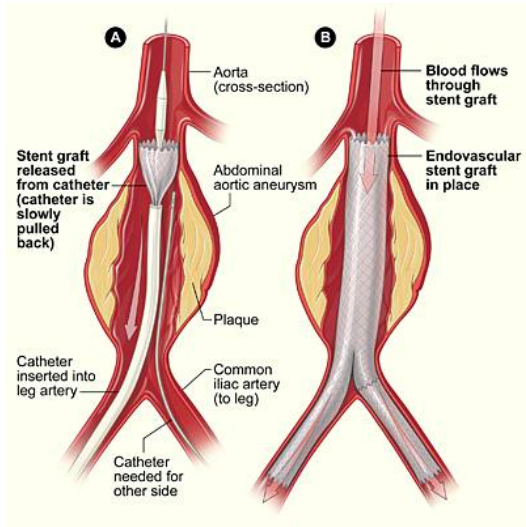


Figure 3. Endovascular aneurysm repair (EVAR). By the National Institutes of Health, Public Domain.

The AJAX (52), IMPROVE (53) and ECAR (54) RCTs compared EVAR with OAR for ruptured AAAs. The study designs differed as AJAX and ECAR only considered patients for randomization if the anatomy of their aneurysms allowed EVAR. Conversely, IMPROVE aimed to compare open and endovascular strategies, randomizing patients before the anatomy was known to an endovascular strategy (EVAR or OAR based on anatomy) or an open strategy (only OAR). There were no differences in overall 30-day mortality between the groups but a meta-analysis observed that at 90 days, EVAR seemed to benefit women and that patients treated with EVAR required shorter hospital stays (46). Further supporting the use of EVAR to treat ruptured AAA, long-term results from IMPROVE showed a lower three-year mortality and higher early quality of life in patients treated with EVAR compared with OAR (56).

The EVAR-2 RCT compared EVAR with no intervention in patients with AAA who reached indication for surgery but were considered too frail for OAR (57). It was shown that there was no difference in aneurysm-related mortality during the first four years, whereas there was decreased aneurysm-related mortality in the EVAR group at later time points. There was no difference between the groups in all-cause mortality. During the course of the trial, 9 patients in the EVAR group suffered rupture before the intervention occurred and, further, ca 30% of patients assigned to no intervention underwent elective AAA surgery nevertheless, complicating and limiting the interpretation of the results.

1.1.6 Surveillance and management of patients with small AAAs

When patients are diagnosed with an AAA that is smaller than the surgical threshold, they are enrolled into a surveillance program. The frequency of surveillance is determined by the

aneurysm diameter, and the patient's aneurysm is measured repeatedly with regular intervals until it reaches the surgical threshold (2,3). The rate of diameter growth correlates with the diameter (18–20,58). A large meta-analysis which included heterogenous studies on patients under surveillance used sophisticated statistical models, which considered individual variation, measurement errors, different imaging modalities and separate growth rates for men and women, in order to determine safe surveillance intervals (58). It recommended safe intervals with respect to the probability of reaching 55 mm and, for men, the risk of rupture. While the growth rates of men and women were similar, the time to reach a 1% risk of rupture was much shorter in women and the authors supported the already existing recommendation to treat female AAA patients at smaller diameter while noting that their risk of perioperative mortality was higher.

Currently, diameter measurements are recommended, preferentially by US, every 3 years for AAAs 3-3.9 cm, every year for AAAs 4-4.9 cm and every 6 months for AAAs ≥ 5.0 cm (2,3). However, the correlation between the baseline diameter and subsequent growth rate is weak and it is difficult to predict the trajectory of an individual patient, resulting in many US examinations required not to miss the few aneurysms that will grow rapidly (59,60). Further, although this protocol is maintained, a small but existing risk of rupture during surveillance remains (61–64). In above mentioned meta-analysis, 1.6% of men and 3.8% of women under surveillance suffered from ruptured AAA, the risk of which increased with increasing diameter (58). Research on the link between sex and AAA is elaborated on in section 1.2.9.

1.1.7 Global outlook

Creating more precise surveillance protocols, gaining a better understanding of pathophysiology and developing future medical treatments would have major implications for the health of patients with AAA globally, including in societies hitherto underserved by vascular surgery. Life expectancy as well as the incidence and prevalence of non-communicable diseases, including cardiovascular disease, are increasing globally (Figure 4) (65–68). The increasing prevalence of cardiovascular disease in low- and middle-income countries (LMIC) can be attributed to increasing life expectancy as well as an increased exposure to behavioral and metabolic risks previously more common in high-income countries (68). However, infectious and environmental risk factors for cardiovascular disease, as well as untreated congenital conditions, remain uncorrected in the poorest parts of the World, resulting in additional risk of morbidity and mortality (68). Further, the underreporting of cardiovascular disease, especially peripheral arterial disease (PAD), is an important impediment to research (65).

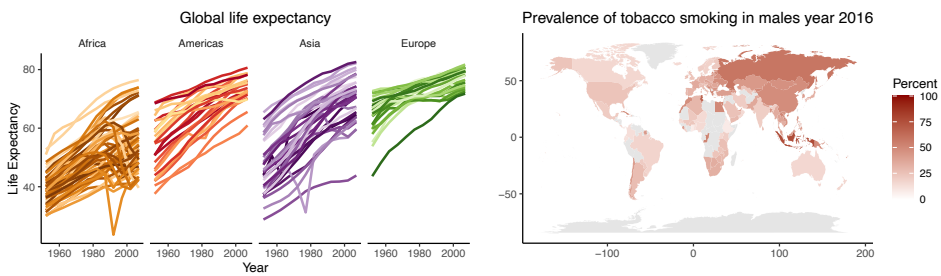


Figure 4: Global life expectancy and tobacco smoking. Data from the World bank and Gapminder. Global life expectancy: each line represents one nation.

Surgery has until recently been a relatively underrecognized aspect of global health. In order to address this gap of knowledge, a Lancet commission on global surgery was formed in 2014 and highlighted five key messages: 5 billion people lack access to safe and affordable surgery and anesthesia, an excess of 140 million additional surgical procedures are required in LMICs, a quarter of people who received surgical care face catastrophic expenditure as a result, improving surgical coverage in LMICs is cost-effective and finally that surgery is an indispensable part of health care (69). Global Surgery is now a rapidly evolving and increasingly recognized discipline. However, while global surgery has been the unrecognized field of global health, vascular surgery is the unrecognized field of global surgery. Vascular surgery is barely mentioned in the report by the Lancet commission and while prevalence of PAD is high and increasing, especially in LMICs, studies suggest that vascular procedures and amputations are performed infrequently and in some settings more often for trauma than for PAD (69,70).

Surgery of AAA requires resources that are scarce in large proportions of the world. Such necessities include access to blood, anesthesia, intensive care and if EVAR is considered, interventional angiography suites and repeated post-operative surveillance (2,3,69). The disease is asymptomatic until rupture occurs and rupture causes death rather than a resource-demanding disability, preferentially in elderly members of society, creating small cost incentives to prioritize treatment of AAA. As such, and without a working medical treatment, the diagnosis of AAA is of little use to a patient who lives in a setting were the above-mentioned infrastructure is missing or inaccessible. Further, the diagnosis itself requires imaging technologies such as US and CT. These reasons cause an underestimation of global AAA prevalence. The true prevalence of AAA, however, is likely to rise due to increasing age and pervasiveness of smoking, hypertension and hyperlipidemia (65,68).

These issues can be attacked from several different angles. In addition to crucial investments in surgical and anesthesiologic infrastructure and providers, increased capability of LMICs to diagnose and treat PAD would be required to meet future needs (69,70). With AAA specifically, improving the specificity and increasing the simplicity of diagnosis and surveillance algorithms, as well as the development of pharmaceutical agents to decrease the diameter growth rate and prevent rupture, would greatly lower the bar for adequate care in low-resource settings.

1.1.8 A brief history of abdominal aortic aneurysm

The first known complete description of the systemic circulation of blood by the beating heart was provided by William Harvey in 1628, before which the respiratory and circulatory systems were considered to be continuous (71). The first preserved account of aneurysms can be found as early as in the Papyrus Ebers (Figure 5), an Egyptian scroll describing diseases and remedies from around 1550 BC, which is thought to be based on even earlier literature (72). The physician Galen and the surgeon Antyllus, both of Greek origin active in Rome in the second century AD, also mentioned aneurysms in their writings and the latter described an operative technique comprising proximal and distal ligation, central incision of the aneurysm, and evacuation of the intraluminal thrombus. This method was used for the next 1000 years (73).

Early development of surgery for AAA beyond simple ligation was undertaken in the eighteenth and nineteenth centuries, as their natural course was described, retroperitoneal aortic exposure was conceived and endovascular repair by use of coiling was attempted. In the end of the nineteenth century, electricity was used to try to thrombose the aneurysm by letting current pass through the packing wires (73). A method tried in early twentieth century

by Rudolf Matas was ‘endoaneurysmorraphy’ where the aneurysm was opened and plicated over a catheter which was removed before closing (74). The reinforcement of an aneurysm by wrapping its outside with cellophane was later proposed by Rea and used to by Rudolf Nissen to treat the likely symptomatic AAA of Albert Einstein which ruptured five years post operation in 1955 (75). These methods were not durable and important strides towards long-lasting solutions were made when Charles Dubost performed aneurysm resection and interposition graft of a cadaveric aortic allograft, the complications of which were drastically reduced when Oscar Creech in 1966 combined this method with Matas’ endoaneurysmorraphy and sutured the graft in place inside the aneurysm sac without sac resection, thus shortening operating time and avoiding damage to adjacent structures such as the vena cava (76,77). The substitution of the aortic allograft with a synthetic dacron graft was described by DeBakey and Cooley in 1958 (78) and this method remains the standard procedure of OAR.

The late twentieth and early twenty-first centuries saw the development and widespread adoption of endovascular repair of AAAs. The first publication describing a successful endovascular placement of an aortic graft was in 1987 by Nicolai Volodos to treat a false aneurysm of the thoracic aorta (79). The breakthrough of EVAR came, however, after Juan C. Parodi and Julio Palmaz in 1991 published a case series of 5 patients with AAA on whom the endovascular placement of an aortic stent-graft was performed (80). In subsequent years, the care of patients with AAA was given a strong evidence base as the diameter threshold for surgical repair, surveillance intervals, screening for AAA and outcomes after OAR and EVAR were established through landmark randomized trials, described above (30,31,33,37–40,49,50,52–54,64).

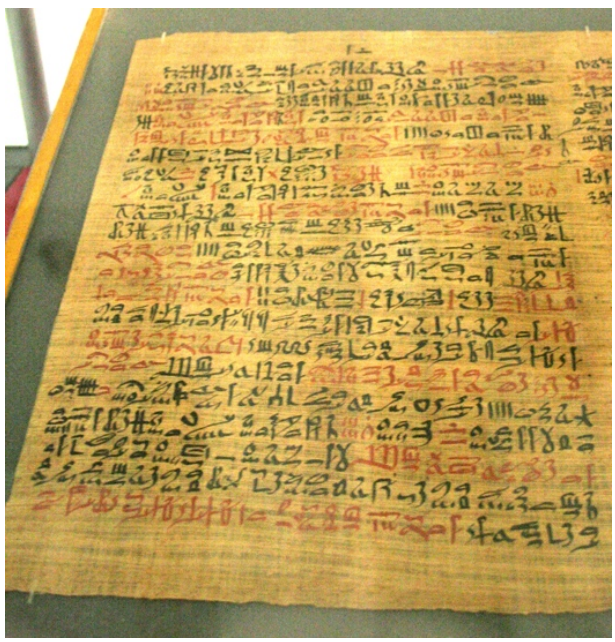


Figure 5. The Papyrus Ebers. By user Einsamer Schütze licensed under CC-BY-SA-3.0,2.5,2.0,1.0.

1.2 PATHOPHYSIOLOGY

1.2.1 The normal aorta

The aorta is the body's major blood vessel and courses from the left ventricle of the heart, in a thoracic arch and descends into the abdomen where it bifurcates into the iliac arteries (Figure 6A). The normal aorta is an elastic artery that consists of intimal, medial and adventitial wall layers (Figure 6B). In simple terms, the infrarenal aorta consists of around 36% collagen, 23% vascular smooth muscle cells (vSMC), 23% elastic fiber and 18% ground substance by dry weight (81).

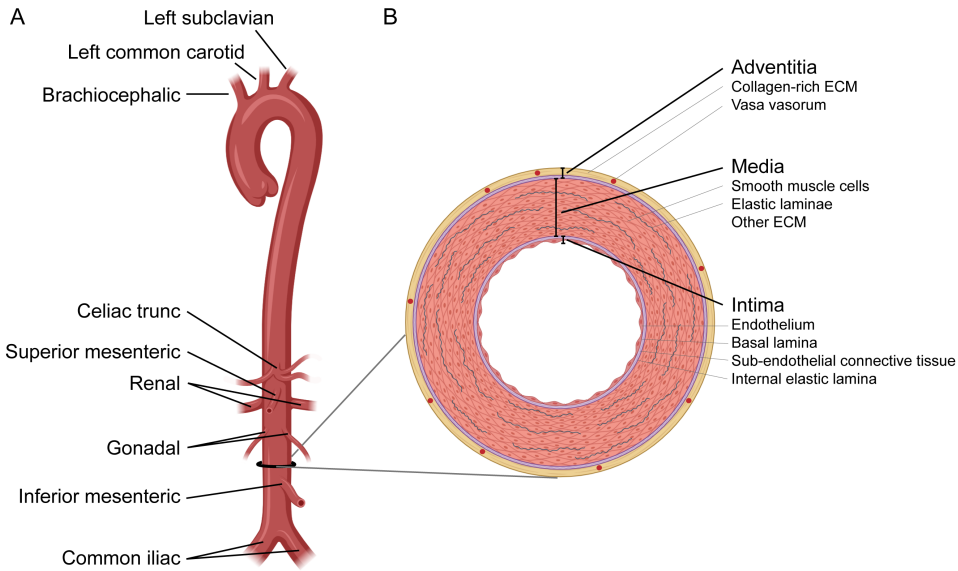


Figure 6: The normal aorta. A: Aortic branches. B: Basic histology of the aorta.

The aortic intima consists of a monolayer of glycocalyx-covered endothelial cells adhering to a basement membrane and loose connective tissue, separated from the media by an internal elastic lamina. The endothelium is the inner lining of the vascular system and plays an active role in the maintenance of vascular homeostasis and health. It regulates the tone of the vSMCs in the underlying media, is selectively permeable to cells and macromolecules while maintaining a non-thrombogenic surface against circulating blood. Glycocalyx refers to the negatively charged glycoproteins and proteoglycans of the endothelial cell membrane which appear to have atheroprotective effects and if the glycocalyx is damaged, increased permeability, platelet aggregation and decreased vascular responsiveness have been shown to follow (82). The endothelium senses changes in biomechanical forces such as shear stress and tensile stress / cyclic stretch by means of mechanoreceptors, which, in addition to the production of NO that relaxes underlying vSMCs and inhibit platelet aggregation and thrombosis, results in production of other vasoactive agents, growth factors, cytokines and affects its permeability (82). The endothelial cells are able to produce elastin and may contribute to the formation of the inner elastic lamina (83). Components of the basement membrane include collagen types IV, XV, XVIII as well as laminins, perlecan and agrin (83–85).

The three-dimensional (3D) structure of the aortic media is complex, with circumferentially oriented elastic lamellae and their radial protrusions, connected via radial intralamellar elastic struts, interspersed with dense collagen fibers and ground substance tightly interwoven with vSMCs, all of which are mainly oriented in the circumferential direction (86). The differentiated vSMC is contractile and typically express markers related to the contractile machinery such as smooth muscle alpha actin, myosin-11 and smoothelin (87). However, the vSMC have been shown to be highly plastic and may de-differentiate in changing conditions (such as an injury) to a less contractile, more synthetic and proliferative phenotype, even transdifferentiating into macrophage-like cells, with emerging significance in several diseases (87). The vSMCs are important to actively distribute stress among aortic wall components and to maintain extracellular matrix (ECM) structure and homeostasis by producing structural proteins and matrix-degrading enzymes (88). However, they do not appear to affect the *passive* mechanics of large, elastic arteries (89). These properties arise from the complex, layered ECM and should include elasticity and compliance so that the aorta distends during systole and recoils in diastole, dampening blood pressure fluctuations and maintaining continuous blood flow, with minimal energy loss, but the aorta should have high strength and its rate of stiffening should also increase with increasing pressure (non-linearly) to avoid rupture. Key constituents include elastic laminae, collagens and proteoglycans (83). The elastic fibers, which are complex structures consisting of polymerized tropoelastin surrounded by microfibrils and over 30 different associated proteins assembled in an intricate, step-wise manner, are highly distensible and yield low tensile strength but lends the aorta its elasticity (83,90,91). Main proteins associated with the elastic fiber are elastin, fibrillins, fibulins, emilins etc as well as lysyl oxidase which cross-links the tropoelastin monomers into polymers (91). Importantly, while its production is tightly regulated during development, elastin is generally not replaced thereafter, and the classic view has been that the same elastic fibers need to last throughout the lifetime of the aorta (92). The elastic fibers are indirectly connected to the contractile machinery of the vSMC and these form together a contractile unit which can respond to aortic stress (88). Collagens are present throughout the aortic wall and generally provide stiffness and strength. Many types of collagens exist and they may be fibril forming, such as types I, II, III and V, while types IV, VIII and X form networks (93). Collagen IV is the major structural protein of the basal lamina. The distribution of main arterial collagens I, III and IV differ depending on the aortic region; in the fetal bovine *abdominal* aorta types I and IV mainly localize to the intima and media wall layers, whereas type III localizes to adventitia. The turnover rate of collagen in the normal aorta far exceeds that of elastin (92,94). Ground substance refers to the non-fibrous aspect of the ECM, where proteoglycans with covalently attached glycosaminoglycans are important constituents. Large proteoglycans present in the aortic wall include versican and aggrecan, which bind the major glucosaminoglycan hyaluronan (83,85). Small leucin rich proteoglycans such as decorin, biglycan and lumican bind to other structural ECM components rather than glucosaminoglycans and are involved in fibrillogenesis but expanding literature also suggests roles in cell growth, differentiation and inflammation (83,85). Vascular glycoproteins include fibronectin and vitronectin, of which the former is well studied with important roles in adhesion, migration, growth and differentiation (95). Biomechanically, ground substance on a whole is insufficiently studied but seems to contribute to aortic mechanics, particularly 'residual stress' (96), which can be explained as the stress a solid material imparts on itself when external stressors are removed. The importance of ground substance and residual stress has long been recognized in for example cartilage tissue (97).

The adventitia is delineated by the outer elastic lamina of the media and encompasses collagen-rich ECM, fibroblasts, immune cells, vasa vasora, lymph vessels and nerves, which gives it high tensile strength and prohibits overstretching of the vessel, but also makes it

capable of responding to insults with fibrosis and recruitment of innate and adaptive immune cells (98). The fibroblast is responsible for the production of the strong ECM but have also been shown to be among the first cells to respond to insult, with activation, participation in ECM remodeling and expression of pro-inflammatory molecules (99). Further, the phenotype of a fibroblast may be altered into that similar to a vSMC (becoming a 'myofibroblast'), including the expression of contractile markers, making it impossible to safely distinguish fibroblasts and vSMCs by simple marker histology (99). Immune cell residents of the normal adventitia include macrophages and dendritic cells which, together with fibroblasts, are able to trigger an inflammatory response and may propagate centripetally (99).

There is significant anatomical heterogeneity of embryology, structure, physiology and susceptibility to disease along the length of the aorta, despite its appearance of a continuous and homogenous conductive tube. The vSMCs of different aortic segments arise from diverse embryologic origins, and boundaries have been approximated such that the thoracic aorta arise from neural crest cells (ascending and arch) and somites (descending) while the abdominal aorta originates from splanchnic mesoderm (100). Further, these cells appear to retain lineage-specific phenotypic characteristics, for example responding differently to transforming growth factor beta (TGF-β), with implications for clinical TGF-β-related, familial aneurysm-causing conditions such as Loeys-Dietz and Marfan syndomes, which more commonly affect the thoracic aorta (101–104). After development, the mature *thoracic* aorta has around 60 layers of medial elastic laminae and a thick vessel wall with a media supported by vasa vasorum. In contrast, the *abdominal infrarenal* aorta conveys a smaller blood volume, since the kidneys take up a significant share of it. As a result, it has a smaller diameter, half as many (around 30) medial elastic laminae and feature few medial vasa vasorum which makes it dependent on transintimal supply of oxygen and nutrients provided by the aortic intraluminal blood flow (105). In patients with AAA, the prevalence of concurrent thoracic aortic aneurysm is 28% in total with male prevalence for ascending and descending aneurysm being 2.2 and 21%, respectively. Female patients with AAA appear to have more aggressive generalized aneurysm disease with corresponding numbers of 7.5% and 46% (29).

1.2.2 Atherosclerosis and aneurysm: two distinct conditions with overlapping risk factors

While the common, degenerative, AAA has been colloquially referred to as an 'atherosclerotic' aneurysm, this term is only half correct. Indeed, atherosclerotic occlusive disease shares many risk factors with AAA, such as age, hypertension and smoking (106–108). Female sex and particularly endogenous estrogen appear to have a protective effect against both conditions (109,110) and, further, PAD is in itself an independent risk factor for AAA (108). There is however clinical, histopathological (Figure 7) and genetic evidence to classify atherosclerosis and AAA as distinct conditions. Clinically, diabetes mellitus is an important driver behind coronary artery disease (106) and aortic occlusive disease (107) whereas it, on the contrary, appears to be protective against AAA prevalence (108) and growth (21). A potential confounder is the use of metformin, which has been proposed as a potential drug target against AAA (111). The histopathological pattern of an atherosclerotic artery is characterized by cholesterol accumulation, inflammation and necrosis that is almost exclusively confined to the tunica intima. In contrast, microscopic evaluation of AAA tissue reveals transmural inflammation with extensive loss of elastic laminae, apoptosis of vSMCs and extensive fibrosis resulting in a thoroughly altered vessel wall structure, compared to the normal artery (112). Finally, the two vascular diseases share some single nucleotide polymorphisms (SNP) but appear to feature distinct gene expression patterns (113,114).

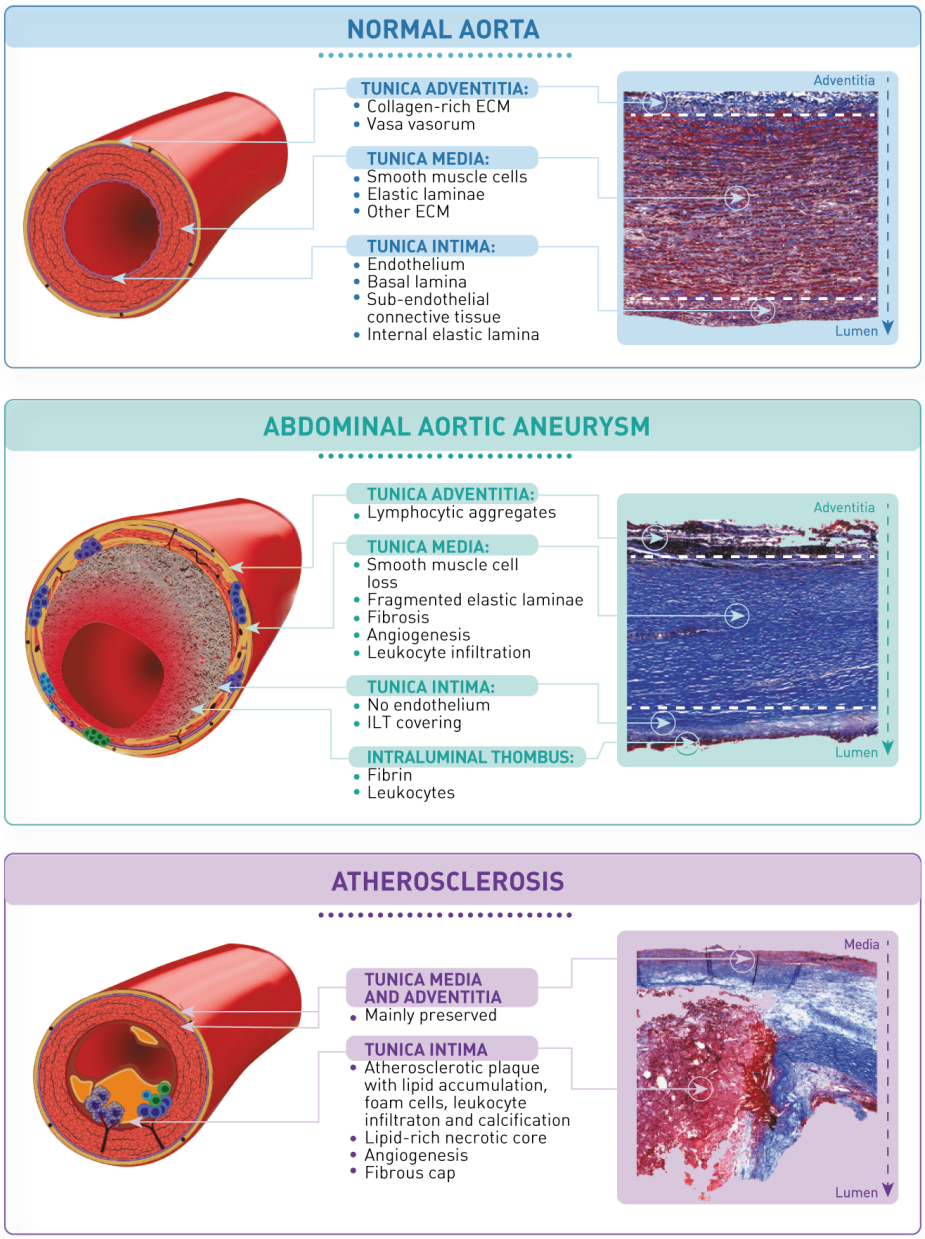


Figure 7: Histopathological characteristics of normal aorta, abdominal aortic aneurysm and atherosclerosis. Left column: schematic diagram of histolpathological characteristics. Right column: Masson's trichrome stains of normal abdominal aorta, abdominal aortic aneurysm and carotid atherosclerosis showing the distribution of cytoplasmic contents (red), fibrous connective tissue (blue) and cell nuclei (black).
Abbreviations; ILT: intraluminal thrombus, ECM: extracellular matrix

1.2.3 Extracellular matrix

Disruption of the medial extracellular matrix is one of the most striking features of aneurysm disease and is characterized by fragmentation of elastin, collagen turnover, reduction of ground substance and apoptosis of vSMC. Specifically, elastin concentrations are lower and elastase activity is higher in AAAs than in both normal and heavily atherosclerotic aortas, the latter from patients of similar age (114–116). The loss of elastin is accompanied by a mechanical compensation by increased production and deposition of collagen, mainly type I and III, by adventitial fibroblasts (81). However, the turnover rate of collagen in aneurysms is increased and the newly produced collagen does not orient in a layered structure as in a normal aorta and, further, display signs of impaired crosslinking (117–119). Consequently, it may be reasonable to assume that the fibrosis observed in AAA is protective and increases aneurysm strength to counter increasing stress but that the fibrillar quality and structure is hampered, predisposing the natural course to rupture. The significance of elastolysis and collagenolysis has been studied by injecting elastase and collagenase into canine aortas. It was found that degradation of elastin caused aneurysmal dilatation of the aorta but that the aortas did not rupture until the collagen was degraded as well (120). These results imply that dilatation, stiffening and widening of the aorta is caused by disruption of the elastic laminae but that imbalanced collagen turnover may underlie the occurrence of rupture. This hypothesis agrees with the material properties of elastin and collagen, which are characterized by high compliance and tensile strength, respectively. The loss of elastic laminae and the subsequent increase of collagen deposition are manifested by an increase in aortic stiffness associated with age and, to an even higher extent, with AAA (121,122).

Ground substance, ie proteoglycans, glucosaminoglycans and glycoproteins, is pathologically remodeled in AAA disease but studied to a lesser extent (81). Proteoglycans previously linked to aneurysm disease include potentially protective syndecan (123), biglycan (124), mast cell secretory granule-associated serglycin and collagen XV, which has been associated with severity of thoracic aortic aneurysm (125), collagen XVIII, which is a circulating marker of AAA diameter (126) and decorin, which when substituted can attenuate dissecting aneurysms in mouse models (127). Large proteoglycans versican and aggrecan have been shown to be decreased in AAA (128). The glycoprotein fibronectin is a fundamental ECM protein which is increased in AAA (128). Mouse experiments of atherosclerosis show that plasma fibronectin-deficiency decreased atherosclerotic plaque size, number and leukocyte recruitment but that lesions in the fibronectin-deficient mice also lacked a protective fibrous cap (129). Aberrant splicing of fibronectin has been suggested as a contributing factor to bicuspid valve-associated thoracic aneurysms (130).

Several molecular and cellular mechanisms important for the aneurysmal destruction of normal vessel architecture have been identified. On the enzyme level, matrix metalloproteases (MMPs) and cathepsins have been implied as culprits in aneurysm disease, while the inhibitors, referred to as tissue inhibitors of metalloproteases (TIMPs) and cystatin C, respectively, are putatively protective. The collagenases MMP-1 (‘interstitial collagenase’), MMP-2 (‘gelatinase A’), MMP-8 (‘neutrophil collagenase’), MMP-9 (‘gelatinase B) and MMP-13 (‘collagenase 3’), the elastolytic MMP-12 (‘macrophage metalloelastase’) as well as MMP-3 (‘stromelysin 1’), the latter able to degrade collagens, ground substance and elastin, have all been suggested as effectors of medial destruction (119,131–133). However, importantly, MMPs are produced in latent form and the level of enzymatic activity does not directly correspond to expression of the MMP gene or zymogen (132). Plasmin is able to activate MMPs but can also degrade pericellular proteins, such as fibronectin. Cathepsins are potent proteases that are primarily active in acidic pH, such as in endosomes and lysosomes, but have been shown to remain active in the extracellular matrix,

given an acidic pericellular environment (134). Cathepsins K, L and S are increased in AAA and cathepsins B, K and S are increased in cerebral aneurysms (135). Some of these peptides, in zymogen or enzyme form, are also increased in the peripheral blood of AAA patients. Circulating MMP-9, TIMP-1, TIMP-2 as well as Cathepsins L, S and V have been shown to be increased in patients with AAA, compared with those without (136). Neutrophil elastase, granzymes as well as mast cell chymase and tryptase have also been implicated in the destruction of ECM and/or activation of other enzymes in AAA (137). A disintegrin and metalloproteinase (ADAM) and ADAM with thrombospondin motifs (ADAMTS) are proteolytic enzymes that have received less attention than MMPs but have been shown to be associated with AAA disease (138–140). Finally, neutrophil elastase can cleave elastin but also fibrillar collagens, fibronectin, laminin and aggrecans and activate MMPs implicating the infiltration of the vessel wall by neutrophils as an important pathophysiologic component (137).

Recent efforts have been made to inhibit the ECM degradation in AAAs to reduce growth rates. Doxycyclin inhibits both inflammation and MMPs and the effect of this drug has been evaluated in three RCTs, one of which was published recently (141). Unfortunately, this treatment did not affect the AAA growth rate.

1.2.4 Loss and phenotypic modulation of vascular smooth muscle cells

Depletion of vSMC is another major hallmark of AAA disease. It has been demonstrated that AAAs contain a lower total number of vSCMs per cross-section, compared to normal aortas, and thus that the scarcity is not only a matter of the same amount of cells spread out on an enlarged vessel (142). The remaining vSMCs express markers of apoptosis (142). Several mechanisms behind the vSMC death have been suggested. Anoïkis, defined as the programmed cell death following loss of cell interactions with the underlying matrix, may be a factor induced in part by the degradation of fibronectin by plasmin and inflammatory cell-derived chymase (143). Other suggested mechanisms include T-cell induced apoptosis and oxidative stress (142,144).

The vSMC is a highly plastic cell type and can switch from a contractile to a synthetic, proliferative and even macrophage-like phenotype (87). This de-differentiation with potential for clonal expansion, for which transcription factor KLF4 has been described as central, has been shown to be important in experimental vascular disease by lineage tracing experiments in mouse models of atherosclerosis and neointima formation (145,146). Downregulation of contractile markers transgelin and smooth muscle alpha actin has previously been shown in vSMCs of a mouse aneurysm model (147). In line with these results, the vSMCs in a lineage-tracing, murine model of dissecting aneurysms were shown to undergo clonal expansion, de-differentiation and expression of phagocytic markers (148).

1.2.5 Immunity and inflammation

The AAA vessel wall is known to be infiltrated by inflammatory cells and masses of lymphocytes were noted upon histological examination already by Dubost et al in their landmark AAA resection paper in 1952 (76). This chronic aortic inflammation is believed to contribute to ECM destruction and vSMC apoptosis (116). The AAA adventitia and outer media are highly infiltrated by T- and B-lymphocytes, plasma cells, monocytes/macrophages, natural killer cells, and mast cells (98,116,149,150) which form activated lymphoid tissue within the aneurysm wall. Several of these leukocyte subsets are also concurrently increased in peripheral blood (151). Several indications of autoimmunity in the common, degenerative, AAA have been published. Infiltrating T-and B-lymphocytes express activation markers and

are flanked with professional and nonprofessional antigen-presenting cells. Further, plasma cells of the aneurysm wall are active and IgGs purified from the aneurysm wall react against a number of auto-proteins (149).

Interferon gamma (IFNγ, IFNG) is an important regulator of immune responses with diverse actions on antigen processing, inflammation and autoimmunity (152). Specifically, IFNγ is produced by activated T-cells, NK-cells and macrophages and activates macrophages, increases expression of major histocompatibility complexes I and II, promote NK-cell activity, induces class switching in B-cells and pushes T-cells into a T_h1 phenotype, ie increasing cell-mediated responses with macrophages and CD8 T-cells as effectors, while suppressing the humoral-response T_h2 phenotype (152,153). The literature pertaining to the role of IFNγ in AAA show divergent results. In human AAA, IFNγ and T_h1-cells have been shown to be increased in both vessel wall and peripheral blood and T_h2-type phenotype and related cytokine IL4 were suppressed (154,155). In animal models, however, results vary such that IFNγ appeared protective against aneurysm in mouse models using angiotensin infusion (which also causes dissection) and aortic allograft transplantation (156) but instead promoted aneurysm in $CaCl_2$-application (157) and elastase perfusion models (158). Macrophages have consistently been found in human AAA and are well-described in mouse models, and a cross-talk between them and T-cells involving IFNγ is likely (137).

Other important pathways of inflammation investigated in AAA disease are that of NF-κB, IL6, and inflammasome. Stimuli of NF-κB include reactive oxygen species, hypertension and MMP9 while target genes include TNF, IL1, IL6, COX2 and several chemokines (159). Suppression of this pathway has been protective against aneurysm in animal models (159). The IL6 pathways can be activated either by activation of a membrane-bound or soluble receptor (IL6R). A common non-synonymous sequence variant of IL6R that results in increased cleavage of membrane-bound IL6R and therefore higher levels of IL6R in circulation but also decreased levels of downstream signaling has been associated with a lower risk of AAA in a Mendelian randomization study and a smaller aneurysm diameter in a recent meta-analysis (160,161). Further, while previous animal models could not see a strong effect of blocking IL6R generally, the latter investigation showed that specific intervention against the soluble receptor increased survival in two murine aneurysm models (159,161). Finally, the multiprotein complex known as ‘inflammasome’, which is a critical mediator of the innate immune system’s response to danger- or pathogen-associated molecular patterns, has been shown to be active in human AAA and its suppression has been shown to be protective in animal models (159).

Many interventions against different branches of the immune system will attenuate experimental aneurysm formation (112). However, these models mostly focus on stopping the formation of, rather than treating, aneurysms that moreover are results of an extreme molecular insult, such as elastase perfusion, $CaCl_2$-application and angiotensin II-infusion. Importantly, these results appear difficult to translate into human disease as worryingly high prevalence, growth and rupture rates of AAAs in association with aggressive immunosuppressive treatment have been observed (162,163). The only completed RCTs testing anti-inflammatory drugs against human AAA were those of mast cell inhibitor pemirolast, motivated by the protection against experimental rodent aneurysms by mast cell-deficiency and -inhibition (150,164), and doxycycline which has anti-inflammatory and MMP-inhibiting properties (141). These interventions, however, did not affect aneurysm growth rate (141,165).

1.2.6 Hypoxia, oxidative stress and angiogenesis

Oxidative stress has been proposed as an important feature in AAA disease, as it is increased in inflammation as well as in AAA, further promotes inflammation, effects of matrix-degrading enzymes and inducing smooth muscle cell apoptosis (144). Neutrophils, macrophages, endothelial cells, vSMCs and fibroblasts are capable of producing oxidant species which in turn may cause upregulation of chemotactic cytokines, activation of MMPs, and induce vSMC apoptosis via NADPH oxidase (166). Antioxidants that have been protective against aneurysms in experimental models include catalase, tamoxifen, reservatrol as well as vitamins C and E (167). Other examples of antioxidants are metallothioneins, which bind trace metals and are downregulated in bicuspid valve-related ascending thoracic aneurysms (168). Oxidative stress induced by cigarette smoke exposure has been shown to induce phenotypic switching in vSMCs and by blocking this pathway, experimental cerebral aneurysms could be attenuated (169). Additionally, the vessel wall of AAA display signs of hypoxia and expression of hypoxia-inducible factors (HIFs) with regulating long non-coding RNA H19 being able to cause matrix degradation and vSMC apoptosis (170–172).

In contrast to the normal abdominal aorta (105), the AAA vessel wall exhibit angiogenesis with increased vasa vasorum density pronounced specifically in the outer media and the adventitia of the aneurysm (173). This angiogenesis is associated with inflammation (174) but also matrix degradation (175), making it highly relevant to aneurysm disease. Specifically, the formation of new vessels in the aneurysm media is dependent on proteolysis of extracellular matrix by MMPs, especially MMP-2 and -9, which have been described as central in AAA pathophysiology, are well studied in this regard (175). Indeed, high levels of medial neovascularization have been observed at the site of rupture (176). It is highly likely that angiogenesis facilitates recruitment of inflammatory cells to the vessel wall and is directly and indirectly involved in mechanical weakening of the same.

1.2.7 Other signaling pathways

Transforming growth factor beta (TGF-β, TGFB) is an important regulator of several biological processes such as immune and stem cell regulation and differentiation. Signaling typically occurs when TGF-β ligands such as TGFB1, -2 and -3 binds to receptors TGFBR1, -2 and co-receptor -3 and proceeds downstream with SMAD-dependent, ‘canonical’, or SMAD-independent, ‘non-canonical’, pathways (159). Mutations in TGF-β-related signaling can cause hereditary aneurysm disease, chiefly Loeys-Dietz syndromes, making it an interesting pathway to study in AAA as well (177). Some TGF-β-related genes are downregulated in human AAA (178) and rodent models have indicated a protective role of overexpression of TGF-β signaling, while its inhibition worsens experimental aneurysms (159,179). Cellular effects of TGF-β includes increased elastin synthesis by vSMCs, increased collagen synthesis by fibroblasts along with decreasing MMP9 and macrophage activity (159). Along those lines, and in line with emerging evidence of phenotypic switching in aneurysm pathogenesis, a recent study demonstrated that SMC-specific TGFB-deletion in mice prone to atherosclerosis caused aneurysms and dedifferentiation of subsets of contractile SMCs (180).

Other signaling pathways examined in AAA include Notch and mitogen-activated protein kinase (MAPK). Notch signaling is highly evolutionarily conserved and acts via cell-cell communication between juxtaposed cells on a vast number of biological processes such as development, including that of vasculature, heart and blood cells, as well as homeostasis with tasks such as regulation of vSMC plasticity, sprouting angiogenesis, B-cell differentiation and T-cell production (181). While human data is contradictory, suppression of Notch

signaling is protective in angiotensin II mouse models of aneurysm/dissection (159). MAPK is another hugely diverse signaling pathway which can be activated by growth factors, cytokines, hormones or cellular stress and regulates processes such as proliferation, differentiation, survival and death (182). The MAPK family comprises kinases Jun amino-terminal kinases (JNK), p38 and extracellular signal-related kinases (ERK). JNK is increased in human AAAs and its inhibition protects against experimental aneurysm, with decreased degradation and increased biosynthesis of ECM among proposed mechanisms. Similarly, ERK is increased in human AAA and can be knocked-out to prevent aneurysm in mouse models (159).

1.2.8 Effect of smoking

Smoking is a strong clinical risk factor for developing AAA, exhibiting a clear dose-response relationship (8). Importantly, this dose-response relationship also extends to cessation of smoking in that a decreasing odds ratio of developing AAA is seen with increasing time after quitting. Causality is further supported by a Mendelian randomization study demonstrating a strong association between AAA and genetic variations that predispose an individual to smoke (183). Cell experiments searching a mechanistic explanation have shown that cigarette smoke extract (CSE) causes decreased collagen and increased MMP production in vSMCs, with the latter being true also for inflammatory cells (184). It has also been shown that conditioned media from CSE-stimulated macrophages stimulate MMP2 and -9 expression in vSMCs and endothelial cells as well as the phosphorylation of JAK2 and STAT3 (185). Blocking JAK3/STAT3 appears to inhibit experimental aneurysm formation (186). While soluble smoke particles *increased* proliferation and survival of vSMCs, this may be in accordance with emerging hypotheses of phenotypic switching as a mechanism behind AAA (145,180,187). Further, vSMCs exposed to CSE expressed increased NADPH-oxidase, reactive oxygen species, proinflammatory and matrix remodeling genes while expression of markers of the contractile phenotype decreased. In this experiment, CSE caused increased formation and rupture of *cerebral* aneurysm in mice, whereas blocking NADPH oxidase diminished this effect, leading the authors to conclude that smoking can cause cerebral aneurysms by oxidative stress-dependent phenotypic modulation of vSMC (169). While cell experiments can give mechanistic information, limiting assumptions reviewed in (184) include that the toxin mixture would elicit the same response when applied in vitro as when inhaled in vivo, that the response is similar in a culture as in a complex in vivo environment and that this exposure would activate known pathways. A process in which inhalation of smoke triggers reactions in other organs that affects the aorta in a second stage is a feasible hypothesis.

A rigorous investigation showed in 2013 that tobacco smoke exposure exacerbated elastase-induced AAA in mice, even after a smoke-free interval up to six weeks before elastase perfusion, and that this exacerbation could not be stopped by MMP-inhibition by doxycycline or the genetic knock-out of MMP9, MMP12, Cathepsin S or Neutrophil elastase (188). Instead, the number of T-lymphocytes increased in smoke-exposed animals and smoke-free animals receiving leukocyte preparations from smoke-exposed animals exhibited an exacerbation of elastase-induced aneurysm, suggesting that durable effects of smoking on immune cells may partake in the pathogenesis of AAA. It seems reasonable, given the strong clinical association and indication of direct causality, together with *in vitro* and *in vivo* data, that the key to developing a working treatment against AAA could lie in the analysis of the effect of smoking on the abdominal aorta. Indeed, the only non-surgical intervention to decrease AAA prevalence, growth rate and risk of rupture appears to be the cessation of smoking (8,21).

1.2.9 Influence of patient sex

It is not yet known why women have a lower risk of developing AAA and why those that do have an increased risk of rupture compared with men. Several experimental studies have been performed to understand these findings (189). Male sex hormones seem to aggravate disease as male rodents with aneurysms produced by Angiotensin II-injection or local elastase infusion get larger and more frequent aneurysms, against which orchidectomy is protective unless testosterone substitution is administered (190–195). Knocking out the androgen receptor in vSMCs and macrophages reduces experimental aneurysm (196). Female sex hormones appear to be protective, as female rodents get smaller experimental aneurysms and estradiol treatment protects against aneurysm in male rodents (191,193,197). Further, an aorta from a female donor rat into a male recipient rat was not protected against aneurysm development by elastase perfusion (191). There is, however, conflicting evidence on the effect of ovariectomy on rodent aneurysm development (190,193,197). Mechanistically, female sex hormones have a suppressive effect on macrophage and neutrophil infiltration, MMP9-activation and -production, inflammatory signaling and seems to increase collagen and TGF-β production (192–194,197,198). Injection of mesenchymal stem cells seem to be protective against elastase-induced aneurysms in mice, with the strongest effect on aneurysm and inflammatory profile seen if the stem cells were collected from female mice (199). Female mice with XY chromosome complement develop severe and rupture-prone angiotensin II-induced aneurysms, with signs of increased inflammation and oxidative stress, compared to female mice without (200). The XY chromosome complement in male mice caused diffuse aneurysmal dilatation whereas XX complement caused focal dilatation (201). *In vitro*, MMP-9 and TIMP-1 expressions are lower in vSMCs explanted from female rat aortas, compared to those from male rat aortas (202).

In humans, women who do develop AAA have an early menopause, which is congruent with above preclinical data (203). Female patients with AAA further have a clinically increased risk of rupture (203), which the observed sex-related differences in elastin content (204) and collagen cross-linking (205) may contribute to. The body surface area (BSA) of women is lower, on average, compared with that of men and it could be argued that the aortic dilatation at a specific diameter threshold is generally larger relative to the rest of the patient in women compared with men, explaining why smaller aneurysms in women behave like larger aneurysms in men (58,206,207).

1.2.10 Genetics

Aortic aneurysms can arise in the context of Mendelian or non-Mendelian forms of inheritance and genetic alteration. While monogenic mutations typically affect the thoracic aorta, some syndromes that may involve AAA include Marfan, Loeys-Dietz and Ehlers-Danlos, resulting from mutations in FBN1, TGF-β-axis and COL3A1, respectively (208). For the common AAA, a large number of genetic studies have been performed and polymorphisms regions located in or near genes related to ECM, inflammation/immunity, TGF-β-pathways and those associated with cardiovascular disease have been implicated (209).

Genome-wide association study (GWAS) is a method used to screen for common genetic variants (allele frequency of > 1%) across the genome for association with phenotypic traits (210). Several GWAS have been included in a meta-analysis by Jones et al (211). The authors found SNPs at 9 loci near the genes PSRC1-CELSR2-SORT1, IL6R, CDKN2BAS1/ANRIL, DAB2IP, LDLR, SMYD2, LINC00540, PCIF1-ZNF335-MMP9 and ERG. With the exception of loci near PSRC1-CELSR2-SORT1, CDKN2BAS1/ANRIL and LDLR, these

seem to be specific for AAA. The identified SNPs were associated with transcription factor binding site affinity variations and eight of them had potential for long-range interactions, with two affecting expression of proximal genes in normal arterial tissue (expression quantitative trait loci, eQTL). Rarer single nucleotide variants have been quantified in patients with AAA and controls by use of whole-genome sequencing, compiled into a genetic risk score and used together with clinical characteristics to diagnose AAA (212).

1.2.11 Transcriptomics

The word transcriptome refers to the entirety of RNA molecules produced by a cell, tissue or organism (213). While the genome of an individual is identical throughout its body and lifetime, with important exceptions such as uncorrected DNA damage or specific rearrangements in for example lymphocytes, the pool of RNA produced by a cell, set of cells, tissue or organ at a certain time and given any type of stimulation is specific. The transcriptome is thus a snapshot; cropped in time and space. Researchers were long forced to study the expression of one gene at a time, which was time-consuming and laborious. However, the mid-90's saw the advent of microarray technology, which for the first time could measure the (relative) expression of all known protein-coding and later non-coding genes at the same time. Further strides were taken with the introduction of RNA-sequencing, which enabled absolute quantification, de novo-sequencing and nucleotide-level differences between transcripts, as well as single cell RNA-sequencing which resolves these features to the transcriptome of single cells (214). Transcriptomics by microarray or RNAseq also allow querying which functional groups of genes, 'gene sets' or 'pathways', are enriched in a sample (215,216). As these technologies became and are becoming accessible, the use of transcriptomic approaches is now widespread (Figure 8). Features and limitations of microarray analyses are discussed in section 3.8

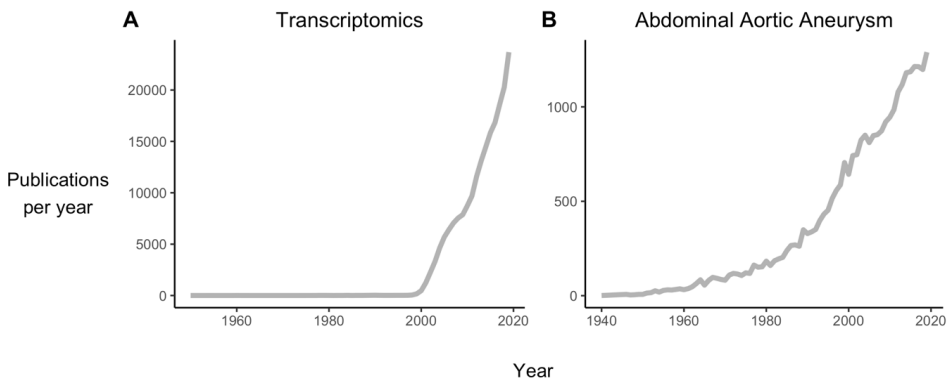


Figure 8: PubMed search results for publications about transcriptomics and abdominal aortic aneurysm. A: search term: (microarray* OR transcriptom* OR "RNA sequencing" OR "RNA seq"). B: search term: "abdominal aortic aneurysm".

As alluded to in previous sections, the common, degenerative AAA seems to be of a highly complex and multifactorial nature and finding a singular disease-causing gene, protein or cell type is an unlikely prospect. Instead, recent investigations into aneurysm disease involved genome-wide expression analyses by use of microarrays on both tissue and peripheral blood (Table 2). Microarray-based comparisons between full-thickness tissue from AAA and control aorta (the latter from autopsies or organ donation) have shown upregulations of pathways related to immune functions such as those related to NK-, T- and B-cells as well as complement cascade, cytokine and chemokine signaling, whereas downregulated genes

included those related to muscle cells and lipid metabolism (113,217–219). These immune pathways seem to be upregulated even in the non-aneurysmal proximal neck just below the renal arteries (220). Other studies have focused on ruptured AAAs, comparing either paired samples from the same patient, ie non-ruptured with ruptured areas in the aneurysm, or comparing tissue from patients treated electively or for rupture. These analyses have pointed to an association between rupture and angiogenesis, immunity/inflammation and adipogenic degeneration (221–223). An intriguing pathophysiological observation was made when vSMCs from AAA, compared with those from carotid endarterectomies and nondilated aorta from organ donors, were shown to be transcriptionally distinct, overexpress MMP2 and -9 mRNA and protein as well as having an increased elastolytic activity (224).

Several studies have looked for non- or minimally invasive correlates with AAA biology, such as imaging and blood tests. The RNA from a blood sample of a patient with AAA differs significantly from that of healthy controls, with increased expression of genes related to apoptosis, immunity and proteolysis, but also differs from blood taken from patients with carotid artery stenosis (225,226). One study examined the association between global gene expression and a signal on positron emission tomography (PET) CT, whereas others have compared regions of high and low biomechanical wall stress or rupture risk (227–229). Finding non-invasive methods (or minimally invasive, such as a blood test) that can reveal active processes in the aneurysm vessel wall is an attractive approach to improving predictions of growth, risk of rupture or complications after EVAR.

Table 2: Transcriptomic investigations of mRNA expression in humans with abdominal aortic aneurysm

Publication, Authors	Year, journal, data availability	Platform(s)	Experiment	Key Results
"Whole Genome-expression Profiling Reveals a Role for Immune and Inflammatory Response in Abdominal Aortic Aneurysm Rupture" **Choke E et al.**	2006 Ann N Y Acad Sci	Affymetrix Human Genome U133A	Paired, full-thickness tissue samples from aneurysm rupture edge and anterior vessel wall of 12 patients. Samples were pooled into triplicates.	Dysregulation of genes related to immune and inflammatory responses
"Global expression profiles in human normal and aneurysmal abdominal aorta based on two distinct whole genome microarray platforms." **Lenk et al.**	2006 Ann N Y Acad Sci NCBI GEO: GSE7084	Affymetrix Human Genome U133 Plus 2.0 Array Illumina Sentrix Human-6 Expression BeadChip	Full-thickness tissue samples of from 7 AAAs and 7 controls from autopsy. Matched for age, sex and ethnicity. Affymetrix: 4 pools. Illumina: 13 induvidual samples and 2 pools. Genes considered expressed in both platforms were analyzed.	Overrepresentation of genes related to immunity and defense, cell communication as well as cell structure and motility

1) "Gene expression profiling of peripheral blood in patients with abdominal aortic aneurysm." **Giusti et al.** **2) "Carotid Artery Disease: Novel Pathophysiological Mechanisms Identified by Gene-expression Profiling of Peripheral Blood"** **Rossi et al.**	1) 2009 Eur J Vasc Endovasc Surg 2) 2010 Eur J Vasc Endovasc Surg ArrayExpress: E-mexp-1346	Human AROS v1.1, Operon Technologies	Total RNA from venous blood 1) 10 AAA patients, 10 healthy controls Analyzed in four pools (2x5 AAA patients and 2x5 healthy controls) 2) 10 carotid artery stenosis (CAS) patients, 10 healthy controls. Analyzed in four pools 2x5 carotid patients and 2x5 healthy controls. Results compared with 1). Two replicates with dye swap.	1) Increased expression of hemoglobin changes and genes involved in erythrocyte stability. 2) Dysregulation of immune response in CAS, lipid metabolic processes in AAA.
"Smooth muscle cells from abdominal aortic aneurysms are unique and can independently and synergistically degrade insoluble elastin." **Airhart et al.**	2014 J Vasc Surg	Affymetrix HG-U133 Plus GeneChip and Illumina Human HT-12 v4 Expression BeadChip	Microdissected vessel wall layers; intima, media and adventitia. vSMC from 22 AAAs, 29 CAS and 17 nondilated abdominal aortas. vSMC lines pos for alpha smooth muscle actin, neg for CD11c, CD20, CD3, CD31 and CD68.	vSMC from AAA have a unique transcriptome and they have an elastolytic phenotype which can be amplified by macrophages.
"Differential gene expression in the proximal neck of human abdominal aortic aneurysm." **Biros et al.**	2014 Atherosclerosis NCBI GEO: GSE47472	Illumina HumanHT-12v4 Expression BeadChip	Full-thickness tissue samples from neck of 14 AAAs and juxtarenal aorta of 8 organ donor controls, no significant difference in age and sex between AAA patients and controls.	Upregulation of immune pathways in the non-aneurysmal aorta proximal to the AAA.
"Gene Expression Study in Positron Emission Tomography–Positive Abdominal Aortic Aneurysms Identifies CCL18 as a Potential Biomarker for Rupture Risk" **Courtois et al.**	2014 Mol Med	Affymetrix U133Plus2.0 chip	12 AAA patients. 6 AAAs patients with ^{18}F-FDG uptake, tissue samples from areas with and without uptake. 6 AAA patients without ^{18}F-FDG uptake. Adventitia and Media were separated, Samples were pooled into groups.	Genes related to matrix remodeling, inflammation and osteochondral development were differentially expressed in areas with ^{18}F-FDG-uptake compared with areas without.
"Differential gene expression in human abdominal aortic aneurysm and aortic occlusive disease." **Biros et al.**	2015 Oncotarget NCBI GEO: GSE57691	Illumina HumanHT-12v4 Expression BeadChip	Full-thickness tissue samples from: 49 AAAs, 9 aortas with atherosclerotic occlusive disease and 10 organ-donor controls, significantly more women in control group but similar ages.	The transcriptomes of AAA and aotherosclerotic occlusive disease are uniqe and there is minimal overlap of dysregulated genes compared with organ donor controls
"Microarray-based Gene Expression Profiling of Abdominal Aortic Aneurysm" **Butt et al.**	2016 Eur J Vasc Endovasc Surg	Illumina Human HT-12 BeadChips	Blood. 12 AAAs 12 healthy age- and sex-matched controls	Genes related to proteolysis, inflammation and apoptosis are dysregulated in the peripheral blood of patients with AAA
"Increased Expression of Lamin A/C Correlate with Regions of High Wall Stress in Abdominal Aortic Aneurysms" **Malkawi et al.**	2015 Aorta (Stamford)	Illumina Human Ref-8 vs. 3.0 Bead Chips	Paired full-thickness tissue samples from 3 AAAs. Samples from high- and low- wall stress regions of the aneurysm.	Lamin A/C is increased in areas of high wall stress in AAA.

"Gene Expression Profiling in Abdominal Aortic Aneurysms After Finite Element Rupture Risk Assessment" **Erhart et al.**	2017 J Endovasc Ther	Illumina HumanHT-12 v4 Expression BeadChip	Paired full-thickness tissue samples from 6 AAAs. Samples from aneurysm regions with high and low rupture risk.	Genes related to ECM were increased in areas with high biomechanical rupture risk.
"Molecular Fingerprint for Terminal Abdominal Aortic Aneurysm Disease." **Gäbel et al.**	2017 J Am Heart Assoc NCBI GEO: GSE98278	Illumina HumanHT-12 v4 BeadChips	Full-thickness tissue samples 31 elective AAAs 17 ruptured AAAs	Upreguation of genes related to angiogenesis and adipogenesis in ruptured AAA, these appear to be coordinated by HIF-1α signaling
"Genome-Wide Expression Profiling Unveils Autoimmune Response Signatures in the Perivascular Adipose Tissue of Abdominal Aortic Aneurysm" **Piacentini et al.**	2019 Arterioscler Thromb Vasc Biol NCBI GEO: GSE119717	Illumina HumanHT-12 v4 BeadChips	30 AAA patients (7 large diameter, 23 small diameter) Samples of periaortic adipose tissue proximal to and around the aneurysm sac, as well as from omentum and subcutaneous tissue.	Innate and adaptive immunity, cell-death pathways and ECM degradation pathways were up-regulated in periaortic adipose tissue close to AAA compared with that near non-dilated aorta.
"Gene Expression in Patients With Abdominal Aortic Aneurysm – More Than Immunological Mechanisms Involved" **Prucha et al.**	2019 Physiol Res	Illumina HumanHT-12 v4 BeadChips	48 AAA patients Paired samples; one from the area of the maximal diameter, one from the proximal neck	Changes in genes related to inflammation and immunity, as well as inter- and intracellular signaling.

Abbreviations; ^{18}F-FDG: fluorodeoxyglucose, AAA: abdominal aortic aneurysm, CAS: carotid artery stenosis, ECM: extracellular matrix, vSMC: vascular smooth muscle cell

1.2.12 Epigenetics and non-coding RNA

The transcription of DNA to mRNA, the fate of transcribed mRNA and to which proteins the mRNA will be translated are regulated by transcription factors and epigenetic factors such as histone modification, DNA methylation, microRNA (miRNA, miR) and long non-coding RNA (lncRNA). These mechanisms are usually studied separately but modern systems biology will try to integrate them to make predictions, understanding disease biology and finding relevant drug targets.

Histone modification refers to the acetylation or deacetylation of the nucleosomic histone core. Acetylation of the histone lysine neutralizes its positive change, decreases its binding to the negatively charged DNA, leading to a more open DNA structure and an increase in transcriptional activity. De-acetylation leads to the opposite. Both mechanisms have been suggested to be active in AAA and inhibition of histone deacetylases decreased experimental aneurysm formation in a mouse model (208). DNA methylation of CpG islands within gene promoters modulate several of the main disease processes implicated in AAA, which makes this epigenetic mechanism an interesting target for study (230). It has been shown that the DNA of regulatory T-cells are methylated to a higher degree and that these cells are less frequent and less suppressive in patients with AAA (231,232).

Influencing the stability and translation of mRNA, miRNAs are short non-coding RNA sequences which, after post-transcriptional modifications, targets mRNA by continuous base-pairing (233). Several miRNAs have been studied in animal aneurysm models and human AAA. miR-21 has been shown to regulate SMC differentiation and proliferation and, further, appears to be protective against aneurysm formation in murine AAA models (234). miR-29, on the other hand, may play a disease-promoting role in AAA as it targets the mRNA of matrix proteins such as collagens, fibrillin-1 and elastin. Blocking miR-29 leads to increased

expression of these matrix proteins, downregulation of MMP-2 and 9 as well as decreased aneurysm expansion in murine models. In human AAA tissue, however, miR-29 was reduced compared with normal aortas (233). miR-24 has been suggested as protective against AAA, since miR-24 overexpression and inhibition attenuated and augmented, respectively, aneurysm development in murine models. Moreover, the miR-24 target chitinase 3-like-1 protein was increased in large compared with small AAAs (235). Other miRNA studied in conjunction with AAA includes miR-143/145, miR-181b, miR-195 as well as miR-205 and miR-712, which inhibit TIMP3, and miR-33, which acts proinflammatory in SMCs and macrophages. The latter three can be inhibited to treat experimental aneurysms in mice (236). There is still a paucity of investigations into the influence of lncRNA and alternative splicing of mRNA in AAA disease. The lncRNA H19 was recently shown to be increased in progressing AAAs and its inhibition prevented aneurysms in two different experimental mouse models (172). Its expression correlated positively with apoptosis and negatively with SMC proliferation and survival, with HIF1-alpha identified as a major target. Further, the HIF 1-alpha-antisense RNA 1 (HIF1a-ASI) is increased in thoracoabdominal aortic aneurysms and silencing it protected vSMC against apoptosis *in vitro* (237). Examples of other lncRNAs of potential interest to AAA disease are SENCR and SMILR, which regulate SMC behavior, lnc-HLTF which correlates with the diameter of the ascending aorta and linc00540, implicated in the GWAS meta-analysis mentioned above (236).

1.3 BIOMECHANICAL ASPECTS

1.3.1 Basic concepts of vascular biomechanics

The aorta is continuously exposed to complex mechanical forces, from the pulsating intraluminal blood flow, perivascular tissue and inherent residual stresses, which can be estimated with increasing accuracy due to recent advances in computation. As summarized by Gasser and Bäck et al (238,239), mechanical load on solid materials can be quantified as *stress*, defined as force per area and measured in Pa, such that $1\ Pa = 1\ N/m^2$. Per definition, the direction of the force relative to the area determines if the stress is considered *shear stress*, force parallel to the area, or *normal stress*, force perpendicular to the area (Figure 9). Stress is a tensorial quantity and the *von Mises stress* allows the expression of a general multiaxial stress state by a single number. It is commonly used to determine when a ductile material will fail. The underlying rational will not be elaborated on in the present thesis.

Stress applied to a material causes deformation, or *strain*, a unitless measurement of the relative change of angle or length for *shear* and *normal strain*, respectively (Figure 9). The degree to which tissue strains when exposed to stress is quantified as *stiffness*. Vascular tissue has non-linear stress-strain properties, where the stiffness increases with increasing strain. If the energy that is needed to deform a material can be fully recovered upon unloading, the material is *elastic*, sometimes also called *hyperelastic*. Given a viscoelastic material, the energy is not fully recovered after unloading but partly dissipated instead. Elastic and viscoelastic bodies return to their initial sizes and shapes after unloading (238,239). Elasticity (or viscoelasticity) remains up to a point when irreversible deformation occurs. The material then yields at its elastic limit, a point that determines its strength. Material failure, such as yielding or rupture, occurs when the stress of a material overcomes its strength, both quantified in Pa. Further, if the material properties are similar in all spatial orientations, the material is said to be *isotropic*, it is otherwise *anisotropic*. A material that preserves its volume during deformation is *incompressible*. In order to estimate the biomechanical behavior of a material, these and other mechanical properties should be described and formulated into a *constitutive model.* An important framework to create and use such models is *continuum mechanics*, which consider a macromaterial as a continuum of small

subvolumes, *representative volume elements*. If the material properties of one such subvolume is known, the macromaterial as a whole can be modeled. While many major structures are heterogenous, successful predictions can generally be made with this approach.

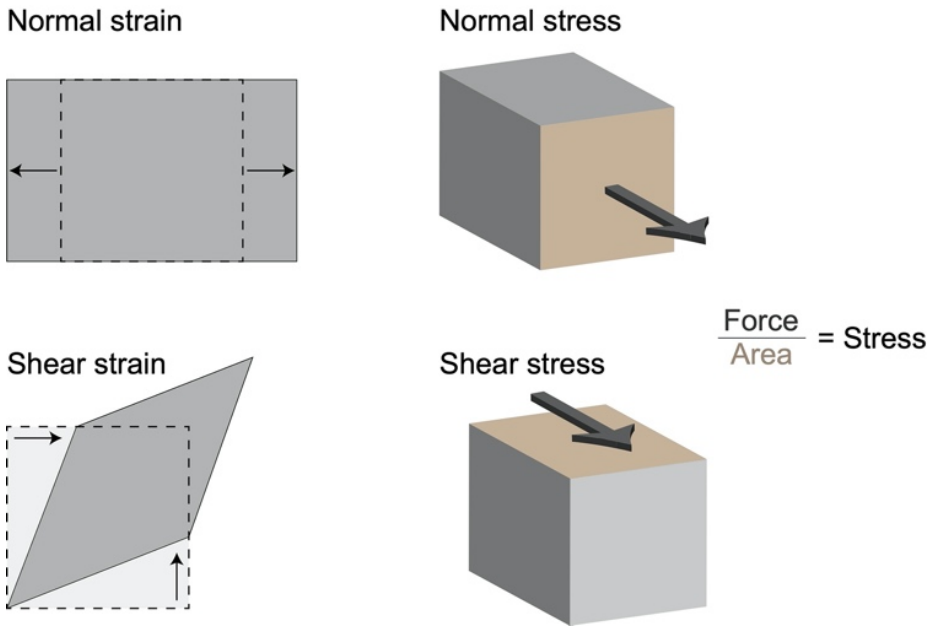


Figure 9: Illustration of normal and shear stress and strain. Dashed line indicates initial configuration.

1.3.2 Biomechanics of the normal aorta and abdominal aortic aneurysm

The biomechanical characteristics of biological tissue can be ascertained by tensile testing whereby a tissue sample is strained with increasing force in a machine and the relationship between applied force and resulting stress and strain is mapped. The force can be applied in one (uniaxial) or two (biaxial) directions. The biomechanical testing results reported on below are from application of perpendicular force (resulting in normal stress). Some literature on shear stress is described but mechanical shear testing on tissue samples are not elaborated on since peak shear stresses measured in such experiments are at least an order of magnitude smaller than normal stresses (240) and, further, that the papers included in this thesis does not consider shear stress from flowing blood. Stress is usually estimated by dividing the applied force with the cross-sectional area of the tissue sample as it measured before the sample was stretched.

The normal artery exhibits a viscoelastic, nonlinear stress-strain response. At low strain levels, elastin dominates with low stiffness but strong recoil. Collagen fibers in an unloaded artery have a wavy and relaxed appearance but as the artery is further strained, more and more collagen fibers are being stretched and contribute to the artery's increasing stiffness, causing a nonlinear stiffening (Figure 10A) (81,241,242). Further, most collagen fibers are aligned circumferentially, explaining why arteries are typically stiffer in this direction and thus anisotropic (86). It has also been shown that the strain of the volume of the artery vessel wall is considerably smaller than the circumferential strain, why incompressibility may be assumed in biomechanical modeling (243). The presence in arteries of residual stress, defined

as the stress a solid material imparts on itself when external stressors are removed, is well-known and apparent from the opening angle that occurs when an (externally unloaded) arterial ring is cut open (244).

The aorta, specifically, is highly compliant and its distention and recoil during systole and diastole is crucial for the maintenance of continuous flow and pressure throughout the vascular tree. From the proximal ascending aorta to the distal infrarenal aorta, the stiffness gradually rises as the thicknesses of media and adventitia decreases and increases, respectively (245,246). There is also an elevated amount of collagen in the media of the abdominal aorta (246). These characteristics change with age and the aging aorta is characterized by elastic fiber fragmentation and collagenous remodeling (with increased stiffness and diameter as a result), as well as a decreased amount of vSMCs (247).

The vessel wall of AAA is weaker compared with that of a normal abdominal aorta and most reports also show increased stiffness in AAA, with some exceptions (248), which seems to be most pronounced in the circumferential direction (81,240,248–250). Further, ruptured AAA had lower a wall strength compared with asymptomatic AAA in one study (251) (Figure 10B), whereas the pooled ruptured and symptomatic AAA had similar wall strength

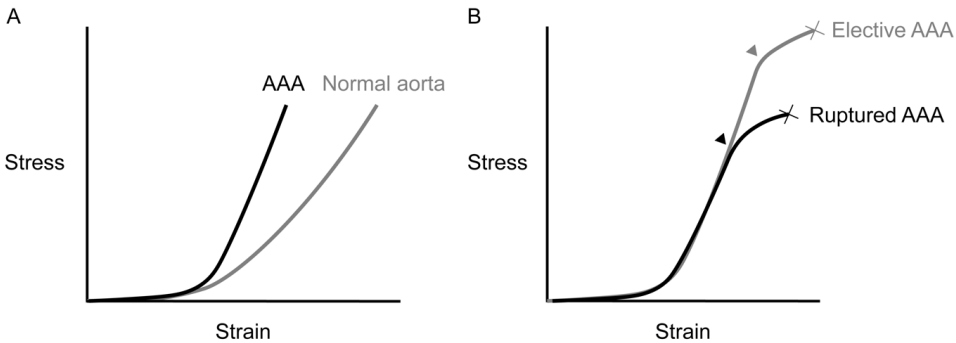


Figure 10: Schematic of uniaxial biomechanics of ex-vivo tissue from aorta and abdominal aortic aneurysm. A: Stress-strain curves from elastic loading of tissue from AAA and normal aorta. B: Stress-strain curves for destructive loading of tissue from elective and ruptured AAA.
Arrowheads denote point where yield (ie irreversible changes in tissue characteristics) occurs, cross denote rupture of tissue.
Abbreviation; AAA: abdominal aortic aneurysm.

as asymptomatic AAA in a larger study (252). Efforts have been made to enable the estimation of wall strength in a patient without access to tissue, which is impossible to obtain without open surgery of the aneurysm. In a multivariable model, it was found that increased local diameter normalized to the expected normal diameter, increased ILT thickness, female sex and positive family history were significantly associated with a decreased wall strength (253). It has also been suggested that diabetes and increased plasma potassium makes the wall weaker (254), whereas vessel wall from AAA patients with chronic obstructive pulmonary disease appears to be stronger than AAA patients without this disease (255) Counterintuitively, wall strength appears to correlate negatively with wall thickness (254). Wall strength (which is the maximal wall stress reached before tissue rupture) is mathematically coupled to wall thickness, as wall stress depends on the cross-sectional area of a specimen. This means that for the same applied force, a lower stress is recorded in thicker walls. Wall thickness can be increased by potentially weakening inflammation and edema, which is supported by significant correlation between wall thickness and metabolic activity as measured by PET CT (254). On the other hand, *failure tension,* which is defined as the maximal applied force before rupture divided by the specimen width and is thus independent

of the specimen (wall) thickness, correlates *positively* with wall thickness (254). These findings complicate the use of wall thickness in biomechanical models. Finally, most but not all, of these investigations have relied on uniaxial tensile testing which is why most models are based on uniaxially derived data whereas anisotropy has been described in biaxial testing (240,256)

Early clinical indications of an interaction between hemodynamics and AAA came in the late 1980's when it was observed that an unexpectedly high proportion of all AAA ruptures occurred in patients who had suffered World War II-related leg amputations (257). A prospective study later revealed that above-the-knee amputees, compared with controls, up to 40 years later had a significantly higher prevalence of AAA and that the aortas of the amputees were axially shifted towards the remaining leg's iliac artery, implicating hemodynamic perturbation as a culprit in AAA disease (258). Further clinical support to this notion came from observations that spinal cord injury is associated with an increased infrarenal aortic diameter (259) and that PAD is an independent risk factor for AAA (108), both conditions causing altered outflow from the aorta. Resistance to aortic outflow has been shown to decrease antegrade and oscillatory wall shear stress in the infrarenal aorta (259). In line with these findings, it has been observed that patients with AAA and occlusion in one or more iliac artery, as well as those with PAD, and thus have an obstructed outflow from the infrarenal aorta, rupture at significantly smaller diameters compared with those without (260).

Shear stress is lower in the distal compared to the proximal aorta and preclinical investigations have revealed an anti-inflammatory phenotype in cells exposed to high shear stress (261), which implies susceptibility of the infrarenal aorta to hemodynamics-substantiated inflammation. The vSMCs senses alterations in hemodynamic forces from endothelial cell signaling and interactions with the vascular extracellular matrix, to which this cell population responds with phenotypic modulation and matrix remodeling (262). Moreover, native murine aorta expresses higher levels of MMP-9 in its abdominal, compared with its thoracic, segment but this association is reversed when the thoracic aorta is transplanted into the infrarenal segment (263). Increased hemodynamic load have further been demonstrated to cause disruption of elastic laminae, possibly mediated by MMP-2 and -9 and it has also been demonstrated that endogenous nitric oxide and reactive oxygen species modulate expression and activity of MMPs in response to altered flow (264,265). In mice of normal aortas and elastase-induced aneurysms, computationally estimated shear stress strongly associated with aortic gene expression and mechanically altered flow markedly affected the aneurysm size, degree of apoptosis and inflammation (266,267). Results from our group also suggest a wall stress-independent association between local diameter growth rate and wall shear stress in AAA (268). However, while flow patterns may contribute to aneurysm formation, it is not clear to what extent they influence the rupture risk of manifest AAA. Areas of aneurysm rupture typically display high normal stress but the shear stress is orders of magnitude lower (269,270). The luminal aspect of the aneurysm vessel wall is also covered by an ILT, which shields it from the frictional force of flowing blood, and the AAA is also lacking endothelium, possibly prohibiting a large share of the shear-stress induced vessel wall signaling (5,238,269,270). For these reasons, rupture of the AAA vessel wall seems to primarily be caused by normal, rather than shear, stress. This motivates the analysis of wall stress and wall strength under blood pressure without including highly complex simulations of blood flow (63,253,270–277).

A number of studies have described molecular responses to normal stress of the vessel wall. In investigations on coronary artery stenosis, computationally estimated stress of the atherosclerotic plaque has been shown to correlate with expression of MMP-1 and important

inflammatory mediators (278,279). Thoracic aortic aneurysms show signs of vSMC phenotypic modulation into a synthetic phenotype at areas of high wall stress (280). An intriguing concept of biomechanical-biological interactions in AAA was that of segmental aortic stiffening increasing the axial tensile stress in adjacent, compliant, segments causing increased inflammation and proteolysis, which also correlated with age-related changes in the human aorta (281). Investigations into associations between gene expression and wall stress or the estimated ratio between wall stress and wall strength have pointed to differences in expression of lamin A/C and genes related to ECM and cytokine signaling, while strong associations surviving correction for multiple testing are difficult to obtain (228,229,282). On the protein/structural/cellular level, high ratio between wall stress and wall strength was associated with decreased vSMCs and elastic fibers as well as more cholesterol and calcified plaques, whereas increased wall stress was associated with increased collagen 1, III and total collagen (283). With respect to local tissue mechanics, stiffness and strength of the AAA vessel wall correlate negatively with MMP2 gene expression (282). Finally, mechanical stretching of vSMCs cause oxidative stress and makes them pro-inflammatory, -fibrotic and -proteolytic (284–286).

1.3.3 Molecular biology and biomechanics of the intraluminal thrombus

The interaction between biology and biomechanics has become apparent in investigations into the role of the ILT. This mesh of fibrin, red blood cells, leukocytes and enzymes was initially disregarded as inert but has through experimental and computational efforts been shown to play dualistic roles in the AAA. The thrombus is preferentially located in the distal half of the aneurysm and its formation is likely related to an environment of vortical flow, recirculation and exposure of the subendothelium (287). Mechanically, the ILT is isotropic, exhibits a mildly non-linear stress-strain curve and is sensitive to mechanical fatigue under pulsatile load (288,289). The formed thrombus appears to act as a cushion, protecting the wall against stress, and is important to include in the biomechanical modeling in order to get accurate rupture predictions (273,290). However, it has been observed that the ILT-covered AAA wall, compared with the ILT-free wall, displays sings of hypoxia, elastin fragmentation, vSMC apoptosis, proteolysis and, consequently, decreased wall strength (171,291,292). Potential explanations have been found in the ILT, which contain high concentrations of neutrophils, capable of releasing gelatinases and serine proteases. Further, the ILT adjacent to the vessel wall display active membrane-bound proteases and high activity of fibrinolysis by plasmin, the latter also able to activate MMPs and degrade fibrinogen (98,140). The ILT has also been shown to contain neutrophils expressing elastase, antitrypsin and MMP9 among others, preferentially in its luminal layer (293). Neutrophils may also increase their elastase activity in the presence of nicotine (294).

In summary then, while it cushions and protects the aneurysm wall against stress, the ILT simultaneously contributes to its destruction. Thus it appears likely that failure of the ILT would be detrimental to the aneurysm, causing increased stress in a weakened vessel wall (295). Clinical data support this hypothesis as radiological signs of ILT crack propagation and failure, such as crescent-like (296), diffuse (297) and localized (298) hyperattenuations in the ILT, are described as risk factors for frank aneurysm rupture.

1.4 LIMITATIONS OF PRESENT-DAY MANAGEMENT

The current management of AAA requires repeated and frequent diameter measurements when the aneurysm is small, as the growth rate of a specific aneurysm is difficult to predict (2,3,59). When the aneurysm reaches 50 mm in women and 55 mm in men, the patient is evaluated for EVAR or OAR, both of which are resource-demanding and associated with significant morbidity and mortality (299). The level of infrastructure, logistics and costs required to adequately follow present guidelines may prohibit treatment for many with a diagnosed or yet undiagnosed AAA (69,70). Further, even in a high-resource setting, some patients with small AAA will suffer from rupture despite being enrolled into surveillance (19,21,61,62). Enhanced precision of surveillance, such as predictions of growth or future need for surgery, would greatly improve management of AAA patients. Establishing an effective biomarker could make AAA diagnosis more accessible and the development of a pharmaceutical treatment would save numerous lives worldwide.

1.5 SUGGESTED METHODS TO MEASURE AND PREDICT GROWTH AND RUPTURE OF ABDOMINAL AORTIC ANEURYSMS

The maximal external diameter of the AAA has been used as the gold standard to predict aneurysm growth and rupture. Current guidelines on timing of surgery and surveillance intervals rely on diameter measurements by US or CT (2,3). As a predictor of rupture, the diameter works well on a population level but is imprecise in the individual perspective with a small but existing share of patients with small AAAs suffering from rupture, while a large proportion of patients with large AAAs does not (18,19,61,63). Further, the correlation between baseline diameter and its future growth rate is weak, necessitating frequent measurements (58,300). Other weakness include variations in technique, with respect to which US “edges” to measure between and in which plane to perform the measurement as well as the inherent weakness of following the evolution of a 3D object with a 2D metric, which can miss rapid changes outside the maximal diameter plane (301,302).

Several geometric indices and tracer imaging methods have been proposed to improve the prediction of AAA outcome. The volume of the AAA is commonly measured between the renal arteries and the aortic bifurcation and encompasses both the effective lumen and the ILT. It is a more sensitive measurement of growth than diameter, with a large share of AAAs expanding in volume despite being stable in diameter (303,304). Our group has also observed that volume growth of AAA is easier to predict and better corresponds with biomechanical deterioration compared with diameter growth (305). The size and structure of the ILT have been connected to both rupture and growth. For example, thicker ILT, asymmetric and anterior position of the ILT, the share of the aneurysm wall arc covered by thrombus and the relative ILT cross-sectional area have all been proposed as predictors of AAA growth (290,300,306–308), whereas the growth rate of the thrombus itself, localized and crescent-shaped hyperattenuations as well as the general attenuation of the thrombus have been associated with rupture (296–298,309). Fluorodeoxyglucose (^{18}F-FDG) is a PET tracer used to detect tissue metabolism and its uptake in the AAA vessel wall has been associated with increased risk of symptomatic AAA and pathological molecular remodeling (310,311) but seems on the other hand to correlate negatively with aneurysm growth rate, with suggested cyclical changes in uptake which could complicate the interpretation of these measurement (312,313). Prospective studies have been performed that evaluated ultrasmall superparamagnetic particles of iron oxide (USPIO) on MRI, corresponding with macrophage presence and cellular inflammation, and 18F–sodium fluoride (^{18}F-NaF) on PET-CT, which can identify early microcalcification (314,315). The signals from both tracers were associated

with an increased growth rate and rate of repair or rupture, independently of baseline diameter in the case of ^{18}F-NaF but not USPIO.

Many circulating biomarkers have been evaluated and have mainly been related to inflammation, ECM turnover, coagulation and lipids (316). Specifically, molecules related to inflammation shown to be associated with AAA growth rate in single studies include IFNγ, neutrophil gelatinase-associated lipocalin, osteopontin and soluble tumor necrosis factor-like weak inducer of apoptosis, with varying results on C-reactive protein and tumor necrosis factor alpha (316–320). Motived by common presence of an ILT in clinically relevant AAA, several hemostasis-related molecules have been proposed as markers of AAA presence, size and growth (316,321). D-dimer is one of the most studied markers and is increased in patients with AAA and correlates with aneurysm size and growth rates (321,322). Other markers include fibrinogen which is increased in patients with AAA, plasmin-antiplasmin complexes which correlates with growth rate and thrombin-antithrombin III-complexes which are increased in AAA and show varying results with respect to size (321). Serum triglycerides seem to be associated with risk of AAA rupture, elastin peptides with AAA growth rate and type XVIII collagen with aneurysm presence and growth rate, although the latter association was not independent of baseline diameter (126,323,324). The levels of several miRNAs are altered in AAA (325).

FEA is a numerical method commonly used to solve complex engineering problems by subdivision of a system into smaller, simpler parts ('finite elements'). These elements can then be reassembled into a whole, providing a simpler estimation of the solution. This method and its application in AAA prediction are further explained in section 3.3. The underlying principle behind predicting rupture of AAA by FEA is that the vessel wall fails when its stress overcomes its strength. Studies to estimate wall stress by FEA in AAA were performed as early as the late 80's and 90's but started to increase in the early 2000's (274,326,327). After initial mapping and modeling of the biomechanical characteristics of the AAA vessel wall (249,328,329), the first successful implementation of FEA to predict emergency surgery of AAA was performed by Fillinger MF et al in 2003 where peak wall stress (PWS) was found to be superior to the maximal diameter (271). A number of similar papers were subsequently published and a meta-analysis from 2014 concluded that PWS is higher in symptomatic or ruptured AAA compared with asymptomatic and intact AAAs (274). In order to further improve rupture predictions, estimations of wall strength have been incorporated into the FEA based on ex vivo tensile testing (253,270,272,273,275,276,330–334). Specifically, the highest estimated ratio between wall stress and wall strength (referred to in this thesis as peak wall rupture risk index – PWRI, Figure 11) is used as a predictor of rupture and can be converted into a

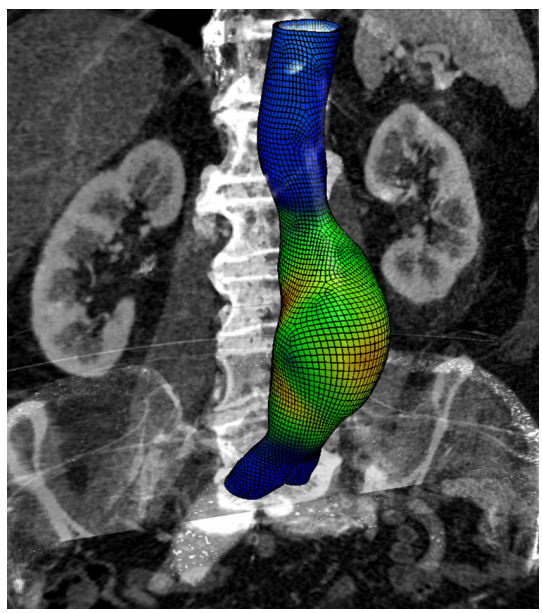


Figure 11: 3D-modeling and finite element analysis to measure volumes and estimate peak wall rupture index. As performed by A4clinics Research Edition (Vascops GmbH).

rupture risk equivalent diameter (RRED) based on diameter-PWRI associations in large studies (334). This approach has been implemented with different software, of which two are widely accessible; the commercial A4clinics (Vascops GmbH) and the partially free/open source software system BioPARR (273,333). Further developments include a probabilistic rupture risk index, developed to account for uncertainties of the modeling (331). Five studies, of which four compared aneurysms of matching diameters, investigated AAAs that had already ruptured with those that had not, and in three of them it was shown that PWRI was increased in ruptured AAA, whereas there was no difference in the other two (63,273,275,332,335). PWRI was also found to be increased in only symptomatic as well as pooled ruptured and symptomatic AAAs compared with diameter-matched asymptomatic AAAs (272,332). In further support of the value of wall stress/wall strength-ratios as rupture predictors, two studies have been published that compare FEA-derived PWRI from images of asymptomatic AAAs that ruptured after the CT image was taken with asymptomatic AAAs that remained intact, both showing an increased PWRI in aneurysms that would later rupture (270,330). A recent prospective study also demonstrated the potential of the wall stress/wall-strength ratio to predict a composite of rupture or elective aneurysm repair independently of diameter (276). Finally, the utilization of machine learning in medical diagnostics is a rapidly evolving field which is starting to encompass AAA research as well. Examples include the use of several anatomical variables in an extreme gradient boosting-algorithm to predict rapid diameter growth rate (336) and Bayesian modeling of AAA growth patterns (337).

2 AIMS

The overall aim of this thesis was to explore and develop new strategies to improve, refine and individualize management of patients with AAA by applying geometric, biomechanical and molecular analyses. Specifically, we aimed to evaluate how clinical rupture risk factors are linked to AAA biomechanics, improve the prediction of AAA progression, find potential circulating biomarkers of AAA as well as to comprehensively map how gene expression is altered in AAA compared with normal aorta, considering smoking and the AAA progression rate.

I. Gender, Smoking, Body Size, and Aneurysm Geometry Influence the Biomechanical Rupture Risk of Abdominal Aortic Aneurysms as Estimated by Finite Element Analysis.

- To investigate if established clinical risk factors for rupture are associated with biomechanical stress and rupture risk in order to gain biomechanical insight into what has been observed in epidemiological studies, as well as to assess the ability of FEA to consider clinically important patient characteristics.

II. Geometric and Biomechanical Prediction Modeling of Growth, Treatment and Outcome of Small Abdominal Aortic Aneurysms Using Machine Learning.

- To determine whether additional geometric or biomechanical indices in small AAAs could improve predictions of which patients would reach the threshold for surgery within four years, their future growth rate and the occurrence of rupture or symptomatic AAA.

III. Neutrophil Elastase-Derived Fibrin Degradation Products Indicate Presence of Abdominal Aortic Aneurysms and Correlate with Intraluminal Thrombus Volume.

- To study E-XDP as a marker of neutrophil-induced degradation of the aneurysm-related ILT as well as to evaluate to what extent this marker is associated the ILT and aneurysm volume, diameter and biomechanical rupture risk.

IV. Tunica-Specific Transcriptome of Abdominal Aortic Aneurysm and the Effect of Intraluminal Thrombus, Smoking and Diameter Growth Rate.

- To detect novel pathophysiologic features of AAA by tunica-specific microarray analysis of the genome-wide expression of genes in ILT-free and -covered aspects of the human AAA wall.

3 MATERIAL AND METHODS

The geometry and biomechanics of AAA were investigated by means of 3D segmentation and FEA based on CT images. Fibrin degradation products in peripheral blood were quantified by enzyme-linked immunosorbent assay (ELISA) and latex-enhanced turbidimetry. Aortic wall biopsies were analyzed with immunohistochemistry and microarrays. Patient data was collected from electronic medical records and information about organ donors was collected from pre-filled forms. Included patients may overlap between the different studies. An overview of methods is shown in Table 3.

Table 3: Overview of study methods.

Study	Study subjects	Design	Principal methods
I.	Patients w AAA of 40 - 60 mm diameter. n = 146	Analysis of one CTA per patient. Simple and multiple regression to test associations between biomechanical rupture risk, geometry and clinical risk factors. FEA was conducted both uninformed 'neutral' and informed 'specific' of patient characteristics.	3D segmentation and FEA. Simple and multiple linear regression.
II.	Patients w AAA of 40 - 50 mm diameter. n = 191	Analysis of one or two CTAs per patient. The precision of multivariable models to predict future indication for surgery, growth rate of diameter and volume as well as rupture or symptomatic aneurysm was evaluated.	3D segmentation and FEA. Simple and multiple linear regression, ridge regression, LASSO and SVM prediction modeling with 10-fold CV. Accuracy tested with ROC, Pearson's and Spearman's rank correlation coefficients.
III.	Patients w AAA of 45-80 mm diameter, n = 37 + 51. Controls from screening, n = 42, and from organ donation.	E-XDP and D-dimer were measured in plasma from patients and controls. Analysis of one CTA per patient. Microscopy of AAA tissue from OAR and and juxtarenal control aorta from organ donors	3D segmentation and FEA. ELISA, latex-enhanced turbidimetry. Immunohistochemistry.
IV.	Patients w AAA, n = 76. Controls from organ donation, n = 13.	Tissue samples from thrombus-free and thrombus-covered AAA and juxtarenal control aorta were dissected into media and adventitia. RNA was purified and analyzed by microarrays. Normalized gene expression data were subjected to the following comparisons: AAA vs controls (adjusted for age and sex), media vs adventitia, smokers vs non-smokers, thrombus-free vs thrombus-covered AAA and correlation with AAA diameter growth rate.	Microarrays. Bioinformatic tools including limma, Enrichr, GSEA, ChEA3, GTEx, CIBERSORT and algorithms tSNE and OPLS-DA

Abbreviations; 3D: Three-dimensional, AAA: Abdominal aortic aneurysm, ChEA3: ChiP-X enrichment analysis 3, CTA: computed tomography angiography, CV: cross-validation, E-XDP: Neutrophil elastase-derived fibrin degradation products, ELISA: Enzyme-linked immunosorbent assay, GSEA: Gene set enrichment analysis (of Broad Institute), GTEx: The genotype-tissue expression project, LASSO: least absolute shrinkage and selection operator, limma: Linear models for microarray and RNA-Seq data, OPLS-DA: orthogonal projection to latent structures discrimination analysis, ROC: receiver operating characteristic, SVM: support vector machine, t-SNE: t-distributed stochastic neighbor embedding.

3.1 STUDY SUBJECTS

Three categories of study subjects are included in this thesis; patients with AAA, control subjects without AAA recruited from screening and organ donors. Patients with AAA were all managed at and included from the Department of Vascular Surgery at the Karolinska University Hospital. Patient data were collected from electronic medical records, CT images were anonymized and downloaded, blood samples were collected preoperatively and tissue samples were collected during elective open AAA surgery from the anterior vessel wall at the approximate level of the maximal aneurysm diameter. Men of 65 years' age invited for screening in Stockholm and not found to have AAA on US were included as controls. After consent, a health questionnaire was answered and blood samples were obtained. Tissue of normal-diameter aorta was collected from juxta-renal aorta during organ harvesting from brain-dead, beating heart organ donors. The patients of study I and II were included retrospectively and did not give informed consent, whereas study III and IV included patients and controls after informed consent had been given. All studies were approved by the local ethics review board and performed according to the declaration of Helsinki. An elaboration on ethical considerations is given in section 3.12.

General exclusion criteria were AAAs resulting from known congenital connective tissue disorders, infections or acute autoimmune conditions as well as rupture, abdominal aortic surgery or symptomatic AAA before or at the time of the baseline CT or sampling. In order to perform FEA, the geometries of ILT and lumen are required, necessitating a CTA. Patients who were unable to undergo such imaging, due to pronounced renal failure for example, were excluded from this type of analysis.

3.2 STAAAB – STOCKHOLM ABDOMINAL AORTIC ANEURYSM BIOBANK

The Department of Vascular Surgery and the Vascular Surgery research group at the Karolinska University Hospital and Karolinska Institutet, respectively, have over the last decade assembled a biobank of tissue and blood samples from patients with AAA and controls without AAA, the Stockholm AAA Biobank (StAAAB, Figure 12). The StAAAB presently comprises blood samples, patient data and CT images from over 600 patients with AAA, tissue of the vessel wall and ILT from over 150 of these patients, control blood samples from over 200 individuals attending AAA screening and control tissue from the abdominal aorta of around 30 organ donors. The recruitment and collection are ongoing and the biobank is continually expanding.

The material is treated according to the following protocols. Plasma samples are anti-coagulated with citrate or ethylenediaminetetraacetic acid and are centrifuged twice in order to obtain platelet-free plasma. Tissue samples are divided into three parts; for analysis of RNA, protein quantification and immunohistochemistry. Tissue samples for RNA are immersed in RNAlater (Thermo Fisher Scientific, Waltham, MA, USA) immediately after extraction, stored in 4° C for 48h, dissected into (intima)/media and adventitia layers and subsequently transferred to long term storage in -80° C. Samples for protein analysis are immediately placed on dry ice and moved to long term storage in -80° C. Biopsies intended for immunohistochemistry are immersed in 4% zinc formaldehyde, transferred to 70% ethanol after 2 days and finally paraffin embedded within approximately 2 weeks.

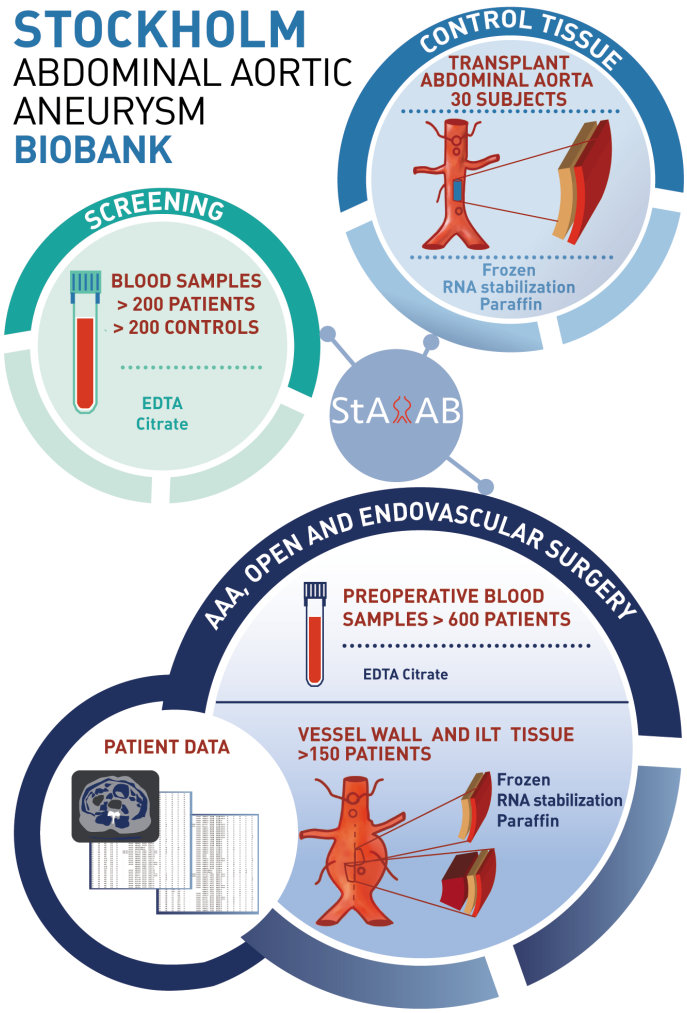


Figure 12: Current state of the StAAAB - Stockholm Abdominal Aortic Aneurysm Biobank

3.3 COMPUTED TOMOGRAPHY, GEOMETRIC MEASUREMENTS AND FINITE ELEMENT ANALYSIS

Geometric and biomechanical analyses of CTAs were conducted by use of the commercial software A4clinics Research Edition produced by VASCOPS GmbH (Graz, Austria), which co-supervisor T. Christian Gasser has developed. Thorough descriptions of core functionality, important assumptions, optimization and clinical validation of this software can be found elsewhere (273,334,338). In short, this software performed 3D segmentation and FEA from CTA images in a semi-automatic, step-wise manner (Figure 13). In a first step, the contrast-enhanced lumen was automatically identified. The lumen geometry then served as the starting point for the software to find the vessel wall of the aneurysm. While this process was automatic, the investigator typically needed to correct the software to ensure

precise identification of the vessel wall. When the lumen and wall model were constructed, space between lumen and the vessel wall was assumed to be ILT and wall thickness was estimated based on the presence of an overlying ILT (291). The finished model was then meshed. The AAA vessel wall was modeled as hyperelastic, incompressible and isotropic. The wall strength was modeled after previous *ex vivo* testing by which female sex and positive family history decreased wall strength globally, whereas the thickness of an overlying ILT and a large local diameter relative to the expected normal diameter decreased wall strength locally (253). The strength of the ILT decreased from the luminal to abluminal layer by 33% and the wall and ILT strengths were further lowered by 50% and 60%, respectively, to account for fatigue mechanisms induced by pulsatile loading (289). The model was subsequently loaded with patient-specific mean arterial pressure (MAP).

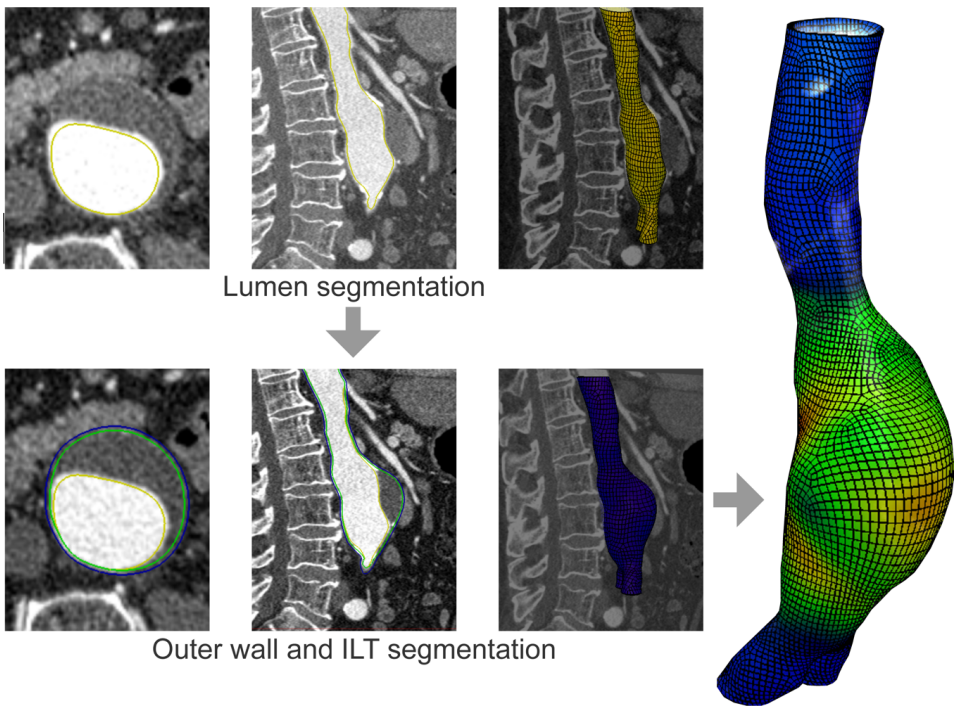


Figure 13: Finite element analysis by A4clinics Research Edition.
Abbreviation; ILT: intraluminal thrombus

All measurements and FEA were performed between the lowest main renal artery and the aortic bifurcation. The output variables were: *semiautomatic diameter*, which was automatically measured perpendicularly to the aneurysm centerline, *aneurysm volume*, *maximal luminal diameter*, *lumen volume*, *maximal ILT thickness*, *ILT volume*, *mean ILT stress*, *mean wall stress (mean WS)*, *PWS* and *PWRI*. Indices PWS and PWRI represented the maximal wall stress and maximal ratio between wall stress and wall strength in the aneurysm, respectively. Mean WS was the average stress estimated in the aneurysm whereas mean ILT stress was the average stress of the ILT. The biomechanical rupture risk index can be converted to an RRED, based on average relationships between PWRI and aneurysm diameters, in order to improve interpretability (334).

3.4 GROWTH RATES

Patients with AAA are monitored by US rather than CTA in most centers world wide, which results in irregular intervals between CTA examinations in retrospective material. Thus, growth rates need to be calculated in a manner that corrects for differing time intervals between patients. Further, with exponential growth patterns suggested for AAA diameter and especially with 3D quantities (339,340), this correction needs to be nonlinear. Martufi G et al proposed a nonlinear growth model in 2013 which was employed in the current thesis (302). This model was used to calculate absolute and relative growth according to :

$$Absolute\ growth\ rate = (Exp(12r) - 1) * M_{baseline}\ \frac{mm\ or\ cm3}{year}$$

and

$$Relative\ growth\ rate = (Exp(12r) - 1) * 100\ \frac{\%}{year}$$

with the logarithmic growth factor:

$$r = \frac{1}{t} * Ln\ (\frac{M_{followup}}{M_{baseline}})$$

Where M is a measurement of either diameter or volume, t denotes the time between the patient's baseline and follow-up CTA, Exp(•) and Ln(•) are the exponential and natural logarithmic functions of (•), respectively. This growth rate correlated to a high degree with a linear growth rate (ie the difference between follow-up and baseline measurement divided by the interval time) with decreasing but still high similarity with longer intervals.

3.5 PREDICTION MODELING

Statistical learning can be used to draw conclusions about to what degree input variables explain an outcome or to simply create a model that predicts the outcome without giving much consideration of causal interpretations. This process can be either supervised or unsupervised and predictions can either be probabilities of class membership, *classification*, or a continuous value, *regression*. While supervised learning is performed to create a model to predict an outcome, unsupervised learning works without an outcome measure and aims to structure or cluster data. A fundamental concern when training a prediction model is its external validity. It is easy to design an 'overfitted' model, especially in the presence of many predictor variables, if one tests the model on the same data it is trained on. In this case, it fits the data in a way that does not correspond to an underlying real structure but may instead be based on noise. Meanwhile, ineffective learning causes 'underfitting', in which real data structures are not captured by the model. Given this problem, a model needs to be carefully trained and then validated on data it has not been trained on. A convenient way to estimate how a modeling approach performs on external data is to divide a data set into training and test data, or to do this iteratively so that predictions can be made for all included subjects based on models trained on data that did not include them. The latter procedure is called 'cross-validation' and is commonly used to estimate test error in order to create robust prediction models. Further, some models have 'hyperparameters' which are model parameters set before the model training process begins. These can be optimized with respect to prediction performance by use of for example additional cross-validation, referred to as 'tuning'. Preferably, the optimal modeling approach should then be trained on the entire dataset and subsequently be validated in external data. An example of this process, as undertaken in paper II and in one analysis of paper IV, is illustrated in Figure 14. Thorough

discussions of above considerations and the models included below are given elsewhere (341–343).

Methods used in this thesis include simple and multiple linear and logistic regression, ridge regression, least absolute shrinkage and selection operator (LASSO), support vector machine (SVM), orthogonal projection to latent structures discrimination analysis (OPLS-DA) and t-distributed stochastic neighbor embedding (t-SNE). Linear regression is an approach to model linear relationships between a continuous dependent and one or several independent variables, usually fitted by use of least squares, where the sum of squares, ie the squared differences between the observed value and the model's fitted value, is minimized. Logistic regression models a binary dependent variable, outputting the probability of a subject belonging to a certain class by use of a logistic function. Ridge regression additionally imposes a penalty function, shrinks the regression coefficients and minimizes the penalized residual sum of squares. In LASSO, this penalty function can shrink some coefficients to zero, effectively performing feature selection. The SVM identifies a hyperplane with maximal margins between two classes, and can be performed with a linear approach or a by use of a non-linear kernel which is a way to achieve the effect of transforming data to a higher dimension feature space without the need to calculate the transformation of all vectors but rather the products between them. Ridge regression, LASSO and SVMs can be used in both classification and regression problems. Partial least squares or projection to latent structures is a method to project Y and X data to a new space, finding a multidimensional direction in the X space that explains maximal variance direction in the Y space. In OPLS-DA, systemic variation in X that does not correlate with/is orthogonal to Y is removed, which improves interpretability. Finally, while all previously mentioned methods were supervised, t-SNE is an unsupervised clustering and visualization technique for nonlinear dimensionality reduction in which each datapoint is given a location in a 2- or 3D map.

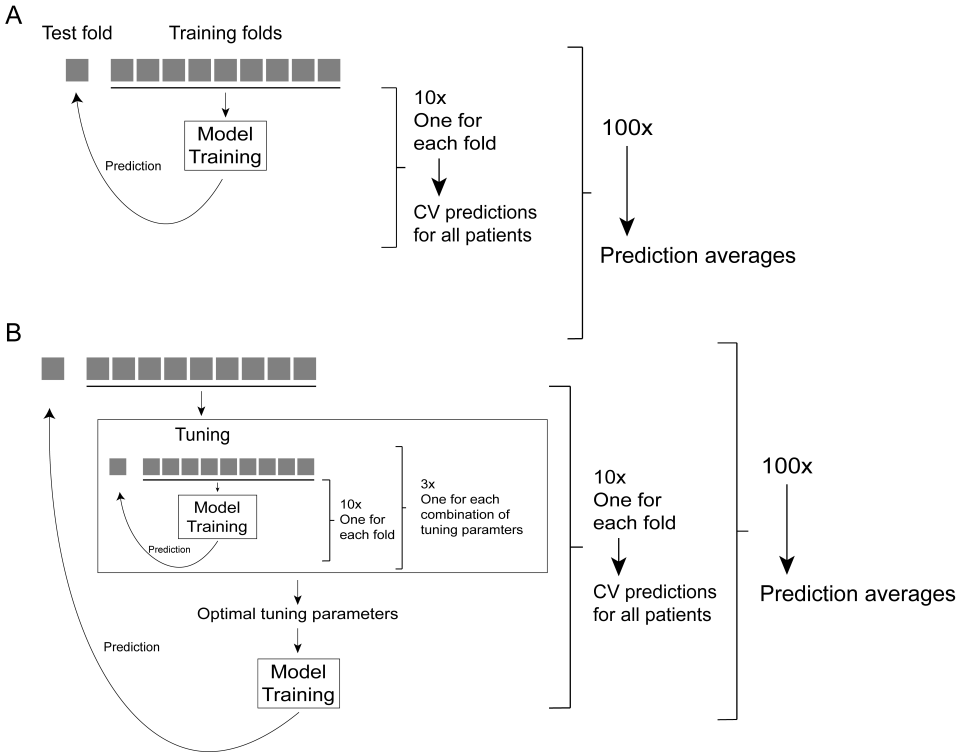


Figure 14: Method used in paper II to cross-validate (CV) predictions. In models that did not (A) and did (B) require parameter tuning.

3.6 E-XDP, ENZYME-LINKED IMMUNOSORBENT ASSAY AND TURBIDIMETRY

Different epitopes are revealed on fibrin degradation products depending on which enzyme is involved. The commonly used fibrin degradation product D-dimer results from degradation of fibrin by plasmin. Neutrophil elastase is also able to digest fibrin. An antibody has been developed that recognizes an epitope specifically unmasked on fibrin fragments produced by neutrophil elastase, and importantly not on those produced by plasmin, cathepsin G, trypsin- or chymotrypsin-type enzymes (344). This antibody, IF-123, was used as capture antibody to detect neutrophil elastase-derived degradation products of fibrin/cross-linked fibrinogen (E-XDP) in study III. ELISA is a method used to detect and quantitate a molecule, typically a protein, in a sample by use of antibodies directed against this molecule (345). In-house sandwich ELISA was used in study III and proceeded by having the capture antibody attached to a solid surface to which the liquid sample was added. The protein of interest subsequently attached to the capture antibody, all non-bound protein was washed away and a secondary antibody was added, causing the antigen to be sandwiched between two antibodies. The secondary antibody was conjugated with horseradish peroxidase which reacted with substrate 3,3′,5,5′-tetramethylbenzidine. The resulting color was quantified and the relationship between color and relative concentration of the protein was calibrated by use of a reference sample. In a validation experiment, fibrinogen was incubated with elastase, plasmin or reaction buffer alone during which samples were taken at 10-minute intervals and were analyzed with described ELISA.

Levels of D-dimer were measured by use of latex-enhanced turbidimetry in an automated blood coagulation analyzer at the Karolinska University Hospital laboratory. Turbidimetry refers to the quantification of light scattering, causing loss of intensity of transmitted light, due to particles being suspended in a sample.

3.7 IMMUNOHISTOCHEMISTRY

Immunohistochemistry is a sensitive and specific method to detect and visualize the presence of a molecule in a tissue section by use of antibodies. This method involves deparaffinization, antigen retrieval (epitopes hidden during the fixation process are demasked with for example heating), blocking of unspecific antibody binding sites, addition of a primary antibody targeted at the protein of interest and subsequently a secondary antibody targeted at the first antibody after which an enzyme attached to the secondary antibody catalyzes a color-producing reaction. The specificity depends on the specificity of the antibody and the blocking of unspecific binding, whereas the sensitivity comes from the performance of the antibody as well as the signal amplification produced by several secondary antibodies attaching to one primary with further amplification being possible in the following reactions. While immunohistochemistry is an excellent qualitative method to detect and localize specific proteins in a tissue section, its use for quantification is complicated by a number of conspiring sources of variation that are independent of the protein concentrations in the sample, from practically all steps of any protocol (346). Excellent guides on this methodology exist and the reader is referred to such for in-depth discussion (347).

In project III, the primary antibodies were directed at E-XDP, neutrophil elastase and CD66acd, the latter used as a neutrophil marker (Table 4). Background blocking was performed by use of the commercial Background Sniper and antigen retrieval achieved with high-pressure boiling in DIVA buffer. Primary and negative control antibodies were diluted in Da Vinci Green solution and applied to tissue sections. Species-specific secondary antibodies followed and a probe-polymer system with alkaline phosphatase and its substrate Warp red, along with haematoxylin, were used for staining. All buffers and reagents were from Biocare (Concord, CA, USA) except for haematoxylin which came from Vector Laboratories (Burlingame, CA, USA). Slides were imaged in a microscope equipped with a digital camera (OPTIPHOT-2, Nikon Corp. Tokyo, Japan), as well as a slide scanner (Nano-Zoomer, Hamamatsu Photonics, Hamamatsu City, Japan).

Table 4: Antibodies used for immunohistochemistry in Paper III.

Antibody	Company	Cat. nr	Dilution	Host
Neutrophil elastase	Neomarker	Ms-1841-p	1/50	mouse
E-XDP	Cosmo Bio	MKM-M16	1/2000	mouse
CD66	BIORAD	Mca1147g	1/50	rat

3.8 MICROARRAYS

A microarray is a solid surface covered with microscopic DNA probes, which can be used to simultaneously study the relative expression of a large number of genes. Two principal microarray methods exist, two-channel and one-channel. In two-channel arrays, two different samples of differing conditions, ie case and control, are labelled with two different fluorophores and are hybridized to the same microarray after which the relative balance between colors determines differential expression. In single-channel arrays, which were used in this thesis, intensity data is provided separately for each sample. In both cases, RNA from a sample is first reverse transcribed into cDNA which is transferred to the microarray where it hybridizes to the complementary probes. Samples are labeled before or after hybridization

depending on the type of microarray. After this step, scanning is performed and signal intensity for each probe is measured. Commonly, several probes targeting the same transcript are randomly distributed across the chip and this group is referred to as a *probe set*. In the case of both two- and one-channel microarrays, the output data intensity does not correlate linearly with the RNA concentration in the original sample. The values are relative to other samples in the same batch. A data post-processing step is thus required in which probes are summarized into probe sets and where data is normalized and transformed. In this thesis, data was processed through probe sets summarization, guanine cytosine count normalization, in which probe affinity variations associated with GC-content was adjusted for, signal space transformation, where the intensity distribution is stretched to counteract fold change compression seen in microarrays, followed by standard robust multi-array average including background correction, quantile normalization, log transformation and median polish (348–350). Advantages of microarray is that it is a fast and increasingly affordable method while able to quantify hundreds of thousands coding and non-coding transcripts, including the analysis of alternative splicing. However, quantification remains relative and transcripts need in principle to been known before-hand in order to design the chip.

In study IV specifically, samples were procured and preserved according to section 3.2. Tissue RNA was extracted using Qiazol Lysis Reagent and subsequently purified by use of RNeasy or miRNeasy minikits (Qiagen, Hilden, Germany). The RNA concentrations were measured with the Nanodrop ND 1000 (Thermo Fisher Scientific, Waltham, MA, USA) and quality assessed as RNA integrity numbers by Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Samples with purified RNA of identical concentrations were analyzed with Human Transcriptome Array 2.0 microarrays (Thermo Fisher Scientific) at the Bioinformatics and Expression Analysis core facility at Karolinska Institutet in Stockholm, Sweden. The resulting raw data were normalized and quality-controlled by use of the Transcriptome Analysis Console software (Thermo Fisher Scientific).

3.9 BIOINFORMATICS

A modern microarray experiment yields the relative quantities of tens of thousands of coding and non-coding transcripts. In order to extract biologically relevant information from this data, bioinformatic tools have been developed to study if there is an underlying structure between arrays and if this may be coupled to the condition of interest. Such analyses include unsupervised and supervised clustering, testing if genes significantly different between cases and controls, which biological processes are overrepresented, ‘enriched’, among these genes and if regulating transcription factors can be identified. Further, the normal tissue expression of a gene is available from databases cataloging large RNA sequencing efforts and the relative abundance of immune cell subsets can be quantified with specific algorithms (351,352).

In paper IV, t-SNE and OPLS-DA, described in section 3.5, were used for unsupervised and supervised clustering, respectively. Differential expression between two conditions was queried with linear models for microarray and RNAseq data (limma), which is a flexible R package that fits a linear model for each gene and utilizes the empirical Bayes procedure by adjusting the variance of an individual gene for the global variance of all genes, increasing the effective degrees of freedom (353). The limma approach also allows for multiple regression models, and comparisons between AAA and controls were thus corrected for age and sex. Paired tests were made between tissues from the same subject. In order to control the number of false positives when performing thousands of comparisons, we used false detection rate (FDR) threshold of less than 0.05, meaning that a maximum of 5% positives are false positives. Two approaches were used for gene set enrichment of the resulting list of

up- and downregulated genes, Enrichr and Broad Institute Gene Set Enrichment Analysis (GSEA). Enrichment was searched for in gene set databases Gene ontology biological processes (GO:BP) and Molecular functions (MF) as well as Molecular signatures database (MSigDb) Hallmark dataset. Enrichr is a threshold-dependent method that looks for overrepresented gene sets in a list of genes by a combination of Fisher exact test and the deviation from the expected rank of a term in a random search of each database (215,354). In GSEA, on the other hand, no threshold is used and the ranked order, based on fold change for instance, of all genes is searched for gene sets overrepresented at the extreme ends, normalized to the size of the gene set (216). Both methods used FDR to correct for multiple comparisons. Relationships between enriched GO:BPs and GO:MFs were visualized with Cytoscape based on the number of genes in common normalized by the size of the smallest gene set (355). Normal tissue expression and immune cell subset enumeration were examined by use of the Genotype-Tissue Expression (GTEx) database (351) and CIBERSORT (352), respectively. Transcription factor enrichment was performed by use of ChIP-X Enrichment Analysis 3 (ChEA3), which ranks transcription factors associated with a supplied gene list based on publicly available libraries including transcription factor-gene coexpression in RNA-seq studies and transcription factor-target associations from chromatin immunoprecipitation-sequencing experiments (356).

3.10 OTHER STATISTICAL METHODS

Unless stated otherwise, continuous data is described as median (interquartile range, IQR, or min-max) and categorical data as number (percent). The nonparametric Mann-Whitney U / Wilcoxon rank-sum or Kruskal-Wallis tests were used when normal distribution could not be assumed. The t-test was used after Pearson-D'Agostino omnibus testing for normality in paper II and to compare ages between groups in paper IV. Differences in distributions of categorical data were evaluated with chi-squared tests. Correlations between continuous variables were generally evaluated by use of Spearman's rank correlation coefficient, which is nonparametric and does not rely on normal distribution while being able to detect both linear and nonlinear monotonic correlations. When the relationships between absolute values themselves were important, such as with continuous predictions in study II, the parametric Pearson correlation coefficient was used. Differences between correlation coefficients were tested with R package cocor (357). Classification performance was studied with receiver operating characteristic (ROC) curves, the areas under which were compared by use of the Delong algorithm as implemented in the pROC R package (358). Reproducibility was evaluated according to Bland and Altman (359). All data analysis, except for that involving bioinformatic tools requiring interaction with a desktop or web application, were conducted in R (360) or Prism (GraphPad Software Inc, San Diego, CA, USA).

3.11 STUDY DESIGNS

3.11.1 Study I

Patients with AAA (n = 146 included from 337 screened patient records) were retrospectively identified after having been managed at our outpatient clinic between 2009-2013 with the inclusion criterion of having undergone 1 CTA of an AAA with a clinical diameter between 40 and 60 mm and the exclusion criteria of incomplete patient characteristics data as well as reasons listed in section 3.1. All CTAs underwent geometric analysis and FEA as described in section 3.3. Two types of FEA were performed. In the *neutral* analysis, the FEA software was supplied uniform data, ie remained uniformed of patient sex, age, family history and blood pressure. In the *specific* analysis, these values were supplied. The PWRI was converted to an RRED according to section 3.3, in order to facilitate interpretation of coefficients.

Simple and multiple linear regression analyses were conducted to study associations between PWS, RRED, patient characteristics and aneurysm geometry. Two and three multiple regression models with PWS and RRED, respectively, as dependent variables were created; model A with patient characteristics, model B with patient characteristics and aneurysm geometry and model C with patient characteristics, aneurysm geometry and PWS as independent variables. Patient characteristics were age, gender, smoking, MAP, height and weight (or BMI and BSA, (361) in separate models due to collinearity), and geometric features were semiautomatic maximal diameter, ILT volume and lumen volume. Diagnostic plots were inspected to detect nonlinearity, non-normality, heteroscedasticity and highly influential outliers in the regression models.

3.11.2 Study II

Patients with AAA (n = 191 included from 1172 screened patient records) were included consecutively by use of two search strategies; 1) a list of all patients with a diagnosis of AAA and a registered CT between 2009-2013 and 2) by going through lists of all patients who had presented to our outpatient clinic with an AAA between 2012 and 2013. Inclusion criterion was an AAA with a maximal clinical diameter at baseline of 40-50 mm imaged by CTA at least four years prior to the record review date and exclusion criteria were according to section 3.1. Of the 191 patients, 173 had complete follow-up data and 163 had two CTAs performed with an 8 month to 8 year interval. Growth predictions were studied in the latter group.

Patients underwent geometric and biomechanical analysis of CTAs as described in section 3.3. Reaching the threshold for surgery was defined as the AAA reaching 55 mm diameter if the patient was male, 52 mm if female (which has been the current practice at our department for the last decade), being surgically treated at smaller diameters or the occurrence of rupture or symptomatic AAA. Conversely, the aneurysm was considered Stable if it was intact and asymptomatic with a diameter below 55 mm in males and 52 mm in females after four years. Patients who had incomplete follow-up due to dying from unrelated causes or considered too frail for surgery were included in the Stable group. Growth rates of the clinical diameter, as measured by radiologists or vascular surgeons, the semiautomatic diameter and vessel volume, as calculated by the FEA software, were calculated in patients with two available CTA by use of growth equations described in section 3.4.

All variables were used in the prediction modeling in addition to the clinical diameter. All predictions of growth and indication for surgery were performed under 10-fold cross-validation, please refer to section 3.5. Multivariable models used were logistic and linear regression for prediction of indication for surgery or growth rates, respectively, as well as ridge regression, LASSO and SVM with a linear or radial basis function kernel for both outcomes. Tuning was performed by 10-fold cross-validation with 3 repeats. All predictions were repeated 100 times and average predictions were used for evaluation. All modeling and variable importance analyses were conducted with R package caret (362). Ruptured and symptomatic AAAs were too few to allow multivariable modeling or cross-validation. Classification was evaluated with ROC analysis and correlations between continuous predicted and observed values were tested with the Pearson's correlation coefficient.

3.11.3 Study III

Blood samples were drawn prior to elective aneurysm repair from 37 male patients with AAA and 42 age- and sex-matched controls, the latter identified via AAA screening as described in section 3.1. Patient data were collected from the electronic medical records and CTA images were download from the hospital picture archiving and communication system. The CT images of this main cohort were analyzed by use of A4clinics Research Edition as described in section 3.3, while also using this software to segment the ILT volume of all coexisting aneurysms. To validate the findings, a separate cohort of 51 patients with AAA was studied and the volumes of their ILTs were measured by standard 'manual' segmentation using the Leonardo Workstation (Siemens AG, Berlin and Munich, Germany). Circulating levels of E-XDP and D-dimer were measured with sandwich ELISA and turbidimetry, respectively, as described in section 3.6.

The relationship between AAA and E-XDP was tested by comparing circulating levels in cases and controls, ROC testing of classification performance, multiple regression to investigate if E-XDP levels were affected by patient characteristics other than the presence of an AAA and correlation tests relating E-XDP levels to aneurysm geometry and biomechanics. We also studied the more traditional marker of fibrinolysis, D-dimer, in parallel and tested which of these two markers that showed the strongest association with AAA. In the validation cohort, E-XDP and D-dimer levels were correlated with the ILT volume and the clinical AAA diameter.

In order to verify that the circulating E-XDP originated from ILT degradation by neutrophil elastase activity, these entities were stained specifically, by use of immunohistochemistry as described in section 3.6, in vessel wall from AAAs and organ donor controls.

3.11.4 Study IV

Tissue samples from AAA and control aorta were compared and the effect of the ILT, patient smoking status and the aneurysm progression rate were investigated, while taking age and sex into account. Patients and controls with the highest RNA integrity number were selected from the biobank. Patients with both ILT-covered and -free vessel wall available and controls in similar age groups as the patients were prioritized. A total of 246 aortic tissue samples, dissected into tunica media and adventitia, from 76 patients with AAA and 13 organ donor controls were collected and preserved as described in section 3.2. These samples were analyzed with microarrays as described in section 3.8. The resulting normalized and log-transformed data was subjected to bioinformatic evaluation according to section 3.9.

Specifically, samples of AAA and controls were compared with adjustment for age and sex, which lead to identification of a large number of differentially expressed genes. These results were compared to those of previously published microarray studies on AAA tissue (113,217). In order to make functional interpretations of the large number of dysregulated genes, gene set enrichment analysis and transcription factor prediction was performed as described in section 3.9. Further, the tunica-specific approach allowed comparison of media-adventitia relationships in AAA and controls. As such, genes upregulated in adventitia compared with media in controls, while to the opposite being upregulated in media compared with adventitia in AAAs, were cross-referenced against genes dysregulated between AAA and controls, revealing a group of disease-associated genes switching tunica predominance from adventitia in normal aorta to media in AAA disease. In the next step, the transcriptomic effects of an overlying ILT, active smoking and the diameter growth rate were examined. The media and adventitia of thrombus-covered and thrombus-free AAA wall were compared in a pairwise

manner, including only patients from whom both ILT-covered and ILT-free wall were available. Comparisons were also made, separately for AAA and control samples, between subjects actively smoking and those that did not. Finally, in patients imaged twice with a 10 to 26 months interval by the same modality (US or CT), with the final imaging instance being pre-operative, the diameter growth rate was correlated with the expression of each gene. Enrichment analysis resources Enrichr (215,354,363) and GSEA (216) were used when the significantly dysregulated genes withstood adjustment for multiple comparisons whereas only GSEA was used when no genes did.

3.12 ETHICAL CONSIDERATIONS

All studies were performed according to the declaration of Helsinki and were approved by the local ethics review board. In study I and II, patient records and coded, anonymized CT images were analyzed without informed consent. This was motivated by on the one hand the minimal intrusion into patient privacy a retrospective patient record review constitutes, and on the other hand the expected loss of material if prior informed consent was needed due to the high age and common comorbidities of the typical AAA patient. In study III and IV, there was a prospective collection of blood and tissue into a biobank, requiring informed consent. The extra blood samples taken for research purposes was considered a minimally invasive procedure. The collection of tissue from the aortic wall could theoretically affect the closure of the aneurysm sac over the graft and care was therefore given to only excise excess vascular tissue. The organ donors agreed to the usage of tissue samples for research purposes at the time of enrollment into the organ donor registry and the extraction of aortic tissue did not significantly affect organ extraction procedures.

4 RESULTS AND DISCUSSION

Table 5: Overview of study results.

Study	Objective	Results
I.	To study the association between clinical rupture risk factors, geometry and biomechanical rupture risk indices.	Female sex, patient height and BSA were positively, and BMI negatively, associated with $RRED_{neutral}$ in a PWS-dependent manner. Adjusting for PWS revealed smoking as a significant covariate. These effects were independent of maximal diameter and in the $RRED_{neutral}$, FEA had not been informed of patient characteristics. Lumen volume showed strong associations with PWS and RRED.
II.	To study if geometric or biomechanical modeling in small AAAs could improve predictions of which patients would reach the threshold for surgery within four years, their aneurysms' future growth rate and the occurrence of rupture or symptomatic AAA.	Increased precision in diameter measurements improve prediction of growth and future indication for surgery in small AAAs. However, including additional geometric and biomechanical indices with regression and machine learning techniques did not improve these predictions. Lumen diameter, PWS and PWRI were the only significant predictors of the few cases of future rupture or symptomatic AAA. Growth and rupture require different prediction models.
III.	To study E-XDP as a marker of neutrophil-induced degradation of the aneurysm-related ILT as well as to evaluate to what extent this marker is associated the ILT and aneurysm volume, diameter and biomechanical rupture risk.	E-XDP is a sensitive marker of AAA, independently of smoking, investigated comorbidities and medication. E-XDP correlated with ILT volume, mean ILT stress and the AAA diameter. It was positively associated with presence of co-existing aneurysms outside the abdominal aorta. The correlation between E-XDP and ILT volume was stronger than between D-dimer and ILT volume.
IV.	To detect novel pathophysiologic features of AAA by tunica-specific microarray analysis of the genome-wide expression of genes in ILT-free and -covered aspects of the human AAA wall.	A large number of genes were differentially expressed between AAA and controls, many of which were unique to either media or adventitia. Pathways related to inflammation were transmurally upregulated whereas a pattern comprising upregulation of proteolysis and angiogenesis with downregulation of vSMC- and differentiation-related pathways were specific for media. The ILT had a pro-inflammatory and -proteolytic effect. Smoking caused aneurysm-like gene expression in control arteries. Growth rate correlated with B- and T-cell-related gene expression in media, and lipid pathways in adventitia.

4.1 STUDY I – GENDER, SMOKING, BODY SIZE, AND ANEURYSM GEOMETRY INFLUENCE THE BIOMECHANICAL RUPTURE RISK OF ABDOMINAL AORTIC ANEURYSMS AS ESTIMATED BY FINITE ELEMENT ANALYSIS.

The AAA maximal diameter is an imperfect risk indicator and it has been observed that patients with AAAs of similar diameter have differing risks of rupture, suggesting that other factors need to be taken into account (19,21,61). Identified clinical risk factors for rupture include female sex, active smoking, high blood pressure, old age and low BMI (19,21). An emerging method proposed to increase precision of rupture prediction is FEA, described in section 1.5, which estimates biomechanical risk indices PWS and PWRI or RRED. In study I, it was investigated to what extent there was a biomechanical explanation for these clinically

observed risk factors. The FEA was performed both uninformed, 'neutral', and informed, 'specific', about patient characteristics. We also aimed to find which geometrical variables were most important for the biomechanical state of the aneurysm.

Results

In multiple regression analysis, it was found that $PWS_{neutral}$ was associated with patient height and female sex, $PWS_{specific}$ with patient height and blood pressure, and that both were positively associated with maximal diameter and lumen volume, while negatively associated with ILT volume (Table 6). These results suggest that the biomechanical stress on the aneurysm wall, not considering its strength, is generally increased in taller patients as well as in females. A larger ILT, a smaller diameter and a smaller lumen decrease the wall stress.

Table 6: Multiple linear regression models for peak wall stress.

	Dependent			
	Model A	Model B	Model A	Model B
Independent	$PWS_{neutral}$	$PWS_{neutral}$	$PWS_{specific}$	$PWS_{specific}$
Age, years old				
Female sex				
Tobacco smoking (Current)				
Family history (Yes)				
Height, cm				
Weight, kg				
MAP, mmHg				
Max diameter of 3D model, mm				
ILT volume, cm^3	-		-	
Lumen volume, cm^3	-		-	
	$R^2 = 0.35$ ***	$R^2 = 0.69$ ***	$R^2 = 0.51$ ***	$R^2 = 0.75$ ***

Covariates included in multiple regression were; model A: patient demographics and diameter, model B: model A, ILT and lumen volume. Stronger shading represents a more significant effect on the model (lower p-value), red represents a positive and blue a negative coefficient.
Abbreviations; 3D: three-dimensional, ILT: intraluminal thrombus, MAP: mean arterial pressure, PWS: peak wall stress

Multiple regression was performed with RRED, which considers both wall stress and wall strength and converts their ratio into a diameter value of corresponding rupture risk, as the dependent variable. Both $RRED_{neutral}$ and $RRED_{specific}$ increased in patients with female sex, tall stature and who were actively smoking (Table 7). Female sex and height elevated $RRED_{neutral}$ in a wall stress-dependent manner. Female sex also remained a significant covariate in $RRED_{specific}$ after adjustment for PWS, corresponding to the way in which wall strength is calculated by the software. Smoking, which is not considered by the software, appeared to decrease wall strength, as it significantly increased $RRED_{neutral}$ and $RRED_{specific}$ after adjustment for wall stress. Geometric factors elevating RRED were maximal diameter and lumen volume. When adjusting for PWS, increasing ILT size contributed to an increased RRED.

When height and weight were replaced by BSA and BMI, it was found that BMI correlated negatively with PWS and RRED whereas the opposite was true for BSA. The effect of lumen volume on RRED can be qualitatively appreciated in Figure 15, which displays two

aneurysms of similar diameter that nevertheless have among the lowest (A) and highest (B) estimated RRED in the study. The effective lumen is small and wall stress is cushioned by a thick circumferential ILT in Figure 15A, whereas the aneurysm in Figure 15B does not benefit from the same geometry.

Table 7: Multiple linear regression models for rupture risk equivalent diameter

	Dependent					
	Model A	Model B	Model C	Model A	Model B	Model C
Independent	**$RRED_{neutral}$**	**$RRED_{neutral}$**	**$RRED_{neutral}$**	**$RRED_{specific}$**	**$RRED_{specific}$**	**$RRED_{specific}$**
Age, years						
Female sex						
Tobacco smoking (Current)						
Family history (Yes)						
Height, cm						
Weight, kg						
MAP, mmHg						
Max diameter of 3D model, mm						
ILT volume, cm^3						
Lumen volume, cm^3						
$PWS_{neutral}$, kPa	-	-		-	-	-
$PWS_{specific}$, kPa	-	-	-	-	-	
	R^2 = 0.39 ***	R^2 = 0.69 ***	R^2 = 0.92 ***	R^2 = 0.65 ***	R^2 = 0.82 ***	R^2 = 0.91 ***

Covariates included in multiple regression were; model A: patient demographics and diameter, model B: model A, ILT and lumen volumes, model C:, model B and peak wall stress. Stronger shading signifies a more significant effect on the model (lower p-value), red represents a positive and blue a negative coefficient.
Abbreviations: 3D: three-dimensional, ILT: intraluminal thrombus, MAP: mean arterial pressure, PWS: peak wall stress, RRED: rupture risk equivalent diameter

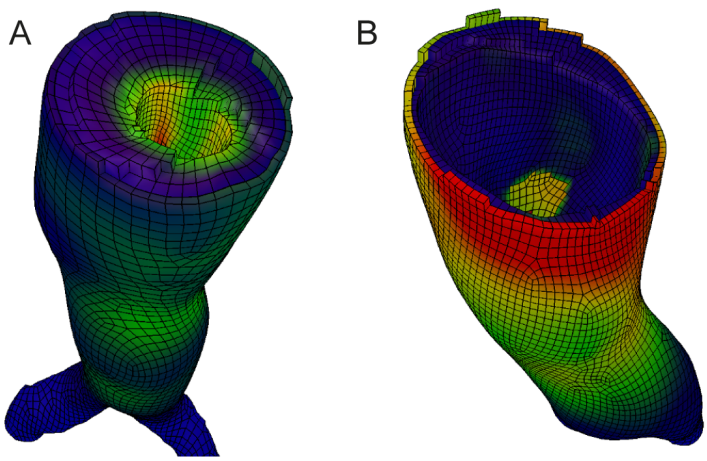


Figure 15: Aneurysms with differing biomechanical rupture risk despite similar maximal diameter. Cross-sections of two AAAs with the lowest (A) and highest (B) biomechanical rupture risks of the study despite both having diameters of approximately 55 mm.

Discussion

Several results of the present study were intriguing. First, female sex is a strong clinical risk factor for rupture of AAA and it has previously been shown that female patients have weaker vessel walls compared with male patients (253). As a result, RRED is increased in women when informing the software of the patient sex (364). However, the present study observed that female sex was associated with increased $PWS_{neutral}$ and $RRED_{neutral}$, ie were the software does not have information about patient characteristics but only the CTA-derived geometry. This implies that female sex is not only associated with a weaker vessel wall but also a biomechanically unfavorable aneurysm and ILT geometry. Patient height or BSA were also associated with an increased $PWS_{neutral}$ and $RRED_{neutral}$ with similar implications that tall or larger patients have a less stable aneurysm geometry compared with their shorter counterparts. While patient height is not typically described as a rupture risk factor for AAA, it has been linked to AAA prevalence and aortic rupture (365,366). The latter study did not specifically examine rupture of the *abdominal* aorta and it was not known to what extent ruptures were associated with diseases like Marfan syndrome, that also cause increased body height, but the sample's mean age of 55 and the much higher prevalence of common AAA make it unlikely that the entire association can be explained by connective tissue disorders. The observed inverse association between BMI and PWS/RRED agrees with clinical observations (21) and it has been proposed that the aneurysm diameter should be considered relative to body size (206,207). While these results and others make a case for taking an interest in the *relative* aneurysm diameter, it is not known if it should be related to height, BMI or BSA. Age showed no correlation with PWS or RRED, despite being associated with rupture in a large meta-analysis but not in the largest included study (19,21). Female patients were significantly older than their male counterparts in the present study and such an underlying confounder was not excluded in the large meta-analysis.

The most important geometric factor of the present analyses was lumen volume. The size of the AAA lumen has since been recognized as a risk modifier in AAA disease, congruent with the findings above (277,367). Further, the dual role of the ILT became apparent as it cushions against wall stress but, given the same stress, weakens the wall and increases the biomechanical risk index. An important assumption when considering lumen and ILT geometry and biomechanics is the structural integrity of the ILT. As discussed in section 1.3.3, mechanical failure of the ILT would increase stress in an already weakened vessel wall (295) and taking radiological signs of ILT crack propagation (296–298) into account could improve estimations of PWS and PWRI/RRED.

Due to the complexity of biological tissue and organs, along with consideration of computational cost as well as our incomplete knowledge about relevant processes and properties, biomechanical modeling requires assumptions and simplifications. The employed FEA software does not consider blood flow, residual stresses, wall calcifications, interactions with surrounding organs or porosity of the ILT while it consider the vessel wall to be isotropic, in contrast to observed anisotropy, and relies on average constitutive properties based on uniaxial tensile testing (240,249,253,273,289,334,338). However, given above constraints, a finite element model should only be complex enough to fulfill its intended application. The intended application of this FEA is to predict rupture and features that increase complexity without improving diagnostic precision should not be included. Blood flow requires extensive fluid-structure interaction modeling but resulting effect on wall stress appears to be negligible compared with FEA without modeling of flow (368). Wall calcifications are easy to recognize on CT but the resulting effect on wall stress depends on how the calcification and its interaction with surrounding wall are modeled, with conflicting biomechanical and clinical results in the literature (369–373). While mild anisotropy has been

observed in AAA tissue, anisotropic and isotropic FEA performs similarly (240,374). Patient-specific constitutive models of vessel wall behavior are intuitively appealing but it has been shown that varying tissue properties within the 95% confidence interval resulted in ≤ 4% change in computed wall stress, motivating the use of average constitutive descriptions (328). Neglecting the impact of surrounding organs, residual stresses and mechanobiological responses to stress could be important limitations to the used FEA and considering these factors may improve stress predictions (375–377). Finally, respecting the non-linear behavior of the vessel wall, having a high level of geometric precision in the 3D reconstruction and including the ILT, which the FEA used in this thesis does, appears to be important for accurate stress predictions (273,378,379).

In summary, these results show that female sex, smoking, increased height or low BMI as well a large lumen volume are associated with significantly increased wall stress and rupture risk indices, giving in part a biomechanical explanation to observed clinical risk factors. Importantly, these associations were found even in analyses where the software was uninformed about patient characteristics.

4.2 STUDY II – GEOMETRIC AND BIOMECHANICAL PREDICTION MODELING OF GROWTH, TREATMENT AND OUTCOME OF SMALL ABDOMINAL AORTIC ANEURYSMS USING MACHINE LEARNING.

After having established that there appeared to be a biomechanical foundation for clinically observed rupture risk factors, and that several geometric variables were independently associated with the risk of rupture, study II focused on using FEA and geometry in multivariable models to try to increase the prediction of outcome of AAAs. The FEA software considers the entire geometry of the AAA and outputs a comprehensive set of geometric and biomechanical variables, most of which have not been studied with respect to AAA outcome prediction. Further, the relationships between these variables could be complex or non-linear and with the use of many variables in the same prediction model there is a risk of over-fitting. In parallel with the development of FEA, algorithms for complex statistical learning have been made increasingly available, shown to be effective and are increasingly used in a wide variety of research applications, including cardiac and AAA imaging (336,337,341,380). As such, multivariable modeling, tuning by machine learning protocols and cross-validation to estimate performance outside the present dataset were used as described in section 3.5 in order to effectively and robustly utilize the available output data from FEA to predict future outcome of AAA.

Results

Of the 191 patients, 103 remained stable and 88 reached the threshold for surgery in four years (Figure 16). Most geometrical and biomechanical measurements differed between these two groups. There were however no differences in age, smoking, blood pressure or the number of female patients.

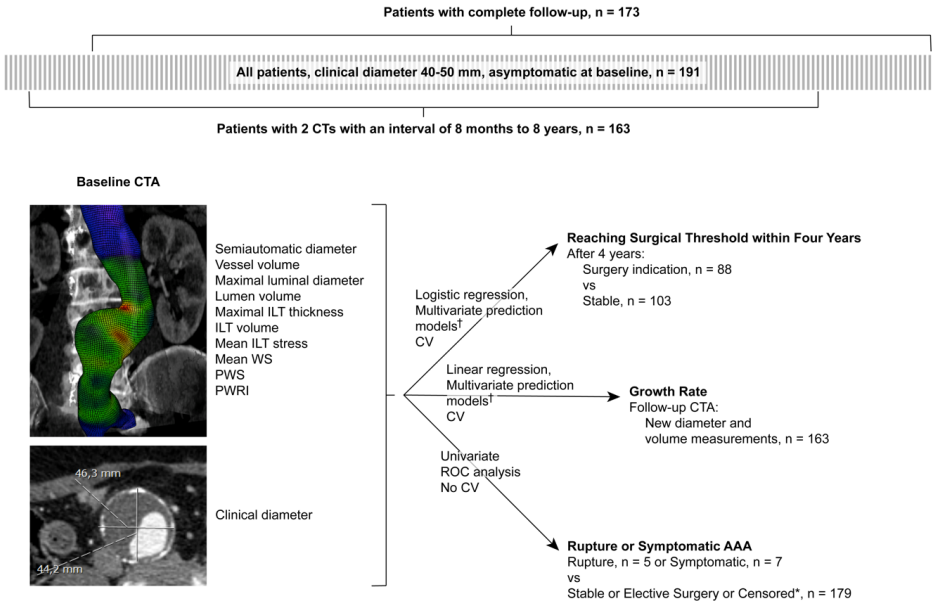


Figure 16: Overview of study design and outcomes.
Abbreviations; CV: cross validation, ILT: intraluminal thrombus, PWRI: peak wall rupture index, PWS: peak wall stress, WS: wall stress.
*: Patients were censored if they died from unrelated causes or follow-up was terminated before surgery.
†: Included prediction models were multiple linear/logistic regression, ridge regression, least absolute shrinkage and selection operator as well as support vector machines with linear and radial basis function kernels.

Multivariable models, utilizing machine learning algorithms and incorporating all geometrical and biomechanical output from the FEA software, were superior compared with the clinically measured diameter at predicting the four-year outcome (Figure 17A). Specifically, the area under curve (AUC) for the best performing multivariable model, compared with that of the reference model, was 0.85 vs 0.80 ($p = 0.031$). As an example, the best model could achieve 100% sensitivity and 21% specificity, whereas the reference model did not reach 100% sensitivity. Only lumen volume, PWS and PWRI could significantly predict which patients would suffer from ruptured or symptomatic AAA (Figure 17B). Multivariable models also improved prediction of diameter growth rate, ($r = 0.38$, $p = 6.1e-07$ for the best-performing model and $r = 0.17$, $p = 0.033$ for clinical diameter reference, correlation comparison $p = 0.0031$, Fig 17C). There was a trend towards improved prediction of volume growth rate (Figure 17D). The most influential variable was the semiautomatic diameter measured from the FEA software and by only using the clinical and the semiautomatic diameters as predictors, similar predictive power for diameter growth rate and four-year outcome and was obtained as with the more complex models (not shown).

Discussion

These findings suggest that a semi-automatic measurement of the aneurysm diameter, based on a 3D model, can significantly improve the precision of AAA growth rate prediction. No support was found for adding additional geometric and biomechanical variables to this end.

The contrasting finding of PWS, PWRI and lumen volume as the only significant rupture or aneurysm symptom predictors imply that predicting growth and rupture of small AAAs require different models.

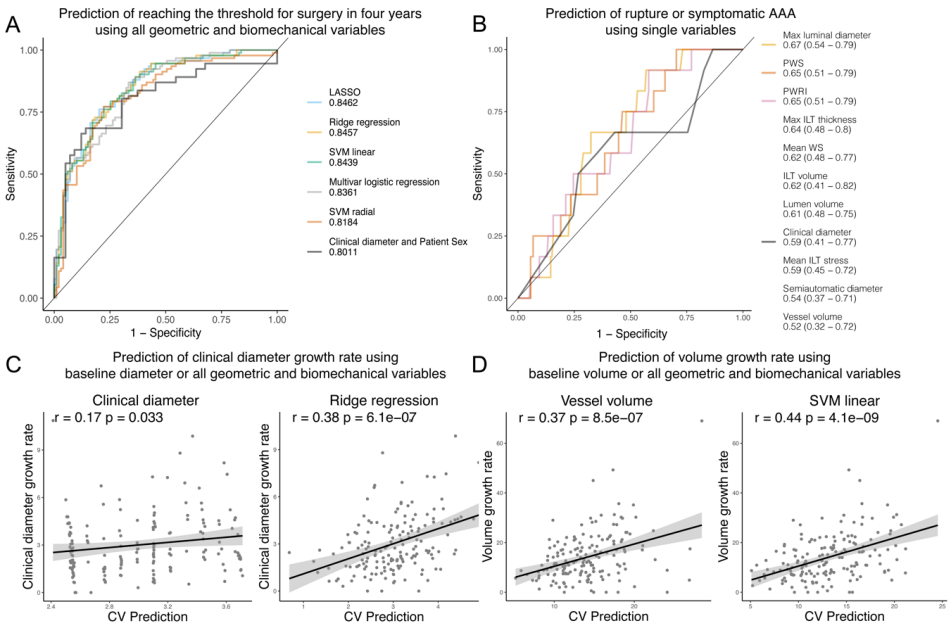


Figure 17. Prediction of reaching surgical threshold within four years, future rupture or symptomatic AAA and growth rate. A: ROC analysis of predictions by multivariable models including all variables, AUC below each variable. B: ROC analysis for prediction of future rupture or symptomatic aneurysm. The AUC (95% confidence interval) is presented below each variable. C: Prediction of diameter and volume growth rate.
Abbreviations; AUC: area under curve, CV: cross-validated, ILT: intraluminal thrombus, LASSO: least absolute shrinkage and selection operator, PWRI: peak wall rupture index, PWS: peak wall stress, ROC: receiver operating characteristic, SVM: support vector machine with linear or radial basis function kernel, WS: wall stress

A large number of promising markers of presence, size, growth and rupture of AAAs have been previously described, as discussed in section 1.5. Employed methods include geometric analysis (290,300,307), FEA (270,271,273–276,332,333), biomarkers (316) and tracers for PET-CT (310,311,315) or MRI (314). However, none these markers have thus far been used clinically as replacement for or addition to diameter and female sex, motivating a critical appraisal. In many studies, the findings have not been validated, for example by division of data into training/test sets, cross-validation or external validation etc and even if the marker itself has been examined in more than one study, specific thresholds have seldom been established. Further, any clinically meaningful prediction study should compare the new, presumably more complex algorithm with the performance of already existing ones (ie clinical diameter measurements). Few AAA prediction studies manages to check both of these boxes with favorable results. So far, most investigations have also been limited to a single or only very few predictors per study.

The present study sought an effective and robust approach to prediction modeling in AAA disease, with principles explained in section 3.5. The results revealed that slight but significant improvements can be achieved to predict growth and future need for surgery compared with the currently used clinical diameter but also that most included variables were

not independently informative. Additional analyses not described in Results were performed in which cross-validation was ignored and prediction algorithms were tested on the same data it was trained on. In these analyses, the multivariable machine learning models achieved near perfect results, with a large share of included variables contributing to the predictive performance. This emphasizes the need for external validation, and may explain why markers that have not been tested outside the dataset they were developed from, often fail to reach clinical relevance. The framework used in the present study could in the future include clinical characteristics, biomarkers available from peripheral blood and imaging tracer markers.

The major limitation of the present study is its sample size and retrospective nature. While it was large compared with previous FEA studies, and at level with several successful cardiac CT studies (380), there is a clear correlation between the size of a training set and the diagnostic performance of resulting algorithms (381). With a larger number of included patients and prospectively determined protocols and imaging intervals, the performance in the current study would most likely increase and some variables would potentially become more informative. The present study also used a simplified approach in which descriptors of geometry and biomechanics were used rather than the geometry itself, such as with training on a 3D model of the aneurysm, a map of spatial distribution of mechanical load or even the entire CT image. With a larger sample size and less reduced descriptions of the AAA, widely applied systems such as artificial neural networks could become effective despite showing poor predictive performance on the present material (not shown).

4.3 STUDY III – NEUTROPHIL ELASTASE-DERIVED FIBRIN DEGRADATION PRODUCTS INDICATE PRESENCE OF ABDOMINAL AORTIC ANEURYSMS AND CORRELATE WITH INTRALUMINAL THROMBUS VOLUME.

The results from study II showed that 3D geometric and biomechanical modeling improves prediction of outcome compared with the currently used clinical diameter. However, most used variables were not independently informative and much room for improvement remained. In study III, the scope was broadened to include biological markers for future use to facilitate diagnosis and improved outcome prediction of AAA. Most clinically relevant AAAs contain an ILT, which traps neutrophils. As elaborated on in section 3.6, the degradation of fibrin by neutrophil elastase results in a specific degradation product, E-XDP. It was hypothesized that E-XDP produced in the ILT is a marker of AAA and ILT presence, size and biomechanical rupture risk, and that it is superior in this respect to the widely available D-dimer.

Results

The employed antibody was shown to specifically bind E-XDP and not plasmin-derived fibrin degradation products. E-XDP was significantly increased in the peripheral blood of patients with AAA compared with controls (Figure 18A) and a ROC analysis showed an excellent accuracy of this potential biomarker, with an AUC of 0.97 and the possibility to attain 98% sensitivity and 86% specificity to diagnose AAA by use of E-XDP (Figure 18B). This difference in E-XDP levels between AAAs and controls remained when only AAA with thin ILTs were included. Multiple regression revealed that E-XDP was independently associated with the diameter of the AAA and the presence of aneurysms outside the infrarenal aorta, while not being affected by smoking, prescription drugs or comorbidity.

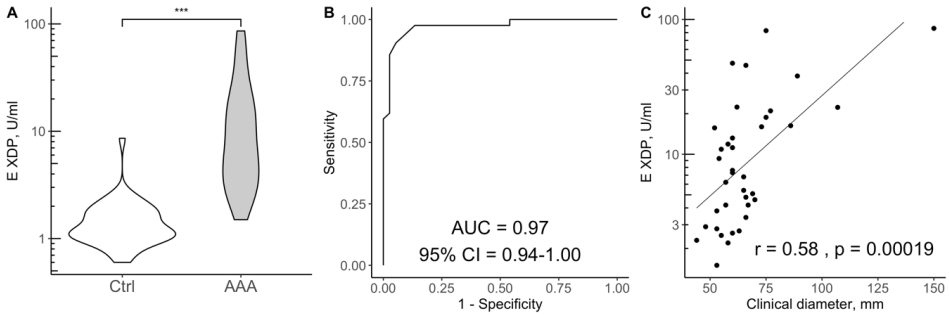


Figure 18. E-XDP as a marker of abdominal aortic aneurysm presence and size. A: Violin plot with logarithmic y-axis of E-XDP levels in controls and patients with AAA, difference tested with Wilcoxon rank-sum test. B: ROC analysis of the ability of E-XDP to distinguish AAA from controls. C: Correlation between E-XDP and the clinical maximal diameter, tested with Spearman's rank correlation coefficient. Abbreviations; AUC: area under ROC curve, E-XDP: neutrophil elastase-derived fibrin degradation products, ROC: receiver operating characteristic.
Notations; ***:p<0.001.

Supporting the ILT as a source of E-XDP as well as its use for prognostic information, there was a strong correlation between E-XDP levels and the total ILT volume (ie the sum of all ILTs of all aneurysms in a patient), AAA ILT, AAA maximal diameter and the mechanical stress in the ILT in the patient group (Figure 19). D-dimer also correlated significantly with ILT of all aneurysms and that of the AAA but to a significantly weaker degree than E-XDP. There was no association between E-XDP and PWS or PWRI. Further, E-XDP was not a marker of general low-grade inflammatory activity, as it did not correlate with C-reactive protein or leukocyte count. The relationship between E-XDP and ILT volume, but not that between E-XDP and AAA diameter, could be validated in the separate cohort. In this cohort, there was a much weaker relationship between ILT volume and diameter. Finally, by use of immunohistochemistry a strong presence of E-XDP in the vessel wall-thrombus and vessel wall-lumen interfaces were observed in AAA but not control specimens.

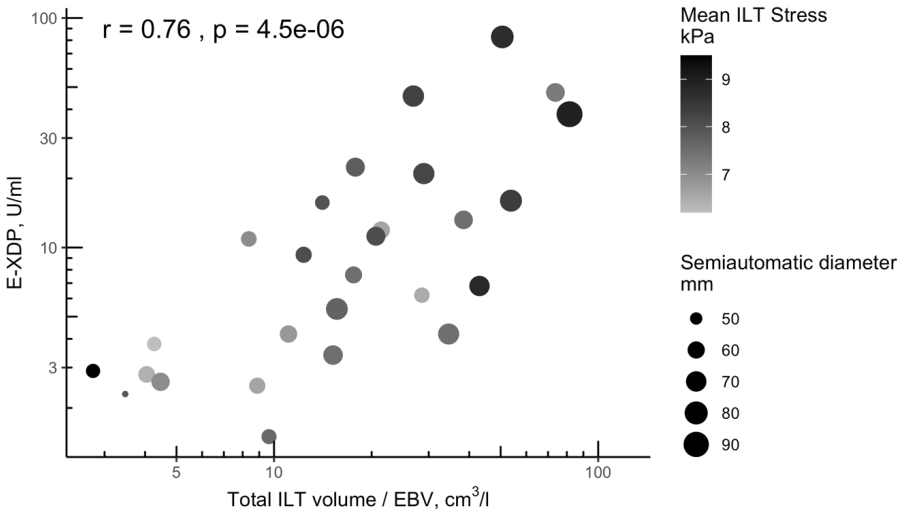


Figure 19. E-XDP as a marker of ILT volume, diameter and mean ILT stress. Sizes of dots correspond to the semiautomatic maximal diameter of the AAA, shades represent mean ILT stress. Volume of the ILT was normalized against estimated blood volume.
Correlation tested with Spearman's rank correlation coefficient.
Abbreviations; EBV: estimated blood volume, E-XDP: neutrophil elastase-derived fibrin degradation products, ILT: intraluminal thrombus.

Discussion

This study is the first to describe E-XDP as a marker of AAA and establishes a close relationship between E-XDP levels and the ILT. The results also imply that E-XDP could be used to detect any large arterial aneurysm and that they could be detected even with thin ILTs. As such, the inner surface area of the aneurysm as well as the volume of the ILT could both important for the production of E-XDP.

The rationale behind the use of E-XDP was that most clinically relevant AAAs contain an ILT, which traps neutrophils expressing elastase. The specific degradation product of fibrin from elastase, E-XDP, was hypothesized to be more specific than that produced by plasmin, D-dimer, which has been studied as an AAA marker previously (322). Above results support this notion, with observed ROC curves of 0.97 and 0.89 as well as correlations between marker concentrations and ILT volume of r = 0.76 and r = 0.64 for E-XDP and D-dimer, respectively.

Some limitations of this study merit consideration. We only had access to male 65-year-old control subjects as this is the only group invited to screening. As such, applying the sensitivities and specificities of the current study to female patients would constitute extrapolation. On the other hand, the validation group included women and showed clear correlations between E-XDP concentration and ILT volume. Mean ILT stress is calculated based on previous ex vivo biomechanical testing, based on the same principles as the well-studied PWS and PWRR/RRED, but this measurement has not been specifically evaluated. It is also worth noting that while the relationship between E-XDP and ILT volume was validated in external data, the thresholds to differentiate between AAA and controls were not, necessitating future investigations before clinical implementation.

While a circulating biomarker of AAA presence and size is easily outcompeted by the widely accessible, non-invasive and precise US examination in a high resource-setting, such a biomarker could find additional uses. A sensitive biomarker could be used as a screening tool in locations were US equipment is not available or as a quick test to exclude the presence of large aneurysms. Further, a biomarker of the contact between ILT and circulating blood could potentially be used to detect endoleaks after EVAR treatment. Future studies should also investigate whether the E-XDP could give information about the mechanical integrity of the ILT, as suggested by the correlation of E-XDP and the mean ILT stress, or if it could predict growth. Importantly, however, AAA is not the only condition affecting E-XDP levels. It has been seen to rise with sepsis, acute leukemia, deep venous thrombosis and disseminated intravascular coagulation (344,382,383). These factors affect the specificity but not sensitivity of E-XDP which was high. As such, this marker seems more poised to rule-out, rather than rule-in, AAA.

4.4 STUDY IV – TUNICA-SPECIFIC TRANSCRIPTOME OF ABDOMINAL AORTIC ANEURYSM AND THE EFFECT OF INTRALUMINAL THROMBUS, SMOKING AND DIAMETER GROWTH RATE.

Study IV was directed at systematically mapping the pathophysiology of AAA, with consideration of growth rate and smoking, to identify the most relevant mechanisms. As the rate of OAR is decreasing in favor of EVAR, the study of processes active in AAA tissue has been made less available. Our research group has previously assembled large biobanks to study vascular disease and the present study made use of StAAAB, detailed in section 3.2. With this resource, the largest transcriptomic analysis of AAA tissue to date was performed. In addition to the presented analyses, the microarray data will be made publicly available and may subsequently be used by the international research community to understand AAA pathophysiology, identify biomarkers and to find human data for drug target development.

Patient characteristics

Patients, median age 69 (IQR 65-75), were significantly older than controls, median age 53 (IQR 44-68), and there was a trend towards a smaller proportion of women among the patients with AAA. All comparisons between AAA and control were therefore adjusted for age and sex. Diameters of included AAA were median 60 (IQR 56-70) mm which meant that included AAA were late-stage and any early transient events in AAA development could thus not be detected. Further, ruptured AAAs were not included due to the inherent obstacles of obtaining informed consent preoperatively.

Bioinformatic overview

All tissues were divided into media and adventitia. The transcriptomes of AAA and control aortas, as well as between tunica media and adventitia, were generally distinct with a large number of differentially expressed genes (Figure 20A) and a tendency to cluster together in unsupervised t-SNE analysis of the genes with highest variance (Figure 20B). Most genes were dysregulated in a specific tunica and the present data identified thousands of additional genes compared with previous microarray studies. Many of the genes upregulated in AAA media were normally predominant in the adventitia of controls while those downregulated in AAA media were predominant in the media of controls. Transcription factors predicted from upregulated genes were mainly related to hematopoietic tissue while those predicted from downregulated genes were related to tissue of mesenchymal origin, such as SMC-rich organs and adipose tissue in the case of media and adventitia, respectively. Further, it was observed that a group of genes related to inflammation, SMC migration, ECM organization and

disassembly that were dysregulated in AAA predominated in the adventitia of controls but instead in the media of AAAs, thus switching their anatomical pattern in AAA disease.

While some biological processes were transmural in AAA, others were tunica-specific. Adaptive and innate immunity were highly upregulated in both media and adventitia of AAA (Figure 21), with the most dominant components being T- and B-cell activity, IFNγ and chemotactic molecules. Specific to the AAA media was the downregulation of muscle cell development and contraction, differentiation and adhesion gene sets along with upregulated ECM disassembly, angiogenesis, apoptosis and phagocytosis (Figure 21A-B). The adventitia specifically showed downregulation of gene sets related to metal ion response and lipid metabolism (Figure 21C-D). There were general agreement between Enrichr and GSEA analyses against GO:BP and MSigDb Hallmark gene sets.

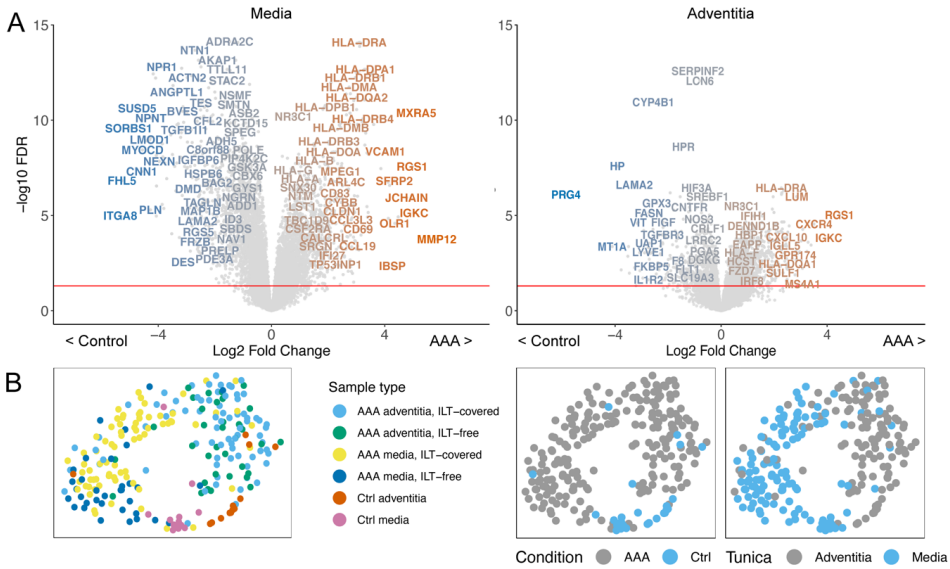


Figure 20. Dysregulated genes and unsupervised clustering. A: Dysregulated genes between AAA and control tissue, divided into media and adventitia. B: Unsupervised clustering by t-distributed stochastic neighbor embedding based on the 100 genes of the highest variance across all samples.
Abbreviations; AAA: abdominal aortic aneurysm, Ctrl: control.

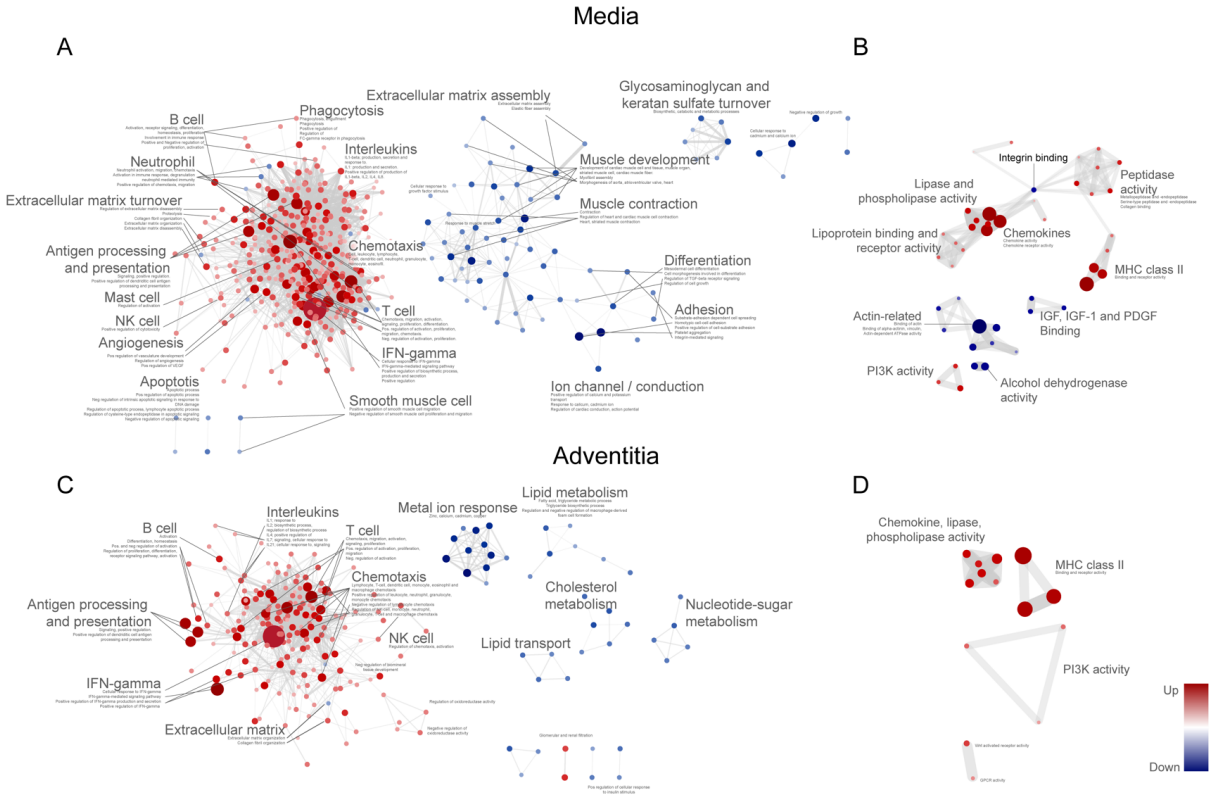


Figure 21. Network of enriched gene ontology biological processes.
Each node represents an enriched gene set as calculated by Enrichr and the edges between nodes are determined by the number of overlapping genes. A red node is upregulated and a blue node is downregulated in AAA. Differentially expressed gene sets were from the following Gene Ontology databases; A and C: biological processes, B and D: molecular functions.

Differentially expressed genes in media and adventitia

Many of the most upregulated genes had not been described in AAA disease. MXRA5 is mainly expressed in fibroblasts and features anti-fibrotic and anti-inflammatory properties (384). LUM is a pro-inflammatory small proteoglycan regulating fibrillogenesis (385). FRZB is a Wnt antagonist that was highly downregulated in AAA media but instead upregulated in adventitia. The Wnt/ β-catenin pathway affects expression of several molecules relevant to AAA disease, including MMPs (386). Several of the most upregulated genes had previously been investigated with regard to aortic aneurysm disease, including MMP12 (128), CXCL8 (387), CXCR4 (388) and RGS1 (389). Macrophage-specific deletion of NTN1 inhibits experimental mice aneurysms (390), it was however highly downregulated in the AAA media of the current data. VCAM1 is important in angiogenesis and increased in the circulation of patients with AAA (136).

The most downregulated genes not previously associated with AAA include PRG4, NPR1 and ADRA2C. PRG4 is a structural ECM protein with important biomechanical roles in cartilage but can also act as an anti-inflammatory buffer and is involved in the resolution of inflammation (391). It is dysregulated in chronic inflammation and upregulated in calcified carotid plaques (391,392). NPR1 is normally most abundant in the aorta (351) and was strongly downregulated together with NPR2, -3 and ligand NPPB/BNP in AAA. The resulting proteins play key roles in cardiovascular homeostasis (393). Transmurally downregulated ADRA2C is also normally predominantly expressed in the aorta and is important for neurotransmitter control (351,394).

Known and emerging pathophysiological processes

Several ECM-related processes described in sections 1.2.1 and 1.2.3 were dysregulated in AAA, preferentially in tunica media. These included upregulated proteolysis, ECM disassembly and peptidase activity whereas ECM and elastic fiber assembly, adhesion and glycosaminoglycan production were downregulated. Specifically, MMP1, -2, -7, -9, -12 and -13, CTSB, -C, -G, -H, -K, -L, -O, -S, -W, -Z, CMA1, ADAM8, -10, -12, -17 and -28 as well as ADAMTS12 and 14 were upregulated in AAA media whereas TIMP3 and -4 were downregulated. In media, MMP12, -1, and -9 were the 1st, 63rd and 79th most upregulated genes. In addition to upregulation of disruptive enzymes, important constituents of the normal aortic wall were downregulated. These include genes coding for components of the elastic lamina such as elastin, fibulin 5 and emilin 1, collagens including IV, VI, VIII, XIV and XVIII, proteoglycans aggrecan, biglycan, syndecan 4, osteoglycin, PRELP, asporin, osteomodulin, chrondroitin sulfate proteoglycan 4, fibromodulin, heparan sulfate proteoglycan 2, SPOCK1, glypican -1 and -4 as well as several integrins. Proteoglycans previously linked to aneurysm disease include potentially protective syndecan (123), biglycan (124), mast cell secretory granule-associated serglycin, collagen XV, which has been associated with severity of thoracic aortic aneurysm (125), and collagen XVIII which is a circulating marker of AAA diameter (126). As important functions in normal arteries have been described, and new interactions with for example inflammation are being elucidated, the role of proteoglycans in AAA requires further attention. Several laminin-coding genes were downregulated, LAMA2 and -5 transmurally, LAMB2, LAMC1- and -2 specifically in media and LAMA3, LAMB1 in adventitia whereas LAMA4 was upregulated in media. Laminin is an integral basement membrane protein, affecting many functions such as cellular survival, migration and differentiation, and elastase-derived fragments of laminin can stimulate macrophages to release plasminogen activator, urokinase, receptor (PLAUR) and MMP9, both upregulated in AAA media (395). Plasmin can in turn activate MMPs and degrade pericellular proteins, causing detachment and inducing death of mesenchymal cells

(98). In the present study, it was observed that both PLAU and PLAUR were upregulated in AAA media whereas potent plasmin inhibitor serine protease inhibitor (SERPIN)-F2 was among the most downregulated transmurally. In summary, the transcriptome of the AAA media displays a strong pro-proteolytic profile.

The AAA media further showed strong downregulation of muscle cell development and contraction, as well as increased apoptosis, in line with AAA pathophysiology described in section 1.2.4. Phenotypic modulation of vSMCs in AAA is an emerging field of research (145,147,148) and the present material paints a compatible picture, with downregulation of TGF-β-, differentiation-, adhesion- and contraction-related gene sets in AAA. Transcription factor KLF4 has been described as central for vSMC differentiation of atherosclerosis (146) and was upregulated in AAA media.

The well-known chronic inflammation of AAA was apparent in both media and adventitia and was aggravated by smoking. One of the most consistent signals in the bioinformatic analyses was that of IFNγ and T- and B-cells, which were upregulated in AAA compared with controls and increased further in the presence of ILT, cigarette smoke exposure and rapid diameter growth rate. These processes also tended to switch tunica predominance from adventitia in controls to media in AAAs. As elaborated on in section 1.2.5, the role of IFNγ in animal models of aneurysm is ambiguous. The strong upregulation of IFNγ-related gene sets in AAA with further augmentation during active smoking and fast diameter growth rate, along with increased AAA expression of IFNG and related genes CXCL10, IFNGR1 and -2, IRF1, and -8 suggests an important role and motivates further study of this pathway. Adaptive immunity by B- and T-cells has long been a suspected culprit in AAA disease, with signs of autoimmunity present in the AAA wall (149). There are however reports of deleterious effects of strong immunosuppressive treatment (162,163), why more specific drugs could potentially be tried in the future. Other enriched gene sets included those of mast cells, NK cells, neutrophils, phagocytosis and pathways such as NF-κB, with upregulation of NFKB1 and TNF, and NLRP3 inflammasome, with upregulated NLRP3, IL1B and IL18.

There was upregulation of angiogenesis and HIF1A in the AAA media, suggesting a hypoxic milieu. Oxidative stress, hypoxia and angiogenesis may aggravate many processes central to AAA pathophysiology, as described in section 1.2.6. Genes coding for the major antioxidants metallothioneins (MTs) were consistently downregulated in adventitia, with several MT1-related genes also downregulated in media. Other downregulated genes were those coding for antioxidants superoxide dismutase 3 and glutathione peroxidase 3. The roles of these molecules are a relevant subject to study further.

Several signaling pathways described in section 1.2.7 were dysregulated in AAA. The TGF-β-axis, including genes TGFB1I1, TGFB3 and SMAD3,-4, -6, -9 were downregulated in media, as was the Notch axis, including genes NOTCH1, -2 and -3. MAPK8 and -9 were upregulated in adventitia but not in media.

Transcription factor prediction and comparison with previous GWAS findings.

Many of the transcription factors predicted from differentially expressed genes were themselves among the most differentially expressed transcription factors. Transcription factors from upregulated genes were generally of hematopoietic origin and related to immune functions, whereas those from downregulated genes were mesenchymal and related to SMC, ECM and adhesion, which correlates with findings in previous reports (396).

Examples of upregulated transcription factors predicted from upregulated genes were ZNF267, a KLF family member of largely unknown function that is upregulated in non-alcoholic fatty liver disease and cirrhosis (397). IRF4 is important for differentiation of immune cells as well as to promote SMC dedifferentiation and to protect against neointima formation in a KLF4-dependent manner (398). IRF8 can be expressed in cardiomyocytes and lymphocytes, promotes macrophage differentiation, is essential for B-cell development and increases vascular hyperplasia (399). STAT4 is of importance for a wide variety of immune cells and is increased in tissue from failing hearts (400). IKZF1/-3 plays major roles in lymphocyte differentiation (401).

Downregulated transcription factors predicted from downregulated genes included TEAD3, among family members required for YAP-binding to DNA and mainly expressed in SMC-rich tissue (351,402). Deficiency of YAP leads to cellular senescence and YAP1 was downregulated in AAA media of the current data. OSR1 was transmurally downregulated in AAA but has a higher expression in normal aorta than in other tissues (351). It has been shown to be regulated by IKZF1 and RUNX2 – both of which were transmurally upregulated in AAA (403). ATOH8 is important in skeletal embryogenesis, among others, and can participate in muscle fiber regeneration (404). TWIST2 is expressed in progenitor cells that contribute to myocardial regeneration (405).

Several genes located close to risk loci identified in a recent meta-analysis (211) were dysregulated in the AAA media. Specifically, MMP9, LDLR and long non-coding CDKN2B-AS1/ANRIL were upregulated whereas DAB2IP, ERG, LDLR, PC1F1, SORT1 were downregulated in AAA media. LINC00540 was significantly upregulated in AAA but filtered due to low expression levels. It is important to note that mere proximity between a SNP and a gene is not sufficient evidence of a functional connection.

Tunica switching

A number of genes upregulated in AAA media, that in AAAs showed higher expression in media than adventitia, were in controls to the opposite expressed to a higher extent in adventitia compared with media, thus switching tunica predominance. These included genes coding for molecules previously implicated as culprits in AAA disease such as MMP1, 2 and 9 (406,407), CTSL and –H (408), PLAU (98), LYZ (409), DPP4 (410) and CHI3L1 (235) as well as the enrichment of gene sets related to proteolysis and inflammation, including T- and B-cells and IFNγ. Others were VCAM1, potentially indicating the increased density of neovessels in ILT-covered AAA media, TREM1 which is an amplifier of innate immune response shown to mediate inflammatory remodeling after myocardial infarction (411), HLA-related genes, IFNγ-inducible lysosomal thiol reductase (IFI30) and AIF1 which is mainly expressed in macrophages and T-cells, promotes inflammation and induce increased VSMC migration and MMP2- and -9 production in response to oxidized low density lipoprotein (412). Considering the high enrichment of genes and processes described as pathological and central to AAA disease among those that switch wall layer predominance from adventitia to media, such genes, proteins and processes not previously investigated in AAA could be important targets for future intervention and investigation.

Effect of ILT

It was observed that an ILT had a transcriptomic effect on the underlying media, but not adventitia. Genes exclusively upregulated in the ILT-covered media of AAA compared with control media were related to transcription, angiotensin maturation, proteolysis and ECM-organization. The ILT-covered compared with ILT-free media displayed higher expression

of genes related to of Hallmark immune gene sets and GO BPs related to gene expression, translation and transcription, ECM organization, angiogenesis and B-/T-cell activation and GO MFs such as vascular endothelial growth factor binding. There was a lower expression of genes included in GO BPs related to metabolism, neutrophil activation, protein folding, cell-matrix adhesion and cellular response to TGF-β. Genes upregulated in AAA media that were increased in ILT-covered compared with ILT-free wall included MS4A1, coding for B-cell surface molecule CD20, SFRP4, a Wnt inhibitor which can but does not always initiate apoptosis (413), immunoglobulin-associated JCHAIN and IGKC as well as CCL19 and CCL20 which are chemotactic for B- and T- cells and activated T-cells, respectively, and IRF4, described above. Examples of genes downregulated in AAA media that were further downregulated in ILT-covered compared with ILT-free media were SORT1, implicated in GWAS of AAA (211), KCNMA1, related to regulation of vSMC contraction as well as adhesion-related ITGB3, CDH13 and PCDH10.

Active smoking and diameter growth rate

To prioritize above mechanisms, associations between gene expression, smoking and diameter growth rate were investigated. All tissues showed increased signs of oxidative stress in response to active smoking. Further, the transcriptomic effect of smoking on control aortas aligned to a large extent with what is known about AAA disease processes, with increased inflammation-, angiogenesis-, apoptosis- and peptidase-related gene sets, along with a decreased expression of those related to muscle cells in tunica media. This presents an explanation of how tobacco smoke could injure the normal vessel wall. Further, AAAs exposed to smoking showed increased inflammation and apoptosis, with increased SMC de-differentiation and proliferation in media and increased lipid-related processes in adventitia. The diameter growth rate correlated with gene sets related to B- and T-cells, adaptive immune response and IFNγ in the AAA media as well as fatty acid/lipid-related gene sets in adventitia.

Comparison with previous studies, technical considerations and limitations

Several previous microarray studies have been published, some of which have compared AAA tissue to that of control aortas, as shown in Table 2. These studies have been performed on full-thickness samples without consideration of the ILT. The present study analyzed media and adventitia separately from ILT-covered and -free aspects of the AAA vessel wall. This approach appears to be important considering that the tunicas were distinct in unsupervised and supervised clustering and that many of the dysregulated genes and several of the enriched processes were specific to one tunica. Further, the present study detected a large number of disease-associated genes that were not detected in the two previous similar studies, while very few genes dysregulated in both previous studies were not also detected in the present study. The transcriptomic changes related to smoking and fast diameter growth rate have not been analyzed previously.

Some technical considerations deserve mentioning. Due to the limited access to non-aneurysmatic abdominal aorta from subjects of similar ages as patients with AAA, the group sizes were unequal. However, the employed statistical method (limma) does not make assumptions about the proportions between cases and controls and performs well with small sample sizes (353,414). Using an adjustment for multiple comparisons is important in order to control the number of false positives but making this adjustment too strict may arguably be misleading as slight variations of gene sets may have biologically relevant effects (415). For this reason, we used the more liberal FDR rather than Bonferroni correction and performed both threshold-based (Enrichr) and threshold-free (GSEA) analyses for gene set

enrichment. Despite this adjustment, a large number of genes were significantly dysregulated between AAA and control tissue, especially in media.

The biobank has been assembled over a long period of time and, therefore, analyses were made to detect batch effects, if any. In these analyses, a strong consistency of dysregulated genes between samples collected in early and recent years was found. Principal component analysis further suggested that AAA vs control, patient age, tunica and ILT-coverage were the most influential variables for between-sample variation in gene expression (not shown). The macroscopic dissection of wall layers into media and adventitia has been used previously by our and others groups and is technically uncomplicated (416–418) but an exact border between media and adventitia is difficult to find in AAA due to transmural fibrosis and vSMC death. Markers of vSMC and adipose tissue (223) were studied and found to predominate in the media and adventitia (not shown), respectively, as would be expected from a working dissection protocol.

The use of transcriptomics technologies has increased explosively due to the powerful results produced with increasing speed and affordability, see section 1.2.11. Some limitations to the technique in general, as well as its use specifically in this project, merit consideration. The gene expression as measured by microarrays is relative to that of other included samples and the expression levels are in a non-linear manner based on the relative concentration of RNA rather than absolute amounts. As such, a highly increased expression of one set of genes will make the other sets of genes appear downregulated despite retaining the same absolute expression level. The gene expression can be investigated on a cellular or bulk level. This project is based on analyses of bulk RNA expression, meaning RNA copies from many different cells are measured at the same time. With this approach it is not possible to discern whether a differential expression is the result of changed transcriptional regulation in the same cells, influx of new cells or absence of previously existing cells. Presumably, the above described perturbations are a combination of all these events. The current project also did not consider differential splicing or non-coding RNA, both highly important for the effect of expressed RNA. It also has to be considered that the underlying assumption from pathway analyses that expressed mRNA correlates with active proteins is simplistic and does not consider important post-translational modification, degradation or activation.

Summary

The described approach allowed the identification and localization of known and emerging disease processes such as ECM degradation, loss and dedifferentiation of vSMCs, inflammation, angiogenesis and oxidative stress, as well as the detection of thousands of genes novel to the understanding of AAA. For the first time, the transcriptomic effects of an overlying ILT, active smoking and rapid diameter growth rate were described. A process was described where sets of genes related to inflammation and proteolysis predominant in the adventitia of controls were overrepresented instead in the media of AAAs. This data will be made publicly available and can aid future research efforts.

5 CONCLUSIONS AND FUTURE DIRECTIONS

5.1 CONCLUSIONS

This thesis was designed to explore and develop new strategies to improve, refine and individualize management of patients with AAA by applying geometric, biomechanical and molecular analyses. From the included projects we made the following conclusions:

- Clinical risk factors of AAA rupture are associated with FEA-derived biomechanical stress and rupture risk indices. This gives biomechanical insights into clinical observations and further motivates the use of 3D modeling and FEA to predict outcomes of AAA.

- 3D modeling improves the ability of diameter measurements to predict growth and future indication for surgery of AAA. Additional biomechanical variables in multivariable models tuned in machine learning did not improve predictions. On the other hand, lumen diameter and biomechanical rupture risk indices were able to predict future rupture or symptomatic AAA. Growth and rupture require different prediction models and additional variables or markers as well as pathophysiological insight, are needed.

- E-XDP is a marker of AAA and ILT presence and size, as well as the mechanical stress of the ILT itself, and could possibly be used to monitor the development of an ILT or the extent to which it is in contact with flowing blood.

- Main pathophysiological features, as inferred from gene expression patterns, include inflammation transmurally and increased proteolysis, angiogenesis, apoptosis as well as decreased muscle contraction, development, differentiation and adhesion in the media. The adventitia displayed downregulation of metal ion response and lipid-related processes. Smoking caused AAA-like transcriptomic changes in control aortas. Growth rate was associated with adaptive immunity in media and lipid-related processes in adventitia. A large number of genes not previously described in AAA disease were detected.

In summary, these projects have found biomechanical explanations for clinical risk factors, made improvements in predictions of AAA outcome and growth by geometric and biomechanical modeling, discovered a new biomarker of AAA and mapped AAA pathogenesis including the molecular fingerprint of smoke exposure and diameter growth. Together, these findings suggest that the care of patients with AAA can be improved and personalized by use of geometric, biomechanical and molecular analyses.

5.2 FUTURE DIRECTIONS

A number of potential directions towards improved management of patients with AAA can be sketched out from these results along with the progress of the field. Future projects should develop and prospectively validate FEA, create more sophisticated machine learning models for AAA outcome prediction and use imaging and biobank resources for high-throughput multi-systems analysis and prediction modeling.

The FEA methodology as applied to AAA has improved in the last twenty years. However, several potential improvements remain. These may include taking signs of a fractured ILT

into account and improving the wall strength model by biaxial testing, more detailed patient descriptions and to factor in vessel wall calcifications. Probabilistic approaches to rupture risk estimation require extensive computation and time but lead to improved predictions (330,331). Histomechanical constitutive descriptions of AAA is a complex but promising prospect under investigation (419). The FEA methodology used in this thesis can predict AAA rupture by retrospective analysis of pre-rupture images (270,330) but it has not been prospectively validated. Our research group is currently performing a prospective study on patients with small AAAs in which patient records are collected in a standardized manner, CTA imaging is systematically performed two times with a two-year interval and a clinical follow-up is completed after five years.

A machine learning framework could be applied to any range of geometric, biomechanical or molecular features to predict outcome of AAA. An ideal future study should include a large number, possibly a thousand or more, patients who undergo at least two CTAs at regular intervals and have their blood sampled at both instances. 3D models of the AAA, spatial maps of biomechanical load as well as data derived from radiomics (defined as the high-throughput analysis of quantitative imaging features) (420) could then be collected from imaging while genomics and plasma proteomics, metabolomics and transcriptomics could be mined from blood samples. These features would then be highly compatible with any machine learning approach to predict growth and rupture of AAA. If made publicly available, this data could be used by international research groups to find the most effective and robust prediction algorithms. In our ongoing prospective study described above, there are also plans to include blood samples in the protocol. This will result in a multi-level dataset on AAA progression aimed to be used with above considerations. Although around 100 patients are treated surgically for AAA at the Karolinska University Hospital every year, the challenge of amassing and analyzing a large-scale dataset comprising thousands of patients described by detailed imaging and biological data is well-suited for international collaborations.

With study IV, a first step in a systems-biology effort to comprehensively map the AAA pathophysiology was made. The most differentially expressed genes and resulting proteins need to undergo mechanistic studies to determine their respective roles. While microarrays yield powerful results, large domains of biological information remain to be explored. Future steps could include epigenomic, genomic, proteomic and single-cell analyses. Integrating these levels of information could uncover eQTLs, clinically relevant heterogeneities within and between patients (421) and, when combined with biomechanical data on the AAAs, open paths toward precision medicine and provide foundational support for future drug target identification. Fortunately, the StAAAB was developed at the outset with these kinds of applications in mind, allowing continued progress in this area.

This thesis implemented 3D analysis, FEA, prediction modeling aided by machine learning and multi-level bioinformatic analyses. Performance and robustness of findings could increase and avenues for improvement of current guidelines may open with the entry of high-throughput technology ("-omics"), rapid biomechanical evaluation, detailed pathophysiological data and machine learning principles into the field of aneurysm research. With the included papers, a foundation for this type of approach has been established.

6 ACKNOWLEDGEMENTS

Joy Roy, main supervisor. What a journey! Thank you for introducing me to vascular surgery research back in 2013 and for giving me great projects but also full freedom to pursue some of my own different research interests under your expert guidance, even when I'm sure you could find more effective use of my time. We have had many rewarding and productive scientific discussions and I have been inspired by your creativity and thorough knowledge in so many diverse subjects. Thank you for flying me out to different parts of the world I could have missed otherwise, including surgery rotations in Kampala and football games in St Petersburg, you made the PhD feel like a second home rather than work. I hope this is just the beginning of a long research collaboration and friendship.

Rebecka Hultgren, co-supervisor. It was your well-written project proposal I initially applied to and you first introduced me to the group. I initially thought there was only one project but was surprised by the multitude of ideas and research plans you and Joy had to offer. Thank you for always looking out for and supporting your PhD students as well as for keeping me and the projects on track. I am inspired by your expertise, efficiency and professionalism, in research and in the clinic, and I aspire to gain at least a fraction of that skillset over the course of my career.

T Christian Gasser, co-supervisor. Thank you for all your help with the engineering aspects of this work, and for educating us medical people in (very) basic solid mechanics. This does not go the other way around, however, as you have such an extensive knowledge base about vascular biology. This fact, together with your talent for explaining complex engineering considerations in ways that children could comprehend (which is the appropriate level for many of us), have made you a truly ideal partner for this interdisciplinary collaboration.

Per Eriksson, co-supervisor. Thank you for your extremely valuable input into the preclinical aspects of the thesis, the last paper especially benefitted from your ideas. I am impressed by your exact memory and thorough comprehension of molecular biology but also your wide knowledge of subjects such as ornithology and African geography.

Ulf Hedin, team leader. I felt welcomed to the vascular surgery group from the first day and it hasn't changed yet. I think a research group that combines ambition and pursuit of excellence with such a friendly atmosphere is rare and hard to come by. Thank you for creating that space.

Thank you, **Mette** and **Malin** for your technical expertise, kind help with experiments and sampling and for always producing high quality results.

Angela, **Christina, Sayid, Giampaolo, Philipp** and **Otto**; thank you for great collaborations and co-authorship.

Ludmila Babina, thank you for your expert assistance with illustrations.

I would like the thank the colleagues at the **Vascular Surgery Department** at the Karolinska University Hospital, and department head **Carl-Magnus Wahlgren**, for supporting all of the different research projects and for taking such care in securing patient consents, tissue and blood samples. Thank you letting me work at your great department, for teaching me about vascular surgery and for always being collegial, helpful and focused on the patients' best.

When first arriving at the CMM annex building L5, there were my classmate **Samuel**, who had started his master's thesis project a few weeks earlier, the engineer **Björn** and the research coordinator **Siw**. We were later joined by **Antti**, **Marko** and **Mareia** and often graced by the presence of the wise PI:s **Anton** and **Joy**. Over the coming years this became a fantastic workplace where profound thought and banal realizations were treated the same. Many discussions about career, life choices and politics were held, not seldom continued at local bars without the need or even capacity to reach any conclusions. The PhD studies would not have been the same without you.

The PhD is a web of different deadlines and hand-ins. Thank you **Siw** and **Ann-Britt** for all your support and for always looking out for the PhD students' best interests.

Over the course of these studies and many other published and un-published projects, we have enlisted the help of several ambitious master's or bachelor's degree project workers; **Antti**, **Emil**, **Mareia**, **Charles**, **Fabian**, **Marko**, **Linda**, **Joya**, **Patricia** and **Andreas**. Thank you for your dedicated work! **Antti**, **Mareia**, **Marko** and **Patricia** continued with our group and I hope to see many high-impact publications from them in the future.

The vascular surgery group; **Ljubica**, **Andrew, Eva, Christina**, **Linnea**, **Mona**, **Urszula**, **Bianca**, **Till**, **Katarina**, **Olga, Alicia, Hong, Suvi, Nikos** and **Philip;** thank you for your support, great collegiality, for kindly including me into your projects and for helping me with the many areas of research in which I am more or less ignorant. And for your great Thursday cakes! Thank you, **Oscar** and **Ali**, for many valuable discussions about work-work balance.

I would like to thank **my friends** for being patient with my absence and for always planning such nice get-togethers.

Thank you, **Mom** and **Dad**, for being such inspiring role models, always projecting positivity about work while teaching me to never give up. You have always been immensely supportive, encouraging and truly showed unconditional love. While politically pessimistic, I think you in general showed an optimistic and can-do attitude that we learned from. Thank you, **Madeleine**, for always being such a fantastic sister, for reminding me of life outside of work and for putting up with your brother for so many years! Thank you, **Suzanne** and **Roland**, for being sources of inspiration and for being such kind and understanding siblings.
You, my family, gave me an ideal upbringing which lies at the base of all that follows.

Victoria, you have been so supportive through this process. It would not have worked without your help. You have shown so much love and patience with me despite all the hours away from you. Thank you for your love, support and for investing in our life together to make us enjoy it as much as possible.

7 REFERENCES

1. Johnston KW, Rutherford RB, Tilson MD, Shah DM, Hollier L, Stanley JC. Suggested standards for reporting on arterial aneurysms. Subcommittee on Reporting Standards for Arterial Aneurysms, Ad Hoc Committee on Reporting Standards, Society for Vascular Surgery and North American Chapter, International Society for Cardiovascular Surgery. J Vasc Surg. 1991 Mar;13(3):452–8.
2. Chaikof EL, Dalman RL, Eskandari MK, Jackson BM, Lee WA, Mansour MA, et al. The Society for Vascular Surgery practice guidelines on the care of patients with an abdominal aortic aneurysm. Journal of Vascular Surgery. 2018 Jan 1;67(1):2-77.e2.
3. Wanhainen A, Verzini F, Van Herzeele I, Allaire E, Bown M, Cohnert T, et al. Editor's Choice - European Society for Vascular Surgery (ESVS) 2019 Clinical Practice Guidelines on the Management of Abdominal Aorto-iliac Artery Aneurysms. Eur J Vasc Endovasc Surg. 2019 Jan;57(1):8–93.
4. Karthaus EG, Tong TML, Vahl A, Hamming JF, Dutch Society of Vascular Surgery, the Steering Committee of the Dutch Surgical Aneurysm Audit and the Dutch Institute for Clinical Auditing. Saccular Abdominal Aortic Aneurysms: Patient Characteristics, Clinical Presentation, Treatment, and Outcomes in the Netherlands. Ann Surg. 2019;270(5):852–8.
5. Hans SS, Jareunpoon O, Balasubramaniam M, Zelenock GB. Size and location of thrombus in intact and ruptured abdominal aortic aneurysms. J Vasc Surg. 2005 Apr;41(4):584–8.
6. Wanhainen A, Hultgren R, Linné A, Holst J, Gottsäter A, Langenskiöld M, et al. Outcome of the Swedish Nationwide Abdominal Aortic Aneurysm Screening Program. Circulation. 2016 Oct 18;134(16):1141–8.
7. Svensjö S, Björck M, Wanhainen A. Current prevalence of abdominal aortic aneurysm in 70-year-old women. Br J Surg. 2013 Feb;100(3):367–72.
8. Kent KC, Zwolak RM, Egorova NN, Riles TS, Manganaro A, Moskowitz AJ, et al. Analysis of risk factors for abdominal aortic aneurysm in a cohort of more than 3 million individuals. Journal of Vascular Surgery. 2010 Sep;52(3):539–48.
9. Wahlgren CM, Larsson E, Magnusson PKE, Hultgren R, Swedenborg J. Genetic and environmental contributions to abdominal aortic aneurysm development in a twin population. Journal of Vascular Surgery. 2010 Jan;51(1):3–7.
10. Fattahi N, Rosenblad A, Kragsterman B, Hultgren R. Risk factors in 50-year-old men predicting development of abdominal aortic aneurysm. J Vasc Surg. 2020 Feb 27;
11. Nyrønning LÅ, Stenman M, Hultgren R, Albrektsen G, Videm V, Mattsson E. Symptoms of Depression and Risk of Abdominal Aortic Aneurysm: A HUNT Study. J Am Heart Assoc. 2019 05;8(21):e012535.
12. Azhar B, Patel SR, Holt PJE, Hinchliffe RJ, Thompson MM, Karthikesalingam A. Misdiagnosis of ruptured abdominal aortic aneurysm: systematic review and meta-analysis. J Endovasc Ther. 2014 Aug;21(4):568–75.
13. Parkinson F, Ferguson S, Lewis P, Williams IM, Twine CP. Rupture rates of untreated large abdominal aortic aneurysms in patients unfit for elective repair. Journal of Vascular Surgery. 2015 Jun 1;61(6):1606–12.
14. Zommorodi S, Bottai M, Hultgren R. Sex differences in repair rates and outcomes of patients with ruptured abdominal aortic aneurysm. Br J Surg. 2019 Aug 12;
15. Bengtsson H, Bergqvist D. Ruptured abdominal aortic aneurysm: a population-based study. J Vasc Surg. 1993 Jul;18(1):74–80.
16. Hultgren R, Zommorodi S, Gambe M, Roy J. A Majority of Admitted Patients With Ruptured Abdominal Aortic Aneurysm Undergo and Survive Corrective Treatment: A Population-Based Retrospective Cohort Study. World J Surg. 2016 Aug 22;
17. Blomgren L, Ledningsgruppen i Swedvasc 2018. Swedvasc's årsrapport 2019 för 2018 års verksamhet.
18. Conway KP, Byrne J, Townsend M, Lane IF. Prognosis of patients turned down for conventional abdominal aortic aneurysm repair in the endovascular and sonographic era: Szilagyi revisited? J Vasc Surg. 2001 Apr;33(4):752–7.
19. Brown LC, Powell JT. Risk factors for aneurysm rupture in patients kept under ultrasound surveillance. UK Small Aneurysm Trial Participants. Ann Surg. 1999 Sep;230(3):289–96; discussion 296-297.
20. Szilagyi DE, Elliott JP, Smith RF. Clinical fate of the patient with asymptomatic abdominal aortic aneurysm and unfit for surgical treatment. Arch Surg. 1972 Apr;104(4):600–6.
21. Sweeting MJ, Thompson SG, Brown LC, Powell JT, RESCAN collaborators. Meta-analysis of individual patient data to examine factors affecting growth and rupture of small abdominal aortic aneurysms. Br J Surg. 2012 May;99(5):655–65.
22. McPhee JT, Hill JS, Eslami MH. The impact of gender on presentation, therapy, and mortality of abdominal aortic aneurysm in the United States, 2001-2004. Journal of Vascular Surgery. 2007 May;45(5):891–9.
23. Grootenboer N, van Sambeek MRHM, Arends LR, Hendriks JM, Hunink MGM, Bosch JL. Systematic review and meta-analysis of sex differences in outcome after intervention for abdominal aortic aneurysm. Br J Surg. 2010 Aug;97(8):1169–79.
24. Giles KA, Wyers MC, Pomposelli FB, Hamdan AD, Ching YA, Schermerhorn ML. The impact of body mass index on perioperative outcomes of open and endovascular abdominal aortic aneurysm repair from the National Surgical Quality Improvement Program, 2005-2007. J Vasc Surg. 2010 Dec;52(6):1471–7.
25. Grant SW, Sperrin M, Carlson E, Chinai N, Ntais D, Hamilton M, et al. Calculating when elective abdominal aortic aneurysm repair improves survival for individual patients: development of the Aneurysm Repair Decision Aid and economic evaluation. Health Technol Assess. 2015 Apr;19(32):1–154, v–vi.
26. Zommorodi S, Leander K, Roy J, Steuer J, Hultgren R. Understanding abdominal aortic aneurysm epidemiology: socioeconomic position affects outcome. J Epidemiol Community Health. 2018;72(10):904–10.
27. Oliver-Williams C, Sweeting MJ, Turton G, Parkin D, Cooper D, Rodd C, et al. Lessons learned about prevalence and growth rates of abdominal aortic aneurysms from a 25-year ultrasound population screening programme. Br J Surg. 2018 Jan;105(1):68–74.
28. Gürtelschmid M, Björck M, Wanhainen A. Comparison of three ultrasound methods of measuring the diameter of the abdominal aorta. Br J Surg. 2014 May;101(6):633–6.
29. Larsson E, Vishnevskaya L, Kalin B, Granath F, Swedenborg J, Hultgren R. High frequency of thoracic aneurysms in patients with abdominal aortic aneurysms. Ann Surg. 2011 Jan;253(1):180–4.
30. Lederle FA. The Last (Randomized) Word on Screening for Abdominal Aortic Aneurysms. JAMA Intern Med. 2016 Dec 1;176(12):1767–8.
31. Guirguis-Blake JM, Beil TL, Senger CA, Whitlock EP. Ultrasonography screening for abdominal aortic aneurysms: a systematic evidence review for the U.S. Preventive Services Task Force. Ann Intern Med. 2014 Mar 4;160(5):321–9.
32. Wanhainen A, Themudo R, Ahlström H, Lind L, Johansson L. Thoracic and abdominal aortic dimension in 70-year-old men and women--a population-based whole-body magnetic resonance imaging (MRI) study. J Vasc Surg. 2008 Mar;47(3):504–12.
33. Scott RAP, Bridgewater SG, Ashton HA. Randomized clinical trial of screening for abdominal aortic aneurysm in women. Br J Surg. 2002 Mar;89(3):283–5.
34. Larsson E, Granath F, Swedenborg J, Hultgren R. A population-based case-control study of the familial risk of abdominal aortic aneurysm. J Vasc Surg. 2009 Jan;49(1):47–50; discussion 51.
35. Hultgren R, Linné A, Svensjö S. Cost-effectiveness of targeted screening for abdominal aortic aneurysm in siblings. Br J Surg. 2019;106(3):206–16.
36. Rughani G, Robertson L, Clarke M. Medical treatment for small abdominal aortic aneurysms. In: The Cochrane Collaboration, Rughani G, editors. Cochrane Database of Systematic Reviews [Internet]. Chichester, UK: John Wiley & Sons, Ltd; 2012 [cited 2013 Sep 3]. Available from: http://summaries.cochrane.org/CD009536/drug-treatment-for-small-abdominal-aortic-aneurysms
37. United Kingdom Small Aneurysm Trial Participants, Powell JT, Brady AR, Brown LC, Fowkes FGR, Greenhalgh RM, et al. Long-term outcomes of immediate repair compared with surveillance of small abdominal aortic aneurysms. N Engl J Med. 2002 09;346(19):1445–52.
38. Lederle FA, Wilson SE, Johnson GR, Reinke DB, Littooy FN, Acher CW, et al. Immediate repair compared with surveillance of small abdominal aortic aneurysms. N Engl J Med. 2002 May 9;346(19):1437–44.
39. Cao P, De Rango P, Verzini F, Parlani G, Romano L, Cieri E, et al. Comparison of surveillance versus aortic endografting for small aneurysm repair (CAESAR): results from a randomised trial. Eur J Vasc Endovasc Surg. 2011 Jan;41(1):13–25.
40. Ouriel K, Clair DG, Kent KC, Zarins CK, Positive Impact of Endovascular Options for treating Aneurysms Early (PIVOTAL) Investigators. Endovascular repair compared with surveillance for patients with small abdominal aortic aneurysms. J Vasc Surg. 2010 May;51(5):1081–7.
41. Filardo G, Powell JT, Martinez MA-M, Ballard DJ. Surgery for small asymptomatic abdominal aortic aneurysms. Cochrane Database Syst Rev. 2015 Feb 8;(2):CD001835.
42. Ricco J-B, Cau J, Biancari F, Desvergnes M, Lefort N, Belmonte R, et al. Outcome After Open and Laparoscopic Aortic Surgery in Matched Cohorts Using Propensity Score Matching. Eur J Vasc Endovasc Surg. 2016 Aug;52(2):179–88.
43. Ma B, Wang Y, Chen K, Zhang Y, Pan H, Yang K. Transperitoneal versus retroperitoneal approach for elective open abdominal aortic aneurysm repair. Cochrane Database of Systematic Reviews [Internet]. 2016 [cited 2020 Mar 18];(2). Available from: https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD010373.pub2/abstract

44. White GH, Yu W, May J. Endoleak--a proposed new terminology to describe incomplete aneurysm exclusion by an endoluminal graft. J Endovasc Surg. 1996 Feb;3(1):124–5.
45. Powell JT, Sweeting MJ, Ulug P, Blankensteijn JD, Lederle FA, Becquemin J-P, et al. Meta-analysis of individual-patient data from EVAR-1, DREAM, OVER and ACE trials comparing outcomes of endovascular or open repair for abdominal aortic aneurysm over 5 years. Br J Surg. 2017;104(3):166–78.
46. Sweeting MJ, Balm R, Desgranges P, Ulug P, Powell JT, Ruptured Aneurysm Trialists. Individual-patient meta-analysis of three randomized trials comparing endovascular versus open repair for ruptured abdominal aortic aneurysm. Br J Surg. 2015 Sep;102(10):1229–39.
47. Becquemin J-P, Pillet J-C, Lescalie F, Sapoval M, Goueffic Y, Lermusiaux P, et al. A randomized controlled trial of endovascular aneurysm repair versus open surgery for abdominal aortic aneurysms in low- to moderate-risk patients. Journal of Vascular Surgery. 2011 May;53(5):1167-1173.e1.
48. van Schaik TG, Yeung KK, Verhagen HJ, de Bruin JL, van Sambeek MRHM, Balm R, et al. Long-term survival and secondary procedures after open or endovascular repair of abdominal aortic aneurysms. J Vasc Surg. 2017;66(5):1379–89.
49. Patel R, Sweeting MJ, Powell JT, Greenhalgh RM, EVAR trial investigators. Endovascular versus open repair of abdominal aortic aneurysm in 15-years' follow-up of the UK endovascular aneurysm repair trial 1 (EVAR trial 1): a randomised controlled trial. Lancet. 2016 Oct 12;
50. Lederle FA, Kyriakides TC, Stroupe KT, Freischlag JA, Padberg FT, Matsumura JS, et al. Open versus Endovascular Repair of Abdominal Aortic Aneurysm. N Engl J Med. 2019 30;380(22):2126–35.
51. Hinchliffe RJ, Bruijstens L, MacSweeney STR, Braithwaite BD. A randomised trial of endovascular and open surgery for ruptured abdominal aortic aneurysm - results of a pilot study and lessons learned for future studies. Eur J Vasc Endovasc Surg. 2006 Nov;32(5):506–13; discussion 514-515.
52. Reimerink JJ, Hoornweg LL, Vahl AC, Wisselink W, van den Broek TAA, Legemate DA, et al. Endovascular repair versus open repair of ruptured abdominal aortic aneurysms: a multicenter randomized controlled trial. Ann Surg. 2013 Aug;258(2):248–56.
53. IMPROVE Trial Investigators, Powell JT, Sweeting MJ, Thompson MM, Ashleigh R, Bell R, et al. Endovascular or open repair strategy for ruptured abdominal aortic aneurysm: 30 day outcomes from IMPROVE randomised trial. BMJ. 2014;348:f7661.
54. Desgranges P, Kobeiter H, Katsahian S, Bouffi M, Gouny P, Favre J-P, et al. Editor's Choice - ECAR (Endovasculaire ou Chirurgie dans les Anévrysmes aorto-iliaques Rompus): A French Randomized Controlled Trial of Endovascular Versus Open Surgical Repair of Ruptured Aorto-iliac Aneurysms. Eur J Vasc Endovasc Surg. 2015 Sep;50(3):303–10.
55. Overview | Abdominal aortic aneurysm: diagnosis and management | Guidance | NICE [Internet]. NICE; [cited 2020 Jul 31]. Available from: https://www.nice.org.uk/guidance/ng156
56. IMPROVE Trial Investigators. Comparative clinical effectiveness and cost effectiveness of endovascular strategy v open repair for ruptured abdominal aortic aneurysm: three year results of the IMPROVE randomised trial. BMJ. 2017 14;359:j4859.
57. Sweeting MJ, Patel R, Powell JT, Greenhalgh RM, EVAR Trial Investigators. Endovascular Repair of Abdominal Aortic Aneurysm in Patients Physically Ineligible for Open Repair: Very Long-term Follow-up in the EVAR-2 Randomized Controlled Trial. Ann Surg. 2017;266(5):713–9.
58. RESCAN Collaborators, Bown MJ, Sweeting MJ, Brown LC, Powell JT, Thompson SG. Surveillance intervals for small abdominal aortic aneurysms: a meta-analysis. JAMA. 2013 Feb 27;309(8):806–13.
59. Brady AR, Thompson SG, Fowkes FGR, Greenhalgh RM, Powell JT, UK Small Aneurysm Trial Participants. Abdominal aortic aneurysm expansion: risk factors and time intervals for surveillance. Circulation. 2004 Jul 6;110(1):16–21.
60. Sweeting MJ, Thompson SG. Joint modelling of longitudinal and time-to-event data with application to predicting abdominal aortic aneurysm growth and rupture. Biom J. 2011 Sep;53(5):750–63.
61. Skibba AA, Evans JR, Hopkins SP, Yoon HR, Katras T, Kalbfleisch JH, et al. Reconsidering gender relative to risk of rupture in the contemporary management of abdominal aortic aneurysms. J Vasc Surg. 2015 Dec;62(6):1429–36.
62. Zommorodi S, Roy J, Steuer J, Hultgren R. High proportion of known abdominal aortic aneurysm in patients with rupture indicates surveillance deficiency. J Vasc Surg. 2016 Oct;64(4):949-955.e1.
63. Siika A, Lindquist Liljeqvist M, Zommorodi S, Nilsson O, Andersson P, Gasser TC, et al. A large proportion of patients with small ruptured abdominal aortic aneurysms are women and have chronic obstructive pulmonary disease. PLoS ONE. 2019;14(5):e0216558.
64. Ashton HA, Buxton MJ, Day NE, Kim LG, Marteau TM, Scott R a. P, et al. The Multicentre Aneurysm Screening Study (MASS) into the effect of abdominal aortic aneurysm screening on mortality in men: a randomised controlled trial. Lancet. 2002 Nov 16;360(9345):1531–9.
65. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015. J Am Coll Cardiol. 2017 Jul 4;70(1):1–25.
66. Bryan J. gapminder: Data from Gapminder [Internet]. 2017. Available from: https://CRAN.R-project.org/package=gapminder
67. Piburn J. wbstats: Programmatic Access to the World Bank API [Internet]. Oak Ridge National Laboratory; 2018. Available from: https://www.ornl.gov/division/csed/gist
68. Kwan Gene F., Mayosi Bongani M., Mocumbi Ana O., Miranda J. Jaime, Ezzati Majid, Jain Yogesh, et al. Endemic Cardiovascular Diseases of the Poorest Billion. Circulation. 2016 Jun 14;133(24):2561–75.
69. Meara JG, Leather AJM, Hagander L, Alkire BC, Alonso N, Ameh EA, et al. Global Surgery 2030: evidence and solutions for achieving health, welfare, and economic development. Lancet. 2015 Aug 8;386(9993):569–624.
70. Stewart BT, Gyedu A, Giannou C, Mishra B, Rich N, Wren S, et al. Consensus recommendations for essential vascular care in low- and middle-income countries. J Vasc Surg. 2016 Dec;64(6):1770-1779.e1.
71. Harvey W, Leake CD. Exercitatio anatomica de motu cordis et sanguinis in animalibus pt. 1. Facsimile of the original Latin ed. (1628)--pt. 2. The English translation. Springfield, III: Thomas; 1928.
72. Bryan CP. The Papyrus Ebers (translated). 1930.
73. Bobadilla JL. From Ebers to EVARs: A Historical Perspective on Aortic Surgery. Aorta (Stamford). 2013 Jul 1;1(2):89–95.
74. Matas R. I. An Operation for the Radical Cure of Aneurism based upon Arteriorrhaphy. Ann Surg. 1903 Feb;37(2):161–96.
75. Rea CE. The surgical treatment of aneurysm of the abdominal aorta. Minn Med. 1948 Feb;31(2):153–6.
76. Dubost C, Allary M, Oeconomos N. RESECTION OF AN ANEURYSM OF THE ABDOMINAL AORTA: Reestablishment of the Continuity by a Preserved Human Arterial Graft, with Result After Five Months. AMA Arch Surg. 1952 Mar 1;64(3):405–8.
77. Creech O. Endo-aneurysmorrhaphy and treatment of aortic aneurysm. Ann Surg. 1966 Dec;164(6):935–46.
78. De Bakey ME, Cooley DA, Crawford ES, Morris GC. Clinical application of a new flexible knitted dacron arterial substitute. Am Surg. 1958 Dec;24(12):862–9.
79. Volodos' NL, Karpovich IP, Shekhanin VE, Troian VI, Iakovenko LF. [A case of distant transfemoral endoprosthesis of the thoracic artery using a self-fixing synthetic prosthesis in traumatic aneurysm]. Grudn Khir. 1988 Dec;(6):84–6.
80. Parodi JC, Palmaz JC, Barone HD. Transfemoral Intraluminal Graft Implantation for Abdominal Aortic Aneurysms. Annals of Vascular Surgery. 1991 Nov 1;5(6):491–9.
81. He CM, Roach MR. The composition and mechanical properties of abdominal aortic aneurysms. Journal of Vascular Surgery. 1994 Jul;20(1):6–13.
82. Cahill PA, Redmond EM. Vascular endothelium – Gatekeeper of vessel health. Atherosclerosis. 2016 May 1;248:97–109.
83. Wagenseil JE, Mecham RP. Vascular Extracellular Matrix and Arterial Mechanics. Physiological reviews. 2009 Jul;89(3):957.
84. M MP. Basement Membrane Proteins: Structure, Assembly, and Cellular Interactions. Critical Reviews in Biochemistry and Molecular Biology. 1992 Jan 1;27(1–2):93–127.
85. Iozzo RV, Schaefer L. Proteoglycan form and function: A comprehensive nomenclature of proteoglycans. Matrix Biology. 2015 Mar 1;42:11–55.
86. O'Connell MK, Murthy S, Phan S, Xu C, Buchanan J, Spilker R, et al. The three-dimensional micro- and nanostructure of the aortic medial lamellar unit measured using 3D confocal and electron microscopy imaging. Matrix Biology. 2008 Apr 1;27(3):171–81.
87. Gomez D, Owens GK. Smooth muscle cell phenotypic switching in atherosclerosis. Cardiovasc Res. 2012 Jul 15;95(2):156–64.
88. Yanagisawa H, Wagenseil J. Elastic fibers and biomechanics of the aorta: Insights from mouse studies. Matrix Biology. 2020 Jan 1;85–86:160–72.
89. Faury G, Pezet M, Knutsen RH, Boyle WA, Heximer SP, McLean SE, et al. Developmental adaptation of the mouse cardiovascular system to elastin haploinsufficiency. Journal of Clinical Investigation. 2003 Nov 1;112(9):1419.
90. Humphrey JD. Possible Mechanical Roles of Glycosaminoglycans in Thoracic Aortic Dissection and Associations with Dysregulated TGF-β. Journal of vascular research. 2013;50(1):1.
91. Yanagisawa H, Davis EC. Unraveling the mechanism of elastic fiber assembly: The roles of short fibulins. Int J Biochem Cell Biol. 2010 Jul;42(7):1084–93.
92. Sherratt MJ. Tissue elasticity and the ageing elastic fibre. Age (Dordr). 2009 Dec;31(4):305–25.
93. Kelleher CM, McLean SE, Mecham RP. Vascular Extracellular Matrix and Aortic Development. In: Current Topics in Developmental Biology [Internet]. Academic Press; 2004 [cited 2020 Apr 2]. p. 153–88. (Developmental Vascular Biology; vol. 62). Available from: http://www.sciencedirect.com/science/article/pii/S0070215304620060

94. Nissen R, Cardinale GJ, Udenfriend S. Increased turnover of arterial collagen in hypertensive rats. Proc Natl Acad Sci U S A. 1978 Jan;75(1):451–3.
95. Xu J, Shi G-P. Vascular wall extracellular matrix proteins and vascular diseases. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. 2014 Nov 1;1842(11):2106–19.
96. Azeloglu EU, Albro MB, Thimmappa VA, Ateshian GA, Costa KD. Heterogeneous transmural proteoglycan distribution provides a mechanism for regulating residual stresses in the aorta. American Journal of Physiology - Heart and Circulatory Physiology. 2008 Mar 1;294(3):H1197–205.
97. Maroudas AI. Balance between swelling pressure and collagen tension in normal and degenerate cartilage. Nature. 1976 Apr 29;260(5554):808–9.
98. Michel J-B, Martin-Ventura J-L, Egido J, Sakalihasan N, Treska V, Lindholt J, et al. Novel aspects of the pathogenesis of aneurysms of the abdominal aorta in humans. Cardiovasc Res. 2011 Apr 1;90(1):18–27.
99. Stenmark KR, Yeager ME, El Kasmi KC, Nozik-Grayck E, Gerasimovskaya EV, Li M, et al. The Adventitia: Essential Regulator of Vascular Wall Structure and Function. In: Julius D, editor. Annual Review of Physiology, Vol 75. Palo Alto: Annual Reviews; 2013. p. 23–47.
100. Majesky MW. Developmental Basis of Vascular Smooth Muscle Diversity. Arteriosclerosis, Thrombosis, and Vascular Biology. 2007 Jun 1;27(6):1248–58.
101. Topouzis S, Majesky MW. Smooth Muscle Lineage Diversity in the Chick Embryo. Developmental Biology. 1996 Sep 15;178(2):430–45.
102. Loeys BL, Dietz HC. Loeys-Dietz Syndrome. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJ, et al., editors. GeneReviews(®) [Internet]. Seattle (WA): University of Washington, Seattle; 1993 [cited 2016 Nov 3]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1133/
103. Dietz HC. Marfan Syndrome. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJ, et al., editors. GeneReviews(®) [Internet]. Seattle (WA): University of Washington, Seattle; 1993 [cited 2016 Nov 3]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1335/
104. Neptune ER, Frischmeyer PA, Arking DE, Myers L, Bunton TE, Gayraud B, et al. Dysregulation of TGF-β activation contributes to pathogenesis in Marfan syndrome. Nat Genet. 2003 Mar;33(3):407–11.
105. Wolinsky H, Glagov S. Comparison of abdominal and thoracic aortic medial structure in mammals. Deviation of man from the usual pattern. Circ Res. 1969 Dec;25(6):677–86.
106. Wilson PWF, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of Coronary Heart Disease Using Risk Factor Categories. Circulation. 1998 May 1;97(18):1837–47.
107. Hirsch AT, Haskal ZJ, Hertzer NR, Bakal CW, Creager MA, Halperin JL, et al. ACC/AHA 2005 Practice Guidelines for the Management of Patients With Peripheral Arterial Disease (Lower Extremity, Renal, Mesenteric, and Abdominal Aortic). Circulation. 2006 Mar 21;113(11):e463–654.
108. Kent KC, Zwolak RM, Egorova NN, Riles TS, Manganaro A, Moskowitz AJ, et al. Analysis of risk factors for abdominal aortic aneurysm in a cohort of more than 3 million individuals. Journal of Vascular Surgery. 2010 Sep;52(3):539–48.
109. Maas AHEM, Appelman YEA. Gender differences in coronary heart disease. Neth Heart J. 2010 Dec;18(12):598–602.
110. Hultgren R. Abdominal Aortic Aneurysms—Gender Aspects on Prevalence, Treatment, and Concurrent Aneurysms. The Thoracic and Cardiovascular Surgeon. 2012 Dec 6;61(01):015–21.
111. Dalman RL, Wanhainen A, Mani K, Modarai B. Top 10 candidate aortic disease trials. Journal of Internal Medicine. 2020;288(1):23–37.
112. Lindeman JH. The pathophysiologic basis of abdominal aortic aneurysm progression: a critical appraisal. Expert Review of Cardiovascular Therapy. 2015 Jul 3;13(7):839–51.
113. Biros E, Gäbel G, Moran CS, Schreurs C, Lindeman JHN, Walker PJ, et al. Differential gene expression in human abdominal aortic aneurysm and aortic occlusive disease. Oncotarget. 2015 May 30;6(15):12984–96.
114. Golledge J, Kuivaniemi H. Genetics of abdominal aortic aneurysm: Current Opinion in Cardiology. 2013 May;28(3):290–6.
115. Campa JS, Greenhalgh RM, Powell JT. Elastin degradation in abdominal aortic aneurysms. Atherosclerosis. 1987 May 1;65(1):13–21.
116. Golledge J. Abdominal aortic aneurysm: update on pathogenesis and medical treatments. Nature Reviews Cardiology. 2019 Apr;16(4):225–42.
117. Gasser TC, Gallinetti S, Xing X, Forsell C, Swedenborg J, Roy J. Spatial orientation of collagen fibers in the abdominal aortic aneurysm's wall and its relation to wall mechanics. Acta Biomaterialia. 2012 Aug;8(8):3091–103.
118. Bode MK, Soini Y, Melkko J, Satta J, Risteli L, Risteli J. Increased amount of type III pN-collagen in human abdominal aortic aneurysms: evidence for impaired type III collagen fibrillogenesis. J Vasc Surg. 2000 Dec;32(6):1201–7.
119. Abdul-Hussien H, Soekhoe RGV, Weber E, von der Thüsen JH, Kleemann R, Mulder A, et al. Collagen Degradation in the Abdominal Aneurysm: A Conspiracy of Matrix Metalloproteinase and Cysteine Collagenases. The American Journal of Pathology. 2007 Mar;170(3):809–17.
120. Dobrin PB, Baker WH, Gley WC. Elastolytic and Collagenolytic Studies of Arteries: Implications for the Mechanical Properties of Aneurysms. Arch Surg. 1984 Apr 1;119(4):405–9.
121. Sonesson B, Hansen F, Stale H, Länne T. Compliance and diameter in the human abdominal aorta--the influence of age and sex. Eur J Vasc Surg. 1993 Nov;7(6):690–7.
122. Derwich W, Wittek A, Pfister K, Nelson K, Bereiter-Hahn J, Fritzen CP, et al. High Resolution Strain Analysis Comparing Aorta and Abdominal Aortic Aneurysm with Real Time Three Dimensional Speckle Tracking Ultrasound. European Journal of Vascular and Endovascular Surgery. 2016 Feb;51(2):187–93.
123. Xiao J, Angsana J, Wen J, Smith SV, Park PW, Ford ML, et al. Syndecan-1 displays a protective role in aortic aneurysm formation by modulating T cell-mediated responses. Arterioscler Thromb Vasc Biol. 2012 Feb;32(2):386–96.
124. Heegaard A-M, Corsi A, Danielsen CC, Nielsen KL, Jorgensen HL, Riminucci M, et al. Biglycan deficiency causes spontaneous aortic dissection and rupture in mice. Circulation. 2007 May 29;115(21):2731–8.
125. Landis BJ, Schubert JA, Lai D, Jegga AG, Shikany AR, Foroud T, et al. Exome Sequencing Identifies Candidate Genetic Modifiers of Syndromic and Familial Thoracic Aortic Aneurysm Severity. J Cardiovasc Transl Res. 2017 Aug;10(4):423–32.
126. Holsti M, Wanhainen A, Lundin C, Björck M, Tegler G, Svensson J, et al. Circulating Vascular Basement Membrane Fragments are Associated with the Diameter of the Abdominal Aorta and Their Expression Pattern is Altered in AAA Tissue. Eur J Vasc Endovasc Surg. 2018;56(1):110–8.
127. Shen Y, Russo V, Zeglinski MR, Sellers SL, Wu Z, Oram C, et al. Recombinant Decorin Fusion Protein Attenuates Murine Abdominal Aortic Aneurysm Formation and Rupture. Sci Rep. 2017 Nov 20;7(1):15857.
128. Didangelos A, Yin X, Mandal K, Saje A, Smith A, Xu Q, et al. Extracellular Matrix Composition and Remodeling in Human Abdominal Aortic Aneurysms: A Proteomics Approach. Molecular & Cellular Proteomics. 2011 Aug 1;10(8):M111.008128.
129. Rohwedder I, Montanez E, Beckmann K, Bengtsson E, Dunér P, Nilsson J, et al. Plasma fibronectin deficiency impedes atherosclerosis progression and fibrous cap formation. EMBO Mol Med. 2012 Jul;4(7):564–76.
130. Paloschi V, Kurtovic S, Folkersen L, Gomez D, Wågsäter D, Roy J, et al. Impaired splicing of fibronectin is associated with thoracic aortic aneurysm formation in patients with bicuspid aortic valve. Arterioscler Thromb Vasc Biol. 2011 Mar;31(3):691–7.
131. Knox JB, Sukhova GK, Whittemore AD, Libby P. Evidence for altered balance between matrix metalloproteinases and their inhibitors in human aortic diseases. Circulation. 1997 Jan 7;95(1):205–12.
132. Annabi B, Shédid D, Ghosn P, Kenigsberg RL, Desrosiers RR, Bojanowski MW, et al. Differential regulation of matrix metalloproteinase activities in abdominal aortic aneurysms. J Vasc Surg. 2002 Mar;35(3):539–46.
133. Tromp G, Gatalica Z, Skunca M, Berguer R, Siegel T, Kline RA, et al. Elevated Expression of Matrix Metalloproteinase-13 in Abdominal Aortic Aneurysms. Annals of Vascular Surgery. 2004 Jul;18(4):414–20.
134. Reddy VY, Zhang QY, Weiss SJ. Pericellular mobilization of the tissue-destructive cysteine proteinases, cathepsins B, L, and S, by human monocyte-derived macrophages. Proc Natl Acad Sci USA. 1995 Apr 25;92(9):3849–53.
135. Sjöberg S, Shi G-P. Cysteine Protease Cathepsins in Atherosclerosis and Abdominal Aortic Aneurysm. Clinical reviews in bone and mineral metabolism. 2011 Jun 1;9(2):138.
136. Stather PW, Sidloff DA, Dattani N, Gokani VJ, Choke E, Sayers RD, et al. Meta-analysis and meta-regression analysis of biomarkers for abdominal aortic aneurysm. Br J Surg. 2014 Oct 1;101(11):1358–72.
137. Liu B, Granville DJ, Golledge J, Kassiri Z. Pathogenic mechanisms and the potential of drug therapies for aortic aneurysm. American Journal of Physiology-Heart and Circulatory Physiology. 2020 Feb 21;318(3):H652–70.
138. Rienks Marieke, Barallobre-Barreiro Javier, Mayr Manuel. The Emerging Role of the ADAMTS Family in Vascular Diseases. Circulation Research. 2018 Dec 7;123(12):1279–81.
139. Zhong S, Khalil RA. A Disintegrin and Metalloproteinase (ADAM) and ADAM with thrombospondin motifs (ADAMTS) family in vascular biology and disease. Biochemical Pharmacology. 2019 Jun 1;164:188–204.
140. Folkesson M, Li C, Frebelius S, Swedenborg J, Wågsäter D, Williams KJ, et al. Proteolytically active ADAM10 and ADAM17 carried on membrane microvesicles in human abdominal aortic aneurysms. Thromb Haemost. 2015 Sep 17;114(6).
141. Baxter BT, Matsumura J, Curci JA, McBride R, Larson L, Blackwelder W, et al. Effect of Doxycycline on Aneurysm Growth Among Patients With Small Infrarenal Abdominal Aortic Aneurysms: A Randomized Clinical Trial. JAMA. 2020 26;323(20):2029–38.

142. Henderson EL, Geng Y-J, Sukhova GK, Whittemore AD, Knox J, Libby P. Death of Smooth Muscle Cells and Expression of Mediators of Apoptosis by T Lymphocytes in Human Abdominal Aortic Aneurysms. Circulation. 1999 Jan 12;99(1):96–104.
143. Michel J-B. Anoïkis in the Cardiovascular System. Arteriosclerosis, Thrombosis, and Vascular Biology. 2003 Dec 1;23(12):2146–54.
144. McCormick ML, Gavrila D, Weintraub NL. Role of Oxidative Stress in the Pathogenesis of Abdominal Aortic Aneurysms. Arteriosclerosis, Thrombosis, and Vascular Biology. 2007 Mar 1;27(3):461–9.
145. Wang Ying, Nanda Vivek, Leeper Nicholas J. Clonal Smooth Muscle Cell Expansion, Autophagy, and Vascular Integrity in Aortic Aneurysm Disease. Arteriosclerosis, Thrombosis, and Vascular Biology. 2019 Jun 1;39(6):982–3.
146. Shankman LS, Gomez D, Cherepanova OA, Salmon M, Alencar GF, Haskins RM, et al. KLF4-dependent phenotypic modulation of smooth muscle cells has a key role in atherosclerotic plaque pathogenesis. Nat Med. 2015 Jun;21(6):628–37.
147. Ailawadi G, Moehle CW, Pei H, Walton SP, Yang Z, Kron IL, et al. Smooth muscle phenotypic modulation is an early event in aortic aneurysms. The Journal of Thoracic and Cardiovascular Surgery. 2009 Dec 1;138(6):1392–9.
148. Clément Marc, Chappell Joel, Raffort Juliette, Lareyre Fabien, Vandestienne Marie, Taylor Annabel L., et al. Vascular Smooth Muscle Cell Plasticity and Autophagy in Dissecting Aortic Aneurysms. Arteriosclerosis, Thrombosis, and Vascular Biology. 2019 Jun 1;39(6):1149–59.
149. Kuivaniemi H, Platsoucas CD, Tilson MD. Aortic Aneurysms. Circulation. 2008 Jan 15;117(2):242–52.
150. Tsuruda T, Kato J, Hatakeyama K, Kojima K, Yano M, Yano Y, et al. Adventitial Mast Cells Contribute to Pathogenesis in the Progression of Abdominal Aortic Aneurysm. Circulation Research. 2008 Jun 6;102(11):1368–77.
151. Langenskiöld M, Smidfelt K, Nordanstig J, Bergström G, Tivesten Å. Leukocyte subsets and abdominal aortic aneurysms detected by screening in men. Journal of Internal Medicine [Internet]. [cited 2020 Mar 24];n/a(n/a). Available from: http://onlinelibrary.wiley.com/doi/abs/10.1111/joim.13040
152. Saha B, Jyothi Prasanna S, Chandrasekar B, Nandi D. Gene modulation and immunoregulatory roles of Interferonγ. Cytokine. 2010 Apr 1;50(1):1–14.
153. Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. Blood. 2008 Sep 1;112(5):1557–69.
154. Galle C, Schandené L, Stordeur P, Peignois Y, Ferreira J, Wautrecht J-C, et al. Predominance of type 1 CD4+ T cells in human abdominal aortic aneurysm. Clin Exp Immunol. 2005 Dec;142(3):519–27.
155. Duftner Christina, Seiler Rüdiger, Klein-Weigel Peter, Göbel Heike, Goldberger Christian, Ihling Christian, et al. High Prevalence of Circulating CD4+CD28− T-Cells in Patients With Small Abdominal Aortic Aneurysms. Arteriosclerosis, Thrombosis, and Vascular Biology. 2005 Jul 1;25(7):1347–52.
156. Shimizu K, Shichiri M, Libby P, Lee RT, Mitchell RN. Th2-predominant inflammation and blockade of IFN-gamma signaling induce aneurysms in allografted aortas. J Clin Invest. 2004 Jul;114(2):300–8.
157. Xiong W, Zhao Y, Prall A, Greiner TC, Baxter BT. Key Roles of CD4+ T Cells and IFN-γ in the Development of Abdominal Aortic Aneurysms in a Murine Model. The Journal of Immunology. 2004 Feb 15;172(4):2607–12.
158. Zhou H, Yan H, Cannon JL, Springer LE, Green JM, Pham CTN. CD43-mediated IFN-γ production by CD8+ T cells promotes abdominal aortic aneurysm in mice. J Immunol. 2013 May 15;190(10):5078–85.
159. Li Z, Kong W. Cellular signaling in Abdominal Aortic Aneurysm. Cell Signal. 2020 Jun;70:109575.
160. Harrison SC, Smith AJP, Jones GT, Swerdlow DI, Rampuri R, Bown MJ, et al. Interleukin-6 receptor pathways in abdominal aortic aneurysm. Eur Heart J. 2013 Dec 21;34(48):3707–16.
161. Paige Ellie, Clément Marc, Lareyre Fabien, Sweeting Michael, Raffort Juliette, Grenier Céline, et al. Interleukin-6 Receptor Signaling and Abdominal Aortic Aneurysm Growth Rates. Circulation: Genomic and Precision Medicine. 2019 Feb 1;12(2):e002413.
162. Abdominal aortic aneurysms (AAA) post heart transplantation: A systematic review of literature [Internet]. Annals of Transplantation. [cited 2016 Nov 13]. Available from: http://www.annalsoftransplantation.com/download/index/idArt/882010
163. Lindeman JHN, Rabelink TJ, Bockel JH van. Immunosuppression and the Abdominal Aortic Aneurysm. Circulation. 2011 Nov 1;124(18):e463–5.
164. Sun J, Sukhova GK, Yang M, Wolters PJ, MacFarlane LA, Libby P, et al. Mast cells modulate the pathogenesis of elastase-induced abdominal aortic aneurysms in mice. J Clin Invest. 2007 Nov;117(11):3359–68.
165. Sillesen H, Eldrup N, Hultgren R, Lindeman J, Bredahl K, Thompson M, et al. Randomized clinical trial of mast cell inhibition in patients with a medium-sized abdominal aortic aneurysm. Br J Surg. 2015 Jul;102(8):894–901.
166. McCormick Michael L., Gavrila Dan, Weintraub Neal L. Role of Oxidative Stress in the Pathogenesis of Abdominal Aortic Aneurysms. Arteriosclerosis, Thrombosis, and Vascular Biology. 2007 Mar 1;27(3):461–9.
167. Emeto TI, Moxon JV, Au M, Golledge J. Oxidative stress and abdominal aortic aneurysm: potential treatment targets. Clinical Science. 2016 Mar 1;130(5):301–15.
168. al PJ et. Basal and oxidative stress-induced expression of metallothionein is decreased in ascending aortic aneurysms of bicuspid aortic valve patients. - PubMed - NCBI [Internet]. [cited 2019 Jun 10]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/?term=Basal+and+Oxidative+Stress%E2%80%93Induced+Expression+of+Metallothionein+Is+Decreased+in+Ascending+Aortic+Aneurysms+of+Bicuspid+Aortic+Valve+Patients
169. Starke RM, Thompson JW, Ali MS, Pascale CL, Lege AM, Ding D, et al. Cigarette Smoke Initiates Oxidative Stress-Induced Cellular Phenotypic Modulation Leading to Cerebral Aneurysm Pathogenesis. Arterioscler Thromb Vasc Biol. 2018 Mar;38(3):610–21.
170. Wang W, Xu B, Xuan H, Ge Y, Wang Y, Wang L, et al. Hypoxia-inducible factor 1 in clinical and experimental aortic aneurysm disease. Journal of Vascular Surgery. 2018 Nov 1;68(5):1538-1550.e2.
171. Vorp DA, Lee PC, Wang DH, Makaroun MS, Nemoto EM, Ogawa S, et al. Association of intraluminal thrombus in abdominal aortic aneurysm with local hypoxia and wall weakening. J Vasc Surg. 2001 Aug;34(2):291–9.
172. Li Daniel Y., Busch Albert, Jin Hong, Chernogubova Ekaterina, Pelisek Jaroslav, Karlsson Joakim, et al. H19 Induces Abdominal Aortic Aneurysm Development and Progression. Circulation. 2018 Oct 9;138(15):1551–68.
173. Thompson MM, Jones L, Nasim A, Sayers RD, Bell PRF. Angiogenesis in abdominal aortic aneurysms. European Journal of Vascular and Endovascular Surgery. 1996 May 1;11(4):464–9.
174. Sunderkötter C, Steinbrink K, Goebeler M, Bhardwaj R, Sorg C. Macrophages and angiogenesis. J Leukoc Biol. 1994 Mar;55(3):410–22.
175. Pepper MS. Extracellular Proteolysis and Angiogenesis. Thromb Haemost. 2001;86(1):346–55.
176. Choke E, Thompson MM, Dawson J, Wilson WRW, Sayed S, Loftus IM, et al. Abdominal Aortic Aneurysm Rupture Is Associated With Increased Medial Neovascularization and Overexpression of Proangiogenic Cytokines. Arteriosclerosis, Thrombosis, and Vascular Biology. 2006 Sep 1;26(9):2077–82.
177. Pinard Amélie, Jones Gregory T., Milewicz Dianna M. Genetics of Thoracic and Abdominal Aortic Diseases. Circulation Research. 2019 Feb 15;124(4):588–606.
178. Biros E, Walker PJ, Nataatmadja M, West M, Golledge J. Downregulation of transforming growth factor, beta receptor 2 and Notch signaling pathway in human abdominal aortic aneurysm. Atherosclerosis. 2012 Apr 1;221(2):383–6.
179. Lareyre F, Clément M, Raffort J, Pohlod S, Patel M, Esposito B, et al. TGFβ (Transforming Growth Factor-β) Blockade Induces a Human-Like Disease in a Nondissecting Mouse Model of Abdominal Aortic Aneurysm. Arterioscler Thromb Vasc Biol. 2017;37(11):2171–81.
180. Chen P-Y, Qin L, Li G, Malagon-Lopez J, Wang Z, Bergaya S, et al. Smooth Muscle Cell Reprogramming in Aortic Aneurysms. Cell Stem Cell. 2020 Apr 2;26(4):542-557.e11.
181. Siebel C, Lendahl U. Notch Signaling in Development, Tissue Homeostasis, and Disease. Physiological Reviews. 2017 Aug 9;97(4):1235–94.
182. Kim EK, Choi E-J. Pathological roles of MAPK signaling pathways in human diseases. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. 2010 Apr 1;1802(4):396–405.
183. Larsson SC, Mason AM, Bäck M, Klarin D, Damrauer SM, Michaëlsson K, et al. Genetic predisposition to smoking in relation to 14 cardiovascular diseases. Eur Heart J [Internet]. [cited 2020 May 1]; Available from: http://academic.oup.com/eurheartj/advance-article/doi/10.1093/eurheartj/ehaa193/5821049
184. Norman PE, Curci JA. Understanding the Effects of Tobacco Smoke on the Pathogenesis of Aortic Aneurysm. Arteriosclerosis, Thrombosis, and Vascular Biology [Internet]. 2013 Jul [cited 2020 May 1]; Available from: http://www.ahajournals.org/doi/abs/10.1161/ATVBAHA.112.300158
185. Ghosh A, Pechota LVTA, Upchurch GR, Eliason JL. Cross-talk between macrophages, smooth muscle cells, and endothelial cells in response to cigarette smoke: the effects on MMP2 and 9. Mol Cell Biochem. 2015 Dec;410(1–2):75–84.
186. Xiao J, Wei Z, Chen X, Chen W, Zhang H, Yang C, et al. Experimental abdominal aortic aneurysm growth is inhibited by blocking the JAK2/STAT3 pathway. International Journal of Cardiology [Internet]. 2020 Apr 15 [cited 2020 May 1]; Available from: http://www.sciencedirect.com/science/article/pii/S016752731935212X
187. Chen Q-W, Edvinsson L, Xu C-B. Cigarette smoke extract promotes human vascular smooth muscle cell proliferation and survival through ERK1/2- and NF-κB-dependent pathways. ScientificWorldJournal. 2010 Nov 4;10:2139–56.
188. Jin J, Arif B, Garcia-Fernandez F, Ennis TL, Davis EC, Thompson RW, et al. Novel Mechanism of Aortic Aneurysm Development in Mice Associated with Smoking and Leukocytes. Arterioscler Thromb Vasc Biol. 2012 Dec;32(12):2901–9.

189. Villard C, Hultgren R. Abdominal aortic aneurysm: Sex differences. Maturitas. 2018 Mar 1;109:63–9.
190. Henriques TA, Huang J, D'Souza SS, Daugherty A, Cassis LA. Orchidectomy, but not ovariectomy, regulates angiotensin II-induced vascular diseases in apolipoprotein E-deficient mice. Endocrinology. 2004 Aug;145(8):3866–72.
191. Ailawadi G, Eliason JL, Roelofs KJ, Sinha I, Hannawa KK, Kaldjian EP, et al. Gender Differences in Experimental Aortic Aneurysm Formation. Arteriosclerosis, Thrombosis, and Vascular Biology. 2004 Nov 1;24(11):2116–22.
192. Sinha I, Cho BS, Roelofs KJ, Stanley JC, Henke PK, Upchurch GR. Female gender attenuates cytokine and chemokine expression and leukocyte recruitment in experimental rodent abdominal aortic aneurysms. Ann N Y Acad Sci. 2006 Nov;1085:367–79.
193. Cho BS, Woodrum DT, Roelofs KJ, Stanley JC, Henke PK, Upchurch GR. Differential Regulation of Aortic Growth in Male and Female Rodents Is Associated With AAA Development. Journal of Surgical Research. 2009 Aug 1;155(2):330–8.
194. Cho BS, Roelofs KJ, Ford JW, Henke PK, Upchurch GR. Decreased collagen and increased matrix metalloproteinase-13 in experimental abdominal aortic aneurysms in males compared with females. Surgery. 2010 Feb;147(2):258–67.
195. DiMusto PD, Lu G, Ghosh A, Roelofs KJ, Sadiq O, McEvoy B, et al. Increased JNK in males compared with females in a rodent model of abdominal aortic aneurysm. J Surg Res. 2012 Aug;176(2):687–95.
196. Huang C-K, Luo J, Lai K-P, Wang R, Pang H, Chang E, et al. Androgen receptor promotes abdominal aortic aneurysm development via modulating inflammatory interleukin-1α and transforming growth factor-β1 expression. Hypertension. 2015 Oct;66(4):881–91.
197. Wu X-F, Zhang J, Paskauskas S, Xin S-J, Duan Z-Q. The role of estrogen in the formation of experimental abdominal aortic aneurysm. Am J Surg. 2009 Jan;197(1):49–54.
198. Laser A, Ghosh A, Roelofs K, Sadiq O, McEvoy B, DiMusto P, et al. Increased estrogen receptor alpha in experimental aortic aneurysms in females compared with males. J Surg Res. 2014 Jan;186(1):467–74.
199. Davis JP, Salmon M, Pope NH, Lu G, Su G, Sharma AK, et al. Attenuation of aortic aneurysms with stem cells from different genders. Journal of Surgical Research. 2015 Nov 1;199(1):249–58.
200. Alsiraj Y, Thatcher SE, Charnigo R, Kuey C, Blalock E, Daugherty A, et al. Female Mice with an XY Sex Chromosome Complement Develop Severe Angiotensin II-Induced Abdominal Aortic Aneurysms. Circulation. 2016 Nov 4;
201. Alsiraj Y, Thatcher SE, Blalock E, Fleenor B, Daugherty A, Cassis LA. Sex Chromosome Complement Defines Diffuse Versus Focal Angiotensin II-Induced Aortic Pathology. Arterioscler Thromb Vasc Biol. 2018;38(1):143–53.
202. Woodrum DT, Ford JW, Ailawadi G, Pearce CG, Sinha I, Eagleton MJ, et al. Gender Differences in Rat Aortic Smooth Muscle Cell Matrix Metalloproteinase-9. Journal of the American College of Surgeons. 2005 Sep;201(3):398–404.
203. Villard C, Swedenborg J, Eriksson P, Hultgren R. Reproductive history in women with abdominal aortic aneurysms. J Vasc Surg. 2011 Aug;54(2):341–5, 345.e1-2.
204. Villard C, Eriksson P, Swedenborg J, Hultgren R. Differences in Elastin and Elastolytic Enzymes between Men and Women with Abdominal Aortic Aneurysm. Aorta (Stamford). 2014 Oct;2(5):179–85.
205. Villard C, Eriksson P, Hanemaaijer R, Lindeman JH, Hultgren R. The composition of collagen in the aneurysm wall of men and women. Journal of Vascular Surgery [Internet]. [cited 2016 Nov 13]; Available from: http://www.sciencedirect.com/science/article/pii/S0741521416003323
206. Länne T, Sandgren T, Sonesson B. A dynamic view on the diameter of abdominal aortic aneurysms. Eur J Vasc Endovasc Surg. 1998 Apr;15(4):308–12.
207. Lo RC, Lu B, Fokkema MTM, Conrad M, Patel VI, Fillinger M, et al. Relative importance of aneurysm diameter and body size for predicting abdominal aortic aneurysm rupture in men and women. Journal of Vascular Surgery. 2014 May 1;59(5):1209–16.
208. Mangum KD, Farber MA. Genetic and epigenetic regulation of abdominal aortic aneurysms. Clinical Genetics [Internet]. [cited 2020 Apr 13];n/a(n/a). Available from: http://onlinelibrary.wiley.com/doi/abs/10.1111/cge.13705
209. Hinterseher I, Tromp G, Kuivaniemi H. Genes and Abdominal Aortic Aneurysm. Annals of Vascular Surgery. 2011 Apr 1;25(3):388–412.
210. Keats BJB, Sherman SL. Chapter 13 - Population Genetics. In: Rimoin D, Pyeritz R, Korf B, editors. Emery and Rimoin's Principles and Practice of Medical Genetics [Internet]. Oxford: Academic Press; 2013 [cited 2020 Apr 13]. p. 1–12. Available from: http://www.sciencedirect.com/science/article/pii/B978012383834600015X
211. Jones GT, Tromp G, Kuivaniemi H, Gretarsdottir S, Baas AF, Giusti B, et al. Meta-Analysis of Genome-Wide Association Studies for Abdominal Aortic Aneurysm Identifies Four New Disease-Specific Risk Loci. Circ Res. 2017 Jan 20;120(2):341–53.
212. Li J, Pan C, Zhang S, Spin JM, Deng A, Leung LLK, et al. Decoding the Genomics of Abdominal Aortic Aneurysm. Cell. 2018 Sep 6;174(6):1361-1372.e10.
213. Piétu G, Mariage-Samson R, Fayein N-A, Matingou C, Eveno E, Houlgatte R, et al. The Genexpress IMAGE Knowledge Base of the Human Brain Transcriptome: A Prototype Integrated Resource for Functional and Computational Genomics. Genome Res. 1999 Feb 1;9(2):195–209.
214. McGettigan PA. Transcriptomics in the RNA-seq era. Current Opinion in Chemical Biology. 2013 Feb 1;17(1):4–11.
215. Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, et al. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinformatics. 2013 Apr 15;14:128.
216. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. PNAS. 2005 Oct 25;102(43):15545–50.
217. Lenk GM, Tromp G, Weinsheimer S, Gatalica Z, Berguer R, Kuivaniemi H. Whole genome expression profiling reveals a significant role for immune function in human abdominal aortic aneurysms. BMC Genomics. 2007;8:237.
218. Lenk GM, Tromp G, Skunca M, Gatalica Z, Berguer R, Kuivaniemi H. Global expression profiles in human normal and aneurysmal abdominal aorta based on two distinct whole genome microarray platforms. Ann N Y Acad Sci. 2006 Nov;1085:360–2.
219. Hinterseher I, Erdman R, Donoso LA, Vrabec TR, Schworer CM, Lillvis JH, et al. Role of complement cascade in abdominal aortic aneurysms. Arterioscler Thromb Vasc Biol. 2011 Jul;31(7):1653–60.
220. Biros E, Moran CS, Rush CM, Gäbel G, Schreurs C, Lindeman JHN, et al. Differential gene expression in the proximal neck of human abdominal aortic aneurysm. Atherosclerosis. 2014 Mar;233(1):211–8.
221. Choke E, Cockerill GW, Laing K, Dawson J, Wilson WRW, Loftus IM, et al. Whole genome-expression profiling reveals a role for immune and inflammatory response in abdominal aortic aneurysm rupture. Eur J Vasc Endovasc Surg. 2009 Mar;37(3):305–10.
222. Gäbel G, Northoff BH, Weinzierl I, Ludwig S, Hinterseher I, Wilfert W, et al. Molecular Fingerprint for Terminal Abdominal Aortic Aneurysm Disease. J Am Heart Assoc. 2017 Nov 30;6(12).
223. Doderer SA, Gäbel G, Kokje VBC, Northoff BH, Holdt LM, Hamming JF, et al. Adventitial adipogenic degeneration is an unidentified contributor to aortic wall weakening in the abdominal aortic aneurysm. J Vasc Surg. 2018;67(6):1891-1900.e4.
224. Airhart N, Brownstein BH, Cobb JP, Schierding W, Arif B, Ennis TL, et al. Smooth muscle cells from abdominal aortic aneurysms are unique and can independently and synergistically degrade insoluble elastin. Journal of Vascular Surgery. 2014 Oct;60(4):1033-1042.e5.
225. Butt HZ, Sylvius N, Salem MK, Wild JB, Dattani N, Sayers RD, et al. Microarray-based Gene Expression Profiling of Abdominal Aortic Aneurysm. Eur J Vasc Endovasc Surg. 2016 Jul;52(1):47–55.
226. Rossi L, Lapini I, Magi A, Pratesi G, Lavitrano M, Biasi GM, et al. Carotid Artery Disease: Novel Pathophysiological Mechanisms Identified by Gene-expression Profiling of Peripheral Blood. European Journal of Vascular and Endovascular Surgery. 2010 Nov 1;40(5):549–58.
227. Courtois A, Nusgens BV, Hustinx R, Namur G, Gomez P, Kuivaniemi H, et al. Gene Expression Study in Positron Emission Tomography-Positive Abdominal Aortic Aneurysms Identifies CCL18 as a Potential Biomarker for Rupture Risk. Mol Med. 2014 Jan;20(1):697–706.
228. Malkawi A, Pirianov G, Torsney E, Chetter I, Sakalihasan N, Loftus IM, et al. Increased Expression of Lamin A/C Correlate with Regions of High Wall Stress in Abdominal Aortic Aneurysms. Aorta (Stamford). 2015 Oct;3(5):152–66.
229. Erhart P, Schiele S, Ginsbach P, Grond-Ginsbach C, Hakimi M, Böckler D, et al. Gene Expression Profiling in Abdominal Aortic Aneurysms After Finite Element Rupture Risk Assessment. J Endovasc Ther. 2017;24(6):861–9.
230. Toghill BJ, Saratzis A, Harrison SC, Verissimo AR, Mallon EB, Bown MJ. The potential role of DNA methylation in the pathogenesis of abdominal aortic aneurysm. Atherosclerosis. 2015 Jul;241(1):121–9.
231. Yin M, Zhang J, Wang Y, Wang S, Böckler D, Duan Z, et al. Deficient CD4+CD25+ T regulatory cell function in patients with abdominal aortic aneurysms. Arterioscler Thromb Vasc Biol. 2010 Sep;30(9):1825–31.
232. Xia Q, Zhang J, Han Y, Zhang X, Jiang H, Lun Y, et al. Epigenetic regulation of regulatory T cells in patients with abdominal aortic aneurysm. FEBS Open Bio. 2019;9(6):1137–43.
233. Maegdefessel L, Dalman RL, Tsao PS. Pathogenesis of Abdominal Aortic Aneurysms: MicroRNAs, Proteases, Genetic Associations. Annual Review of Medicine. 2014;65(1):49–62.
234. Maegdefessel L, Azuma J, Toh R, Deng A, Merk DR, Raiesdana A, et al. MicroRNA-21 blocks abdominal aortic aneurysm development and nicotine-augmented expansion. Sci Transl Med. 2012 Feb 22;4(122):122ra22.

235. Maegdefessel L, Spin JM, Raaz U, Eken SM, Toh R, Azuma J, et al. miR-24 limits aortic vascular inflammation and murine abdominal aneurysm development. Nature Communications. 2014 Oct 31;5:5214.
236. Kumar Sandeep, Boon Reinier A., Maegdefessel Lars, Dimmeler Stefanie, Jo Hanjoong. Role of Noncoding RNAs in the Pathogenesis of Abdominal Aortic Aneurysm. Circulation Research. 2019 Feb 15;124(4):619–30.
237. He Q, Tan J, Yu B, Shi W, Liang K. Long noncoding RNA HIF1A-AS1A reduces apoptosis of vascular smooth muscle cells: implications for the pathogenesis of thoracoabdominal aorta aneurysm. Pharmazie. 2015 May;70(5):310–5.
238. Bäck M, Gasser TC, Michel J-B, Caligiuri G. Biomechanical factors in the biology of aortic wall and aortic valve diseases. Cardiovasc Res. 2013 Jul 15;99(2):232–41.
239. Gasser TC. Biomechanical Rupture Risk Assessment. Aorta (Stamford). 2016 Apr 1;4(2):42–60.
240. Vande Geest JP, Sacks MS, Vorp DA. The effects of aneurysm on the biaxial mechanical behavior of human abdominal aorta. J Biomech. 2006;39(7):1324–34.
241. Wolinsky Harvey, Glagov Seymour. Structural Basis for the Static Mechanical Properties of the Aortic Media. Circulation Research. 1964 May 1;14(5):400–13.
242. Samila ZJ, Carter SA. The effect of age on the unfolding of elastin lamellae and collagen fibers with stretch in human carotid arteries. Can J Physiol Pharmacol. 1981 Oct;59(10):1050–7.
243. Carew Thomas E., Vaishnav Ramesh N., Patel Dali J. Compressibility of the Arterial Wall. Circulation Research. 1968 Jul 1;23(1):61–8.
244. Rachev A, Greenwald SE. Residual strains in conduit arteries. Journal of Biomechanics. 2003 May;36(5):661–70.
245. Tanaka TT, Fung YC. Elastic and inelastic properties of the canine aorta and their variation along the aortic tree. J Biomech. 1974 Aug;7(4):357–70.
246. Sokolis DP. Passive mechanical properties and structure of the aorta: segmental analysis. Acta Physiol (Oxf). 2007 Aug;190(4):277–89.
247. Collins JA, Munoz J-V, Patel TR, Loukas M, Tubbs RS. The anatomy of the aging aorta. Clinical Anatomy. 2014;27(3):463–6.
248. Vorp DA, Raghavan ML, Muluk SC, Makaroun MS, Steed DL, Shapiro R, et al. Wall Strength and Stiffness of Aneurysmal and Nonaneurysmal Abdominal Aorta. Annals of the New York Academy of Sciences. 1996;800(1):274–6.
249. Raghavan ML, Webster MW, Vorp DA. Ex vivo biomechanical behavior of abdominal aortic aneurysm: Assessment using a new mathematical model. Ann Biomed Eng. 1996 Sep 1;24(5):573–82.
250. Xiong J, Wang SM, Zhou W, Wu JG. Measurement and analysis of ultimate mechanical properties, stress-strain curve fit, and elastic modulus formula of human abdominal aortic aneurysm and nonaneurysmal abdominal aorta. Journal of Vascular Surgery. 2008 Jul;48(1):189–95.
251. Di Martino ES, Bohra A, Vande Geest JP, Gupta N, Makaroun MS, Vorp DA. Biomechanical properties of ruptured versus electively repaired abdominal aortic aneurysm wall tissue. Journal of Vascular Surgery. 2006 Mar 1;43(3):570–6.
252. Tavares Monteiro JA, da Silva ES, Raghavan ML, Puech-Leão P, de Lourdes Higuchi M, Otoch JP. Histologic, histochemical, and biomechanical properties of fragments isolated from the anterior wall of abdominal aortic aneurysms. J Vasc Surg. 2014 May;59(5):1393-1401.e1-2.
253. Vande Geest JP, Wang DHJ, Wisniewski SR, Makaroun MS, Vorp DA. Towards a noninvasive method for determination of patient-specific wall strength distribution in abdominal aortic aneurysms. Ann Biomed Eng. 2006 Jul;34(7):1098–106.
254. Reeps C, Maier A, Pelisek J, Härtl F, Grabher-Meier V, Wall WA, et al. Measuring and modeling patient-specific distributions of material properties in abdominal aortic aneurysm wall. Biomech Model Mechanobiol. 2013 Aug;12(4):717–33.
255. Forsell C, Swedenborg J, Roy J, Gasser TC. The quasi-static failure properties of the abdominal aortic aneurysm wall estimated by a mixed experimental-numerical approach. Ann Biomed Eng. 2013 Jul;41(7):1554–66.
256. Pancheri FQ, Peattie RA, Reddy ND, Ahamed T, Lin W, Ouellette TD, et al. Histology and Biaxial Mechanical Behavior of Abdominal Aortic Aneurysm Tissue Samples. J Biomech Eng. 2017 Mar 1;139(3).
257. Vollmar JF, Paes EH, Pauschinger P, Gross P. [Aneurysm of the abdominal aorta and leg amputation. A chance coincidence or a pathogenetic correlation?]. Dtsch Med Wochenschr. 1988 Nov 18;113(46):1795–800.
258. Vollmar JF, Pauschinger P, Paes E, Henze E, Friesch A. AORTIC ANEURYSMS AS LATE SEQUELAE OF ABOVE-KNEE AMPUTATION. The Lancet. 1989 Oct 7;334(8667):834–5.
259. Yeung JJ, Kim HJ, Abbruzzese TA, Vignon-Clementel IE, Draney-Blomme MT, Yeung KK, et al. Aortoiliac hemodynamic and morphologic adaptation to chronic spinal cord injury. J Vasc Surg. 2006 Dec;44(6):1254–65.
260. Crawford JD, Chivukula VK, Haller S, Vatankhah N, Bohannan CJ, Moneta GL, et al. Aortic outflow occlusion predicts rupture of abdominal aortic aneurysm. J Vasc Surg. 2016 Dec;64(6):1623–8.
261. Dua MM, Dalman RL. Hemodynamic Influences on abdominal aortic aneurysm disease: Application of biomechanics to aneurysm pathophysiology. Vascular Pharmacology. 2010 Jul;53(1–2):11–21.
262. Lehoux S, Castier Y, Tedgui A. Molecular mechanisms of the vascular responses to haemodynamic forces. Journal of Internal Medicine. 2006 Apr 1;259(4):381–92.
263. Ailawadi G, Knipp BS, Lu G, Roelofs KJ, Ford JW, Hannawa KK, et al. A nonintrinsic regional basis for increased infrarenal aortic MMP-9 expression and activity. J Vasc Surg. 2003 May;37(5):1059–66.
264. Tronc F, Mallat Z, Lehoux S, Wassef M, Esposito B, Tedgui A. Role of matrix metalloproteinases in blood flow-induced arterial enlargement: interaction with NO. Arterioscler Thromb Vasc Biol. 2000 Dec;20(12):E120-126.
265. Castier Y, Brandes RP, Leseche G, Tedgui A, Lehoux S. p47phox-Dependent NADPH Oxidase Regulates Flow-Induced Vascular Remodeling. Circulation Research. 2005 Sep 16;97(6):533–40.
266. Björck HM, Renner J, Maleki S, Nilsson SFE, Kihlberg J, Folkersen L, et al. Characterization of shear-sensitive genes in the normal rat aorta identifies Hand2 as a major flow-responsive transcription factor. PLoS ONE. 2012;7(12):e52227.
267. Hoshina K, Sho E, Sho M, Nakahashi TK, Dalman RL. Wall shear stress and strain modulate experimental aneurysm cellularity. Journal of Vascular Surgery. 2003 May;37(5):1067.
268. Stevens RRF, Grytsan A, Biasetti J, Roy J, Lindquist Liljeqvist M, Gasser TC. Biomechanical changes during abdominal aortic aneurysm growth. PLoS ONE. 2017;12(11):e0187421.
269. Boyd AJ, Kuhn DCS, Lozowy RJ, Kulbisky GP. Low wall shear stress predominates at sites of abdominal aortic aneurysm rupture. J Vasc Surg. 2015 Mar 6;
270. Erhart P, Roy J, de Vries J-PPM, Liljeqvist ML, Grond-Ginsbach C, Hyhlik-Dürr A, et al. Prediction of Rupture Sites in Abdominal Aortic Aneurysms After Finite Element Analysis. J Endovasc Ther. 2015 Oct 23;
271. Fillinger MF, Marra SP, Raghavan ML, Kennedy FE. Prediction of rupture risk in abdominal aortic aneurysm during observation: wall stress versus diameter. J Vasc Surg. 2003 Apr;37(4):724–32.
272. Maier A, Gee MW, Reeps C, Pongratz J, Eckstein H-H, Wall WA. A comparison of diameter, wall stress, and rupture potential index for abdominal aortic aneurysm rupture risk prediction. Ann Biomed Eng. 2010 Oct;38(10):3124–34.
273. Gasser TC, Auer M, Labruto F, Swedenborg J, Roy J. Biomechanical rupture risk assessment of abdominal aortic aneurysms: model complexity versus predictability of finite element simulations. Eur J Vasc Endovasc Surg. 2010 Aug;40(2):176–85.
274. Khosla S, Morris DR, Moxon JV, Walker PJ, Gasser TC, Golledge J. Meta-analysis of peak wall stress in ruptured, symptomatic and intact abdominal aortic aneurysms. Br J Surg. 2014 Oct 1;101(11):1350–7.
275. Leemans EL, Willems TP, Slump CH, van der Laan MJ, Zeebregts CJ. Additional value of biomechanical indices based on CTa for rupture risk assessment of abdominal aortic aneurysms. PLoS ONE. 2018;13(8):e0202672.
276. Doyle BJ, Bappoo N, Syed MBJ, Forsythe RO, Powell JT, Conlisk N, et al. Biomechanical Assessment Predicts Aneurysm Related Events in Patients with Abdominal Aortic Aneurysm. European Journal of Vascular and Endovascular Surgery [Internet]. 2020 Apr 4 [cited 2020 Apr 22]; Available from: http://www.sciencedirect.com/science/article/pii/S1078588420301581
277. Siika A, Lindquist Liljeqvist M, Hultgren R, Gasser TC, Roy J. Aortic Lumen Area Is Increased in Ruptured Abdominal Aortic Aneurysms and Correlates to Biomechanical Rupture Risk. J Endovasc Ther. 2018 Oct 24;1526602818808292.
278. Lee RT, Schoen FJ, Loree HM, Lark MW, Libby P. Circumferential stress and matrix metalloproteinase 1 in human coronary atherosclerosis. Implications for plaque rupture. Arterioscler Thromb Vasc Biol. 1996 Aug;16(8):1070–3.
279. Hallow KM, Taylor WR, Rachev A, Vito RP. Markers of inflammation collocate with increased wall stress in human coronary arterial plaque. Biomech Model Mechanobiol. 2009 Dec;8(6):473–86.
280. Branchetti E, Poggio P, Sainger R, Shang E, Grau JB, Jackson BM, et al. Oxidative stress modulates vascular smooth muscle cell phenotype via CTGF in thoracic aortic aneurysm. Cardiovasc Res. 2013 Nov 1;100(2):316–24.
281. Raaz U, Zöllner AM, Schellinger IN, Toh R, Nakagami F, Brandt M, et al. Segmental aortic stiffening contributes to experimental abdominal aortic aneurysm development. Circulation. 2015 May 19;131(20):1783–95.

282. Reeps C, Kehl S, Tanios F, Biehler J, Pelisek J, Wall WA, et al. Biomechanics and gene expression in abdominal aortic aneurysm. J Vasc Surg. 2014 Dec;60(6):1640-1647.e1-2.
283. Tanios F, Gee MW, Pelisek J, Kehl S, Biehler J, Grabher-Meier V, et al. Interaction of biomechanics with extracellular matrix components in abdominal aortic aneurysm wall. Eur J Vasc Endovasc Surg. 2015 Aug;50(2):167–74.
284. Hishikawa Keiichi, Oemar Barry S., Yang Zhihong, Lüscher Thomas F. Pulsatile Stretch Stimulates Superoxide Production and Activates Nuclear Factor-κB in Human Coronary Smooth Muscle. Circulation Research. 1997 Nov 1;81(5):797–803.
285. Grote Karsten, Flach Inna, Luchtefeld Maren, Akin Elvan, Holland Steven M., Drexler Helmut, et al. Mechanical Stretch Enhances mRNA Expression and Proenzyme Release of Matrix Metalloproteinase-2 (MMP-2) via NAD(P)H Oxidase–Derived Reactive Oxygen Species. Circulation Research. 2003 Jun 13;92(11):e80–6.
286. Leung DY, Glagov S, Mathews MB. Cyclic stretching stimulates synthesis of matrix components by arterial smooth muscle cells in vitro. Science. 1976 Feb 6;191(4226):475–7.
287. Biasetti J, Spazzini PG, Swedenborg J, Gasser TC. An Integrated Fluid-Chemical Model Toward Modeling the Formation of Intra-Luminal Thrombus in Abdominal Aortic Aneurysms. Front Physiol [Internet]. 2012 [cited 2016 Nov 16];3. Available from: http://journal.frontiersin.org/article/10.3389/fphys.2012.00266/abstract
288. O'Leary SA, Kavanagh EG, Grace PA, McGloughlin TM, Doyle BJ. The biaxial mechanical behaviour of abdominal aortic aneurysm intraluminal thrombus: classification of morphology and the determination of layer and region specific properties. J Biomech. 2014 Apr 11;47(6):1430–7.
289. Gasser TC, Görgülü G, Folkesson M, Swedenborg J. Failure properties of intraluminal thrombus in abdominal aortic aneurysm under static and pulsating mechanical loads. J Vasc Surg. 2008 Jul;48(1):179–88.
290. Speelman L, Schurink GWH, Bosboom EMH, Buth J, Breeuwer M, van de Vosse FN, et al. The mechanical role of thrombus on the growth rate of an abdominal aortic aneurysm. J Vasc Surg. 2010 Jan;51(1):19–26.
291. Kazi M, Thyberg J, Religa P, Roy J, Eriksson P, Hedin U, et al. Influence of intraluminal thrombus on structural and cellular composition of abdominal aortic aneurysm wall. J Vasc Surg. 2003 Dec;38(6):1283–92.
292. Kazi M, Zhu C, Roy J, Paulsson-Berne G, Hamsten A, Swedenborg J, et al. Difference in matrix-degrading protease expression and activity between thrombus-free and thrombus-covered wall of abdominal aortic aneurysm. Arterioscler Thromb Vasc Biol. 2005 Jul;25(7):1341–6.
293. Houard X, Touat Z, Ollivier V, Louedec L, Philippe M, Sebbag U, et al. Mediators of neutrophil recruitment in human abdominal aortic aneurysms. Cardiovasc Res. 2009 Jun 1;82(3):532–41.
294. Murphy EA, Danna-Lopes D, Sarfati I, Rao SK, Cohen JR. Nicotine-stimulated elastase activity release by neutrophils in patients with abdominal aortic aneurysms. Ann Vasc Surg. 1998 Jan;12(1):41–5.
295. Polzer S, Gasser TC, Swedenborg J, Bursa J. The impact of intraluminal thrombus failure on the mechanical stress in the wall of abdominal aortic aneurysms. Eur J Vasc Endovasc Surg. 2011 Apr;41(4):467–73.
296. Mehard WB, Heiken JP, Sicard GA. High-attenuating crescent in abdominal aortic aneurysm wall at CT: a sign of acute or impending rupture. Radiology. 1994 Aug 1;192(2):359–62.
297. Roy J, Labruto F, Beckman MO, Danielson J, Johansson G, Swedenborg J. Bleeding into the intraluminal thrombus in abdominal aortic aneurysms is associated with rupture. J Vasc Surg. 2008 Nov;48(5):1108–13.
298. Talvitie M, Lindquist Liljeqvist M, Siika A, Hultgren R, Roy J. Localized Hyperattenuations in the Intraluminal Thrombus May Predict Rupture of Abdominal Aortic Aneurysms. J Vasc Interv Radiol. 2018;29(1):144–5.
299. Paravastu SCV, Jayarajasingam R, Cottam R, Palfreyman SJ, Michaels JA, Thomas SM. Endovascular repair of abdominal aortic aneurysm. Cochrane Database Syst Rev. 2014;1:CD004178.
300. Behr-Rasmussen C, Grøndal N, Bramsen MB, Thomsen MD, Lindholt JS. Mural thrombus and the progression of abdominal aortic aneurysms: a large population-based prospective cohort study. Eur J Vasc Endovasc Surg. 2014 Sep;48(3):301–7.
301. Long A, Rouet L, Lindholt JS, Allaire E. Measuring the maximum diameter of native abdominal aortic aneurysms: review and critical analysis. Eur J Vasc Endovasc Surg. 2012 May;43(5):515–24.
302. Martufi G, Auer M, Roy J, Swedenborg J, Sakalihasan N, Panuccio G, et al. Multidimensional growth measurements of abdominal aortic aneurysms. J Vasc Surg. 2013 Sep;58(3):748–55.
303. Parr A, Jayaratne C, Buttner P, Golledge J. Comparison of volume and diameter measurement in assessing small abdominal aortic aneurysm expansion examined using computed tomographic angiography. European Journal of Radiology. 2011 Jul;79(1):42–7.
304. Kauffmann C, Tang A, Therasse E, Giroux M-F, Elkouri S, Melanson P, et al. Measurements and detection of abdominal aortic aneurysm growth: Accuracy and reproducibility of a segmentation software. Eur J Radiol. 2012 Aug;81(8):1688–94.
305. Lindquist Liljeqvist M, Hultgren R, Gasser TC, Roy J. Volume growth of abdominal aortic aneurysms correlates with baseline volume and increasing finite element analysis-derived rupture risk. J Vasc Surg. 2016 Jun;63(6):1434-1442.e3.
306. Zhu C, Leach JR, Wang Y, Gasper W, Saloner D, Hope MD. Intraluminal Thrombus Predicts Rapid Growth of Abdominal Aortic Aneurysms. Radiology. 2020 Mar;294(3):707–13.
307. Metaxa E, Kontopodis N, Tzirakis K, Ioannou CV, Papaharilaou Y. Effect of intraluminal thrombus asymmetrical deposition on abdominal aortic aneurysm growth rate. J Endovasc Ther. 2015 Jun;22(3):406–12.
308. Wolf YG, Thomas WS, Brennan FJ, Goff WG, Sise MJ, Bernstein EF. Computed tomography scanning findings associated with rapid expansion of abdominal aortic aneurysms. J Vasc Surg. 1994 Oct;20(4):529–35; discussion 535-538.
309. Stenbaek J, Kalin B, Swedenborg J. Growth of thrombus may be a better predictor of rupture than diameter in patients with abdominal aortic aneurysms. Eur J Vasc Endovasc Surg. 2000 Nov;20(5):466–9.
310. Sakalihasan N, Van Damme H, Gomez P, Rigo P, Lapiere CM, Nusgens B, et al. Positron emission tomography (PET) evaluation of abdominal aortic aneurysm (AAA). Eur J Vasc Endovasc Surg. 2002 May;23(5):431–6.
311. Reeps C, Essler M, Pelisek J, Seidl S, Eckstein H-H, Krause B-J. Increased 18F-fluorodeoxyglucose uptake in abdominal aortic aneurysms in positron emission/computed tomography is associated with inflammation, aortic wall instability, and acute symptoms. J Vasc Surg. 2008 Aug;48(2):417–23; discussion 424.
312. Kotze CW, Groves AM, Menezes LJ, Harvey R, Endozo R, Kayani IA, et al. What is the relationship between ^{18}F-FDG aortic aneurysm uptake on PET/CT and future growth rate? Eur J Nucl Med Mol Imaging. 2011 Aug;38(8):1493–9.
313. Morel O, Mandry D, Micard E, Kauffmann C, Lamiral Z, Verger A, et al. Evidence of Cyclic Changes in the Metabolism of Abdominal Aortic Aneurysms During Growth Phases: ^{18}F-FDG PET Sequential Observational Study. J Nucl Med. 2015 Jul;56(7):1030–5.
314. The MA3RS Study Investigators, Newby David, Forsythe Rachael, McBride Olivia, Robson Jennifer, Vesey Alex, et al. Aortic Wall Inflammation Predicts Abdominal Aortic Aneurysm Expansion, Rupture, and Need for Surgical Repair. Circulation. 2017 Aug 29;136(9):787–97.
315. Forsythe RO, Dweck MR, McBride OMB, Vesey AT, Semple SI, Shah ASV, et al. 18F–Sodium Fluoride Uptake in Abdominal Aortic Aneurysms: The SoFIA3 Study. Journal of the American College of Cardiology. 2018 Feb 6;71(5):513–23.
316. Groeneveld ME, Meekel JP, Rubinstein SM, Merkestein LR, Tangelder GJ, Wisselink W, et al. Systematic Review of Circulating, Biomechanical, and Genetic Markers for the Prediction of Abdominal Aortic Aneurysm Growth and Rupture. J Am Heart Assoc. 2018 Jun 30;7(13).
317. Juvonen J, Surcel HM, Satta J, Teppo AM, Bloigu A, Syrjälä H, et al. Elevated circulating levels of inflammatory cytokines in patients with abdominal aortic aneurysm. Arterioscler Thromb Vasc Biol. 1997 Nov;17(11):2843–7.
318. Ramos-Mozo P, Madrigal-Matute J, Vega de Ceniga M, Blanco-Colio LM, Meilhac O, Feldman L, et al. Increased plasma levels of NGAL, a marker of neutrophil activation, in patients with abdominal aortic aneurysm. Atherosclerosis. 2012 Feb;220(2):552–6.
319. Golledge J, Muller J, Shephard N, Clancy P, Smallwood L, Moran C, et al. Association between osteopontin and human abdominal aortic aneurysm. Arterioscler Thromb Vasc Biol. 2007 Mar;27(3):655–60.
320. Martín-Ventura JL, Lindholt JS, Moreno JA, Vega de Céniga M, Meilhac O, Michel JB, et al. Soluble TWEAK plasma levels predict expansion of human abdominal aortic aneurysms. Atherosclerosis. 2011 Feb 1;214(2):486–9.
321. Sidloff DA, Stather PW, Choke E, Bown MJ, Sayers RD. A systematic review and meta-analysis of the association between markers of hemostasis and abdominal aortic aneurysm presence and size. Journal of Vascular Surgery. 2014 Feb 1;59(2):528-535.e4.
322. Golledge J, Muller R, Clancy P, McCann M, Norman PE. Evaluation of the diagnostic and prognostic value of plasma D-dimer for abdominal aortic aneurysm. European Heart Journal. 2011 Feb 1;32(3):354–64.
323. Lindholt JS, Heickendorff L, Vammen S, Fasting H, Henneberg EW. Five-year results of elastin and collagen markers as predictive tools in the management of small abdominal aortic aneurysms. Eur J Vasc Endovasc Surg. 2001 Mar;21(3):235–40.
324. Watt HC, Law MR, Wald NJ, Craig WY, Ledue TB, Haddow JE. Serum triglyceride: a possible risk factor for ruptured abdominal aortic aneurysm. Int J Epidemiol. 1998 Dec;27(6):949–52.

325. Iyer V, Rowbotham S, Biros E, Bingley J, Golledge J. A systematic review investigating the association of microRNAs with human abdominal aortic aneurysms. Atherosclerosis. 2017 Jun 1;261:78–89.
326. Stringfellow MM, Lawrence PF, Stringfellow RG. The influence of aorta-aneurysm geometry upon stress in the aneurysm wall. J Surg Res. 1987 Apr;42(4):425–33.
327. Mower WR, Quiñones WJ, Gambhir SS. Effect of intraluminal thrombus on abdominal aortic aneurysm wall stress. J Vasc Surg. 1997 Oct;26(4):602–8.
328. Raghavan ML, Vorp DA. Toward a biomechanical tool to evaluate rupture potential of abdominal aortic aneurysm: identification of a finite strain constitutive model and evaluation of its applicability. J Biomech. 2000 Apr;33(4):475–82.
329. Holzapfel GA, Gasser TC, Ogden RW. A New Constitutive Framework for Arterial Wall Mechanics and a Comparative Study of Material Models. Journal of elasticity and the physical science of solids. 2000;61(1):1–48.
330. Polzer S, Gasser TC, Vlachovský R, Kubíček L, Lambert L, Man V, et al. Biomechanical indices are more sensitive than diameter in predicting rupture of asymptomatic abdominal aortic aneurysms. J Vasc Surg. 2020 Feb;71(2):617-626.e6.
331. Polzer S, Gasser TC. Biomechanical rupture risk assessment of abdominal aortic aneurysms based on a novel probabilistic rupture risk index. J R Soc Interface. 2015 Dec 6;12(113):20150852.
332. Erhart P, Hyhlik-Dürr A, Geisbüsch P, Kotelis D, Müller-Eschner M, Gasser TC, et al. Finite element analysis in asymptomatic, symptomatic, and ruptured abdominal aortic aneurysms: in search of new rupture risk predictors. Eur J Vasc Endovasc Surg. 2015 Mar;49(3):239–45.
333. Joldes GR, Miller K, Wittek A, Forsythe RO, Newby DE, Doyle BJ. BioPARR: A software system for estimating the rupture potential index for abdominal aortic aneurysms. Sci Rep [Internet]. 2017 Jul 5 [cited 2019 Feb 9];7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5498605/
334. Gasser TC, Nchimi A, Swedenborg J, Roy J, Sakalihasan N, Böckler D, et al. A novel strategy to translate the biomechanical rupture risk of abdominal aortic aneurysms to their equivalent diameter risk: method and retrospective validation. Eur J Vasc Endovasc Surg. 2014 Mar;47(3):288–95.
335. Vande Geest JP, Di Martino ES, Bohra A, Makaroun MS, Vorp DA. A biomechanics-based rupture potential index for abdominal aortic aneurysm risk assessment: demonstrative application. Ann N Y Acad Sci. 2006 Nov;1085:11–21.
336. Hirata K, Nakaura T, Nakagawa M, Kidoh M, Oda S, Utsunomiya D, et al. Machine Learning to Predict the Rapid Growth of Small Abdominal Aortic Aneurysm. J Comput Assist Tomogr. 2020 Feb;44(1):37–42.
337. Akkoyun E, Kwon ST, Acar AC, Lee W, Baek S. Predicting abdominal aortic aneurysm growth using patient-oriented growth models with two-step Bayesian inference. Comput Biol Med. 2020 Feb;117:103620.
338. Auer M, Gasser TC. Reconstruction and finite element mesh generation of abdominal aortic aneurysms from computerized tomography angiography data with minimal user interactions. IEEE Trans Med Imaging. 2010 Apr;29(4):1022–8.
339. Hirose Y, Hamada S, Takamiya M. Predicting the growth of aortic aneurysms: a comparison of linear vs exponential models. Angiology. 1995 May;46(5):413–9.
340. Gharahi H, Zambrano BA, Lim C, Choi J, Lee W, Baek S. On growth measurements of abdominal aortic aneurysms using maximally inscribed spheres. Med Eng Phys. 2015 Jul;37(7):683–91.
341. Hastie T, Tibshirani T, Friedman J. The Elements of Statistical Learning - Data Mining, Inference, and Prediction. 2nd edition-Corrected 12th printing. 2017.
342. Trygg J, Wold S. Orthogonal projections to latent structures (O-PLS). Journal of Chemometrics. 2002;16(3):119–28.
343. van der Maaten L, Hinton G. Visualizing Data using t-SNE. J Mach Learn Res. 2008 Nov;9:2579–605.
344. Kohno I, Inuzuka K, Itoh Y, Nakahara K, Eguchi Y, Sugo T, et al. A monoclonal antibody specific to the granulocyte-derived elastase-fragment D species of human fibrinogen and fibrin: its application to the measurement of granulocyte-derived elastase digests in plasma. Blood. 2000 Mar 1;95(5):1721–8.
345. Engvall E, Perlmann P. Enzyme-linked immunosorbent assay (ELISA) quantitative assay of immunoglobulin G. Immunochemistry. 1971 Sep 1;8(9):871–4.
346. Taylor CR, Levenson RM. Quantification of immunohistochemistry—issues concerning methods, utility and semiquantitative assessment II. Histopathology. 2006;49(4):411–24.
347. Taylor CR, Rudbeck L, Sjørup AH. Education Guide Immunohistochemical Staining Methods Sixth Edition. 2013;218.
348. Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics. 2003 Apr;4(2):249–64.
349. Affymetrix. Copy Number Algorithm with Built-in GC Waviness Correction in Genotyping Console™ Software. Afffymetrix; 2009.
350. Affymetrix. Microarray normalization using Signal Space Transformation with probe Guanine Cytosine Count Correction. Affymetrix;
351. GTEx Consortium. The Genotype-Tissue Expression (GTEx) project. Nat Genet. 2013 Jun;45(6):580–5.
352. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, et al. Robust enumeration of cell subsets from tissue expression profiles. Nature Methods. 2015 May;12(5):453–7.
353. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015 Apr 20;43(7):e47.
354. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res. 2016 Jul 8;44(Web Server issue):W90–7.
355. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. Genome Res. 2003 Nov;13(11):2498–504.
356. Keenan AB, Torre D, Lachmann A, Leong AK, Wojciechowicz ML, Utti V, et al. ChEA3: transcription factor enrichment analysis by orthogonal omics integration. Nucleic Acids Res. 2019 May 22;
357. Diedenhofen B, Musch J. cocor: A Comprehensive Solution for the Statistical Comparison of Correlations (vol 10, e0121945, 2015). PLoS One. 2015 Jun 26;10(6):e0131499.
358. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez J-C, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics. 2011;12:77.
359. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986 Feb 8;1(8476):307–10.
360. R Core Team. R: A Language and Environment for Statistical Computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2020. Available from: https://www.R-project.org/
361. Du BOIS D, Du BOIS EF. Clinical calorimetry: Tenth paper a formula to estimate the approximate surface area if height and weight be known. Arch Intern Med (Chic). 1916 Jun 1;XVII(6_2):863–71.
362. Kuhn M. caret: Classification and Regression Training [Internet]. 2020. Available from: https://CRAN.R-project.org/package=caret
363. Jawaid W. enrichR: Provides an R Interface to "Enrichr" [Internet]. 2017. Available from: https://CRAN.R-project.org/package=enrichR
364. Larsson E, Labruto F, Gasser TC, Swedenborg J, Hultgren R. Analysis of aortic wall stress and rupture risk in patients with abdominal aortic aneurysm with a gender perspective. J Vasc Surg. 2011 Aug;54(2):295–9.
365. Lederle FA, Johnson GR, Wilson SE, Chute EP, Littooy FN, Bandyk D, et al. Prevalence and associations of abdominal aortic aneurysm detected through screening. Ann Intern Med. 1997 Mar 15;126(6):441-.
366. Collaboration* TERF. Adult height and the risk of cause-specific death and vascular morbidity in 1 million people: individual participant meta-analysis. Int J Epidemiol. 2012 Oct 1;41(5):1419–33.
367. Spanos K, Nana P, Kouvelos G, Mpatzalexis K, Matsagkas M, Giannoukas AD. Anatomical Differences Between Intact and Ruptured Large Abdominal Aortic Aneurysms. J Endovasc Ther. 2020;27(1):117–23.
368. Leung JH, Wright AR, Cheshire N, Crane J, Thom SA, Hughes AD, et al. Fluid structure interaction of patient specific abdominal aortic aneurysms: a comparison with solid stress models. Biomed Eng Online. 2006;5:33.
369. Speelman L, Bohra A, Bosboom EMH, Schurink GWH, van de Vosse FN, Makaorun MS, et al. Effects of wall calcifications in patient-specific wall stress analyses of abdominal aortic aneurysms. J Biomech Eng. 2007 Feb;129(1):105–9.
370. Li Z-Y, U-King-Im J, Tang TY, Soh E, See TC, Gillard JH. Impact of calcification and intraluminal thrombus on the computed wall stresses of abdominal aortic aneurysm. J Vasc Surg. 2008 May;47(5):928–35.
371. Maier A, Gee MW, Reeps C, Eckstein H-H, Wall WA. Impact of calcifications on patient-specific wall stress analysis of abdominal aortic aneurysms. Biomech Model Mechanobiol. 2010 Oct;9(5):511–21.
372. Lindholt JS. Aneurysmal wall calcification predicts natural history of small abdominal aortic aneurysms. Atherosclerosis. 2008 Apr;197(2):673–8.
373. Buijs RVC, Willems TP, Tio RA, Boersma HH, Tielliu IFJ, Slart RHJA, et al. Calcification as a risk factor for rupture of abdominal aortic aneurysm. Eur J Vasc Endovasc Surg. 2013 Nov;46(5):542–8.
374. Vande Geest JP, Schmidt DE, Sacks MS, Vorp DA. The effects of anisotropy on the stress analyses of patient-specific abdominal aortic aneurysms. Ann Biomed Eng. 2008 Jun;36(6):921–32.

375. Wilson JS, Baek S, Humphrey JD. Importance of initial aortic properties on the evolving regional anisotropy, stiffness and wall thickness of human abdominal aortic aneurysms. J R Soc Interface. 2012 Sep 7;9(74):2047–58.
376. de Putter S, Wolters BJBM, Rutten MCM, Breeuwer M, Gerritsen FA, van de Vosse FN. Patient-specific initial wall stress in abdominal aortic aneurysms with a backward incremental method. J Biomech. 2007;40(5):1081–90.
377. Petterson NJ, van Disseldorp EMJ, van Sambeek MRHM, van de Vosse FN, Lopata RGP. Including surrounding tissue improves ultrasound-based 3D mechanical characterization of abdominal aortic aneurysms. J Biomech. 2019 06;85:126–33.
378. Man V, Polzer S, Gasser TC, Novotny T, Bursa J. Impact of isotropic constitutive descriptions on the predicted peak wall stress in abdominal aortic aneurysms. Med Eng Phys. 2018;53:49–57.
379. Hua J, Mower WR. Simple geometric characteristics fail to reliably predict abdominal aortic aneurysm wall stresses. J Vasc Surg. 2001 Aug;34(2):308–15.
380. Singh G, Al'Aref SJ, Van Assen M, Kim TS, van Rosendael A, Kolli KK, et al. Machine learning in cardiac CT: Basic concepts and contemporary data. J Cardiovasc Comput Tomogr. 2018 Jun;12(3):192–201.
381. Figueroa RL, Zeng-Treitler Q, Kandula S, Ngo LH. Predicting sample size required for classification performance. BMC Medical Informatics and Decision Making. 2012 Feb 15;12(1):8.
382. Kamikura Y, Wada H, Nobori T, Matsumoto T, Shiku H, Ishikura K, et al. Elevated plasma levels of fibrin degradation products by granulocyte-derived elastase in patients with deep vein thrombosis. Thrombosis Research. 2005;115(1–2):53–7.
383. Kamikura Y, Wada H, Sase T, Yamaguchi M, Kaneko T, Sakaguchi A, et al. Hemostatic abnormalities and leukocyte activation caused by infection in patients with malignant lymphoma during chemotherapy. Thrombosis Research. 2006;117(6):671–9.
384. Poveda J, Sanz AB, Fernandez-Fernandez B, Carrasco S, Ruiz-Ortega M, Cannata-Ortiz P, et al. MXRA5 is a TGF-β1-regulated human protein with anti-inflammatory and anti-fibrotic properties. J Cell Mol Med. 2017;21(1):154–64.
385. Frey H, Schroeder N, Manon-Jensen T, Iozzo RV, Schaefer L. Biological interplay between proteoglycans and their innate immune receptors in inflammation. FEBS J. 2013 May;280(10):2165–79.
386. Krishna SM, Seto S-W, Jose RJ, Li J, Morton SK, Biros E, et al. Wnt Signaling Pathway Inhibitor Sclerostin Inhibits Angiotensin II-Induced Aortic Aneurysm and Atherosclerosis. Arterioscler Thromb Vasc Biol. 2017 Mar;37(3):553–66.
387. Kokje VBC, Gäbel G, Dalman RL, Koole D, Northoff BH, Holdt LM, et al. CXCL8 hyper-signaling in the aortic abdominal aneurysm. Cytokine. 2018;108:96–104.
388. Michineau Stéphanie, Franck Grégory, Wagner-Ballon Orianne, Dai Jianping, Allaire Eric, Gervais Marianne. Chemokine (C-X-C Motif) Receptor 4 Blockade by AMD3100 Inhibits Experimental Abdominal Aortic Aneurysm Expansion Through Anti-Inflammatory Effects. Arteriosclerosis, Thrombosis, and Vascular Biology. 2014 Aug 1;34(8):1747–55.
389. Patel J, McNeill E, Douglas G, Hale AB, de Bono J, Lee R, et al. RGS1 regulates myeloid cell accumulation in atherosclerosis and aortic aneurysm rupture through altered chemokine signalling. Nat Commun. 2015 Mar 18;6:6614.
390. Hadi T, Boytard L, Silvestro M, Alebrahim D, Jacob S, Feinstein J, et al. Macrophage-derived netrin-1 promotes abdominal aortic aneurysm formation by activating MMP3 in vascular smooth muscle cells. Nature Communications. 2018 Nov 27;9(1):5022.
391. Das N, Schmidt TA, Krawetz RJ, Dufour A. Proteoglycan 4: From Mere Lubricant to Regulator of Tissue Homeostasis and Inflammation. BioEssays. 2019;41(1):1800166.
392. Karlöf E, Seime T, Dias N, Lengquist M, Witasp A, Almqvist H, et al. Correlation of computed tomography with carotid plaque transcriptomes associates calcification with lesion-stabilization. Atherosclerosis. 2019 May 11;
393. Arise KK, Kumar P, Garg R, Samivel R, Zhao H, Pandya K, et al. Angiotensin II represses Npr1 expression and receptor function by recruitment of transcription factors CREB and HSF-4a and activation of HDACs. Sci Rep. 2020 Mar 9;10(1):4337.
394. Hein L, Altman JD, Kobilka BK. Two functionally distinct alpha2-adrenergic receptors regulate sympathetic neurotransmission. Nature. 1999 Nov 11;402(6758):181–4.
395. Faisal Khan KM, Laurie GW, McCaffrey TA, Falcone DJ. Exposure of cryptic domains in the alpha 1-chain of laminin-1 by elastase stimulates macrophages urokinase and matrix metalloproteinase-9 expression. J Biol Chem. 2002 Apr 19;277(16):13778–86.
396. Pahl MC, Erdman R, Kuivaniemi H, Lillvis JH, Elmore JR, Tromp G. Transcriptional (ChIP-Chip) Analysis of ELF1, ETS2, RUNX1 and STAT5 in Human Abdominal Aortic Aneurysm. International Journal of Molecular Sciences. 2015 May;16(5):11229–58.
397. Schnabl B, Czech B, Valletta D, Weiss TS, Kirovski G, Hellerbrand C. Increased expression of Zinc finger protein 267 in non-alcoholic fatty liver disease. Int J Clin Exp Pathol. 2011 Sep 22;4(7):661–6.
398. Cheng W-L, She Z-G, Qin J-J, Guo J-H, Gong F-H, Zhang P, et al. Interferon Regulatory Factor 4 Inhibits Neointima Formation by Engaging Krüppel-Like Factor 4 Signaling. Circulation. 2017 Oct 10;136(15):1412–33.
399. Zhang X, Jiang D, Li H. The interferon regulatory factors as novel potential targets in the treatment of cardiovascular diseases. Br J Pharmacol. 2015 Dec;172(23):5457–76.
400. Das S, Frisk C, Eriksson MJ, Walentinsson A, Corbascio M, Hage C, et al. Transcriptomics of cardiac biopsies reveals differences in patients with or without diagnostic parameters for heart failure with preserved ejection fraction. Scientific Reports. 2019 Feb 28;9(1):3179.
401. Awwad MHS, Kriegsmann K, Plaumann J, Benn M, Hillengass J, Raab MS, et al. The prognostic and predictive value of IKZF1 and IKZF3 expression in T-cells in patients with multiple myeloma. OncoImmunology. 2018 Oct 3;7(10):e1486356.
402. Xie Q, Chen J, Feng H, Peng S, Adams U, Bai Y, et al. YAP/TEAD–Mediated Transcription Controls Cellular Senescence. Cancer Res. 2013 Jun 15;73(12):3615–24.
403. Yamauchi M, Kawai S, Kato T, Ooshima T, Amano A. Odd-skipped related 1 gene expression is regulated by Runx2 and Ikzf1 transcription factors. Gene. 2008 Dec 15;426(1–2):81–90.
404. Ejarque M, Mir-Coll J, Gomis R, German MS, Lynn FC, Gasa R. Generation of a Conditional Allele of the Transcription Factor Atonal Homolog 8 (Atoh8). PLOS ONE. 2016 Jan;11(1):e0146273.
405. Min Y-L, Jaichander P, Sanchez-Ortiz E, Bezprozvannaya S, Malladi VS, Cui M, et al. Identification of a multipotent Twist2-expressing cell population in the adult heart. Proc Natl Acad Sci USA. 2018 04;115(36):E8430–9.
406. Kuivaniemi H, Ryer EJ, Elmore JR, Tromp G. Understanding the pathogenesis of abdominal aortic aneurysms. Expert Review of Cardiovascular Therapy. 2015 Sep 2;13(9):975–87.
407. Ghosh A, Pechota A, Coleman D, Upchurch GR, Eliason JL. Cigarette smoke-induced MMP2 and MMP9 secretion from aortic vascular smooth cells is mediated via the Jak/Stat pathway. Hum Pathol. 2015 Feb;46(2):284–94.
408. Abisi S, Burnand KG, Waltham M, Humphries J, Taylor PR, Smith A. Cysteine protease activity in the wall of abdominal aortic aneurysms. J Vasc Surg. 2007 Dec;46(6):1260–6.
409. Hinterseher I, Erdman R, Elmore JR, Stahl E, Pahl MC, Derr K, et al. Novel Pathways in the Pathobiology of Human Abdominal Aortic Aneurysms. PAT. 2013;80(1):1–10.
410. Lindquist Liljeqvist M, Eriksson L, Villard C, Lengquist M, Kronqvist M, Hultgren R, et al. Dipeptidyl peptidase-4 is increased in the abdominal aortic aneurysm vessel wall and is associated with aneurysm disease processes. PLoS ONE. 2020;15(1):e0227889.
411. Boufenzer A, Lemarié J, Simon T, Derive M, Bouazza Y, Tran N, et al. TREM-1 Mediates Inflammatory Injury and Cardiac Remodeling Following Myocardial Infarction. Circulation Research [Internet]. 2015 May [cited 2019 Jun 12]; Available from: http://www.ahajournals.org/doi/abs/10.1161/CIRCRESAHA.116.305628
412. Zhao Y-Y, Yan D-J, Chen Z-W. Role of AIF-1 in the regulation of inflammatory activation and diverse disease processes. Cellular Immunology. 2013 Jul 1;284(1):75–83.
413. Pohl S, Scott R, Arfuso F, Perumal V, Dharmarajan A. Secreted frizzled-related protein 4 and its implications in cancer and apoptosis. Tumor Biol. 2015 Jan 1;36(1):143–52.
414. Jeanmougin M, Reynies A de, Marisa L, Paccard C, Nuel G, Guedj M. Should We Abandon the t-Test in the Analysis of Gene Expression Microarray Data: A Comparison of Variance Modeling Strategies. PLOS ONE. 2010 Sep;5(9):e12336.
415. Abedi M, Fatehi R, Moradzadeh K, Gheisari Y. Big data to knowledge: common pitfalls in transcriptomics data analysis and representation. RNA Biology. 2019 Nov 2;16(11):1531–3.
416. Björck HM, Du L, Pulignani S, Paloschi V, Lundströmer K, Kostina AS, et al. Altered DNA methylation indicates an oscillatory flow mediated epithelial-to-mesenchymal transition signature in ascending aorta of patients with bicuspid aortic valve. Scientific Reports. 2018 Feb 9;8(1):2777.
417. Battle T, Arnal JF, Challah M, Michel JB. Selective isolation of rat aortic wall layers and their cell types in culture--application to converting enzyme activity measurement. Tissue Cell. 1994 Dec;26(6):943–55.
418. Gomes D, Louedec L, Plissonnier D, Dauge MC, Henin D, Osborne-Pellegrin M, et al. Endoluminal smooth muscle cell seeding limits intimal hyperplasia. J Vasc Surg. 2001 Oct;34(4):707–15.

419. Martufi G, Christian Gasser T. Review: the role of biomechanical modeling in the rupture risk assessment for abdominal aortic aneurysms. J Biomech Eng. 2013 Feb;135(2):021010.
420. Lambin P, Leijenaar RTH, Deist TM, Peerlings J, de Jong EEC, van Timmeren J, et al. Radiomics: the bridge between medical imaging and personalized medicine. Nat Rev Clin Oncol. 2017 Dec;14(12):749–62.
421. Aguirre AJ, Hruban RH, Raphael BJ. Integrated Genomic Characterization of Pancreatic Ductal Adenocarcinoma. Cancer Cell. 2017 Aug 14;32(2):185-+.

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