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Vascular remodelling and circulating basement membrane fragments in abdominal aortic aneurysm

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Abstract

An abdominal aortic aneurysm (AAA) is a degenerative disease, characterized by advanced inflammation and extracellular matrix (ECM) remodelling. Enhanced protease activity mediated by cytokines results in the degradation of ECM proteins, leading to the generation of different bioactive fragments. Some of these generated fragments are released from the vascular basement membrane (VBM), a highly specialized ECM. VBM provides mechanical and structural stability and regulates many important cellular functions of the vascular system. Type IV and XVIII collagens are two structural proteins in VBM, with crucial roles in maintaining of the VBM integrity and vascular architecture. Circulating levels of type IV and XVIII collagen fragments are found physiologically, but have also been associated with many diseases. Remodelling of VBM and expression of its components has not been as well studied in AAA as that of the interstitial ECM.

Here we investigate these VBM collagens, their expression and possible association with aortic diameter and expansion rate in individuals with an AAA in comparison with different control groups. Further we study whether there is a link between the circulating VBM collagen fragments and several inflammatory markers, all highly involved in AAA pathogenesis. Lastly, we study the impact of surgical intervention on plasma levels of VBM collagens in patients treated by either open surgical repair (OSR) or endovascular aortic aneurysm repair (EVAR).

Methods

Circulating levels of type IV and XVIII collagen fragments were analysed in individuals with an AAA and compared with healthy controls and patients with peripheral artery disease (**paper I**). A possible association between VBM collagen fragments and the aortic diameter and expansion was studied in a large population-based cohort of 615 men stratified into three aortic diameter groups based on initial maximum aortic diameter (**paper II**). Furthermore, 159 individuals were followed up over time with repeated measurements of aortic

diameter and blood samples. The follow up cohort were divided into two subgroups based on expansion rate of AAA. Moreover, the location of VBM collagens in tissue from aortic wall in individuals with an AAA was characterized and the expression pattern was compared with normal aorta (**paper II**). In **paper III**, the association between the plasma levels of VBM collagens and inflammatory markers; IL-1 (IL-1α and IL-1β), IL-6, IL-8, TNF-α INF-γ and hs-CRP were studied in same cohort as paper II. Finally, the effect of surgical intervention on circulating levels of VBM collagen fragments was investigated in AAA patients who had undegone either OSR or EVAR by comparison of plasma levels before and after AAA repair.

Ultrasound technique was used for measurements of aortic diameter (**paper I, II, III and IV**). Analysis of circulating VBM collagens and inflammatory markers were performed by ELISA-assay (**Paper I, II, III and IV**) and Multiplex-assays, respectively (**paper III**). Aortic wall tissues were analysed by haematoxylin and eosin (H&E) and immunofluorescence staining (**Paper II**).

Results

There were significantly increased plasma levels of VBM collagen fragments in individuals with an AAA, compared with healthy controls and individulas with a peripheral artery disease (PAD), (**Paper** I). The levels of type IV collagen in AAA patients did not differ from the group with PAD, and there were no significant differences between the control groups regarding plasma levels of both VBM collagen fragments (**Paper I**). The increased levels of VBM collagen fragments were significantly associated with aortic diameter with highest levels in the group with an AAA (**Paper II**). Altered expression of the VBM collagens and fragmentation of elastic fibres were observed in tissue from AAA patients (**Paper II**). A significant association between the levels of pro-inflammatory cytokines IL-6 and IL-8, and VBM collagens was found. Additionally, there were a significant association between the plasma levels of IL-8, TNF-α and hs-CRP and an AAA (**Paper III**). Aneurysms with faster expansion rate had significantly higher levels of IL-6, IL-1β, and type XVIII/endostatin collagen. Additionally, IL-6, type

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XVIII/endostatin collagen and baseline-aortic diameter were significantly associated with expansion rate (**Paper III**). AAA repair was associated with changes in plasma levels of VBM collagens (**Paper IV**).

Conclusion

Circulating levels of VBM collagens were increased in patients with an AAA, and significantly associated with aortic diameter and expansion rate. The expression of VBM collagens was altered in AAA tissue compared with normal aorta. In addition, plasma levels of several inflammatory markers were associated as with VBM collagens, aortic diameter and expansion rate. The levels of both VBM collagens were altered at short and long time after AAA repair.

List of Abbreviations

AAA	Abdominal aortic aneurysm
AP	Antero-posterior
ANOVA	Analysis of variance
ASA	Acetylsalicylic acid
BM	Basement membrane
CAD	Cardiac artery disease
Cats	Cathepsins
CD31	Cluster of differentiation 31
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
CT	Computed Tomography
CVD	Cerebrovascular disease
DM	Diabetes mellitus
ECs	Endothelial cells
ECM	Extracellular matrix
EDTA	Etylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EVAR	Endovascular aortic aneurysm repair
IL	Interleukins
ILT	Intra-luminal thrombus
IFN-γ	Interferon gamma
hs-CRP	High sensitivity C-reactive protein
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
NA	Normal aorta
PAD	Peripheral artery disease
RCT	Randomized controlled trials
SAA	Sub-aneurysmal aorta
SD	Standard deviation
SEP	Serum elastin peptides

SMCs	Smooth muscle cells
TIMP	Tissue inhibitor of matrix metalloproteases
TNF-α	Tumour necrosis factor-alpha
t-PA	Tissue plasminogen activator
u-PA	Urokinase plasminogen activator
VBM	Vascular basement membrane

List of original papers

Paper I

Ramazani (Holsti) M, Lundin C, Sund M
Increased circulating levels of basement membrane components in patients with abdominal aortic aneurysms- a pilot study, Eur J Vascular Endovasc Surg 2011; 42 (4); 484-487

Paper II

Holsti M, Wanhainen A, Lundin C, Björck M, Tegler G, Svensson J, Sund M
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 Vascular Endovas Surg 2018; 56(1); 110-118

Paper III

Holsti M, Wanhainen A, Lundin C, Björck M, Svensson J, Sund M
Association of inflammatory cytokines to vascular wall remodelling and the aortic diameter, Submitted

Paper IV

Holsti M, Wanhainen A, Lundin C, Mani K, Svensson J, Sund M
Effect of aortic aneurysm treatment on circulating levels of vascular basement fragments-a pilot study, Manuscript

I. Introduction

1. AAA Definition

An aneurysm is a permanent and localized widening of the vessel wall. Normal abdominal aortic diameter for men and women >50 years is estimated to be 16.8 mm (2.9 SD) and 14.6 mm (1.9 SD), respectively (Pedersen et al., 1993). The definition of an abdominal aortic aneurysm is based on the aortic diameter. Many definitions have been proposed (McGregor et al., 1975), (Sterpetti et al., 1987), (Collin, 1988), (Johnston et al., 1991). These definitions are based on different imaging methods, and with consideration regarding age, gender and body surface area, in the respective populations, since all these factors affect the aortic diameter. In a Swedish evaluation, some issues with the different definitions and their impact on the prevalence of abdominal aortic aneurysms (AAAs) have been highlighted (Wanhainen, 2008).

The most recognized and widespread used definition of an AAA is as a maximum infrarenal aortic diameter of 30 mm or more. This definition has been confirmed to be well above the average (2 SD) regardless of age and body surface area for both men and women. It is therefore a clinically relevant cut-off between the non-aneurysmal aorta and an AAA (Lederle et al., 1997b), (Wanhainen, 2008).

2. AAA epidemiology

2.1 Prevalence

Clinical data from the early 1990s to the beginning of the 2000s, including randomized screening programs and autopsy material, indicated high and rising prevalence of AAAs (Eickhoff, 1993), (Wanhainen et al., 2001), (Choke et al., 2012b). Old age, male sex, smoking, heredity, hypertension and dyslipidaemia were shown to be the most important risk factors (Brown and Powell, 1999), (Brady et al., 2004), (Chun et al., 2014), (Gianfagna et al., 2016), which also accounted for the variation in reported AAA prevalence (4-18%). However, recent

data from the Western world indicate declining prevalence of AAA, and a change in AAA epidemiology (Svensjo et al., 2011), (Anjum and Powell, 2012), (Choke et al., 2012a).

The prevalence of AAA is strongly correlated to the risk factor profile in a population, but also ethnicity and geography matter (Lederle et al., 1997a). The observed reduction in AAA prevalence in the Western world is considered largely to be due to a changing risk factor profile in populations, in particular related to smoking habits, but also improvements in the treatment of cardiovascular disease (Lederle, 2011), (Ullery et al., 2018).

According to a population based study, the current prevalence of AAA among 65 year old men in Sweden is 2.2%, of which 1.7% detected at screening (Svensjo et al., 2011).

2.2 Natural course and expansion

The natural course of an AAA is gradual expansion, and eventually rupture. Overall mortality is as high as 80-90% at rupture, and the worldwide AAA rupture related death has been estimated to 150000-200000 patients annually (Golledge, 2018). Currently, a decision for elective repair of an AAA is mainly based on the aneurysm diameter, which is considered to be the strongest predictor of the expansion rate (Lederle et al., 2002a).

An AAA is commonly asymptomatic and mostly with staccato growth but can also present with a continuous linear growth pattern (Michel et al., 2011), (Kurvers et al., 2004). The expansion rate and pattern is unpredictable and with a large individual variability.

Annual growth for an AAA is about 5-10% of the initial aneurysm diameter (Collaborators et al., 2013), (Wanhainen et al., 2016b). While small aneurysms have a slow average growth, larger aneurysms tend to grow faster (Powell and Brady, 2004), although with heterogeneity in reported studies. The rupture risk

increases with larger size (Brown et al., 2003a), (Lederle et al., 2002a), and a average diameter of 70-80 mm at rupture has been reported (Lederle et al., 2002a), (Heikkinen et al., 2002). In addition to aneurysm size, also rapid growth influences rupture risk, with aneurysms having grown >10mm/year being more prone to rupture. A faster mean expansion rate has been reported in ruptured vs. unruptured AAAs of equivalent size (8.4mm/year vs. 3.9mm/year in AAA ≥60mm), (Brown et al., 2003b).

2.3 Risk factors for growth and rupture

Among risk factors for an AAA, current smoking has been associated with faster aneurysm growth by 15-20% and rupture rate is two times higher in smokers (Brady et al., 2004). An increase in blood pressure has furthermore been associated to growth (Santilli et al., 2002) and rupture (Sweeting et al., 2012) although there is a heterogeneity in reported studies regarding the association between hypertension and expansion rate and rupture (Brady et al., 2004). In contrary, diabetes mellitus and chronic limb ischemia more consistently have shown a negative and reverse association with AAA diameter, expansion and rupture rate (Vega de Ceniga et al., 2006), (Brady et al., 2004), (Lederle et al., 2000).

Although there is a 4-6 fold higher prevalence of AAA in men, the growth rate and rupture risk is larger in women, even for aneurysms of equal size (Brown and Powell, 1999), (Brown et al., 2003b), (Mofidi et al., 2007), (Villard and Hultgren, 2018).

The fact that small aneurysms do rupture and large aneurysm can remain stable during patient's lifetime (Baxter et al., 2008), (Thompson et al., 2002) indicates that aneurysm diameter merely is a surrogate marker of AAA growth and rupture. Patient-specific risk factors as well as haemodynamic conditions, aneurysm-specific geometry and morphology, presence and the thickness of intraluminal thrombus (ILT), appear to be of importance to the growth and fate of the aneurysm (Golledge, 2018).

3. AAA management

3.1 Diagnostic techniques

The detection and measurement of an AAA by manual palpation of the abdomen is accurate, but has low sensitivity and specificity. The use of ultrasound imaging has thus become the golden choice in AAA surveillance and screening programs (Fink et al., 2000). Despite high intra- and inter-observation variability from 2-7 mm to 2-10 mm, respectively (Wanhainen et al., 2016b) ultrasound is preferred to other modalities such as CT and MRI. CT-scan is most suitable in elective preoperative evaluation or when AAA rupture is suspected.

3.2 Surgical treatment

Several large randomized controlled trials (RCTs), demonstrated that early repair of small AAAs was neither beneficial nor safe. Based on these findings a cutoff at 55 mm was recommended for repair of an asymptomatic AAA in surgery-suitable individuals, while a surveillance program with repeated measurements until time for surgery for the smaller AAAs was advocated (United Kingdom Small Aneurysm Trial et al., 2002), (Lederle et al., 2002b). Current guidelines thus recommend elective repair of asymptomatic AAA in men with a maximum diameter of ≥55 mm and ≥50 mm in women (Chaikof et al., 2018). In addition, repair is recommended for individuals with an AAA expansion rate of >10mm/year, presence of symptoms with or without rupture.

In appropriate cases, intervention is offered either by conventional open surgical reconstruction (OSR; resection of the aneurysm sack and replacement by a synthetic graft) or endovascular aortic aneurysm repair (EVAR) with placement of a stent graft. OSR, introduced in the 1950s, was the dominating choice of therapy for more than four decades until the beginning of 1990s (Dubost et al., 1952), (Volodos et al., 1986).

EVAR has become the most utilized method in AAA repair in the recent decades and shown to be superior in terms of short-term outcomes (Lederle et al., 2007), (Becquemin et al., 2011). Despite the many advantages for EVAR, the technique is associated with a high level of re-intervention due to serious complications such as endoleak, sack expansion and even rupture (Stather et al., 2012). According to the annual report from the national Swedish vascular registry (Swedvasc 2017), EVAR accounted for 60% of all AAA repairs, with a lower 30-day mortality compared to OSR (0,8% versus 2,0% in intact AAA).

3.3 Medical therapy and modification of risk factors

Currently there is no novel medical therapy targeting growth and expansion rate of small AAAs with the aim to prevent progression and eventually rupture. It has thus been suggested that potential medication should be directed at AAA pathogenesis (Golledge, 2018).

Atherosclerosis might have an important role in development of AAA (Lederle et al., 2000), (Golledge, 2018). Medical therapeutic strategies for AAA have focused on cardiovascular risk factors, as patients with an AAA have a high prevalence of cardiovascular comorbidity. Having an AAA is associated to the degree of carotid artery stenosis (Alund et al., 2008), and coronary heart disease is a strong predictor of future AAA related events (Hernesniemi et al., 2015). Improved protective medical treatment and secondary preventive treatment of coexisting cardiovascular disease is of major importance in AAA patients.

A substantial proportion of individuals with AAA have a smoking history, whereas the prevalence of AAA in non-smokers is very low (Ahmed et al., 2016). A meta-analysis on clinical risk factors for AAA, reported that current smoking is strongly associated to rapid expansion, with a mean increase in AAA expansion rate of 0,35 mm/year in current smoker compared with non-smoker or former smoker (Sweeting et al., 2012). Smoking is the only modifiable and strongest evident risk factor associated to development, progression and rupture of an AAA (Lederle et al., 2000), (Powell et al., 2011), (Golledge, 2018). The prevalence of AAA is

declining, and it occurs in parallel with simultaneous decrease in smoking prevalence (Svensjo et al., 2011), (Lederle, 2011).

Furthermore, higher mean arterial blood pressure was associated with rupture (Sweeting et al., 2012), indicating the importance of smoking cessation and blood pressure control in patients with AAA.

Experimental and cohort studies have shown that the cholesterol-reducing agents statins reduce inflammation and stabilize plaques in vessel walls (Kurosawa et al., 2013), but there is no evidence for influence of statins on AAA growth. Recently published guidelines thus do not suggest using statins with the purpose to reduce risk of AAA growth or rupture (Chaikof et al., 2018).

4. AAA Screening

4.1 Benefit of AAA screening

In order to detect AAAs at an early stage and to prevent mortality in ruptured AAA, screening with ultrasound was initiated in many countries (Scott et al., 1995), (Lindholt et al., 1998), (Lederle et al., 2000). Based on estimations of AAA prevalence of 4-7.6 % in men aged 64-83 year and ≈ 1.3% in women, no evidence of benefit from screening women was found (Fleming et al., 2005). Several RCTs have demonstrated that single scan screening with ultrasound of men aged ≥65 can significantly reduce AAA specific mortality, and that it is feasible, easy to implement and cost-effective (Lindholt et al., 2005), (Ashton et al., 2002), (Fleming et al., 2005), (Svensjo et al., 2014). Those with normal aortic diameter (≤25 mm) are discharged from the screening programs, and individuals with an AAA <55 mm are followed up according to surveillance programs. There are however no clear guidelines for the group with aortic diameters between 26-29 mm, also recognized as ectatic or sub-aneurysmal aorta (SAA). Based on results from many screening studies a significant proportion (>50 %) of individuals with a SAA developed an AAA over a median of 5 years (Hafez et al., 2008), (Svensjo et al., 2014). Moreover,

individuals with an abnormal aortic diameter of 26-29 mm have a similar risk factor profiles and aortic diameter growth pattern as those with an AAA (Basnyat et al., 2003). Individuals with a SAA also have a higher all cause mortality compared with individuals with a normal aortic diameter (Svensjo et al., 2011). Re-screening those with a SAA after 5 year has thus been recommended (d'Audiffret et al., 2002), (Lindholt et al., 2005), (Thompson et al., 2005), (Wild et al., 2013).

The psychological side effects of screening and impact on health related quality of life (HRQoL) have been debated from the start (Lindholt et al., 2002), (Brown and Powell, 1999), but recently also cost-effectiveness of AAA screening is questioned as increasing evidence suggests that the epidemiology of AAA has changed during last decades and the prevalence is falling. Instead screening of high-risk subgroups, such as men aged >65 who ever smoked, has been advocated (Lederle, 2011). Recently results from some nationwide screening programs confirmed persisting cost-effectiveness of AAA screening, with reduced AAA-specific and all-cause mortality, in line with previous reports from screening trials (Wanhainen et al., 2016a). However, the cost-effectiveness and psychological effects of screening should be further investigated.

4.2 Screening in Sweden

In Sweden, a general screening programme, targeting men aged ≥65 was launched in Uppsala County in 2006, and implemented in all of Sweden by 2015. All individuals are identified through the National Population registry, with regular updates of data. Individuals with a previous history of AAA surveillance or surgery are excluded. All participants are asked to complete a standardized health questionnaire about medical history and medications. The diameter of the abdominal aorta is measured with a single ultrasound scan (Svensjo et al., 2011). A maximum of anteroposterior (AP) diameter of the abdominal aorta ≥30 mm is defined as AAA. Individuals with an AAA are referred to a vascular surgeon or specialized nurse.

The time for follow up within the surveillance programme is based on initial aortic

diameter and individuals with an aortic diameter of 26-29 mm are followed up every 5 years, 30-39 mm every 2 years, 40-44 mm every year, 45-49 mm every 6 months and 50-55 mm every 3 months. Recently publishes guidelines have advocated extended ranges between these examinations (Chaikof et al., 2018).

Recently, the outcome of the Swedish nationwide screening programme during 2006 to 2014 was evaluated. In a total of 302957 men aged 65, invited for an one time scan by ultrasound, with an attendance rate of 84%, the study reported a prevalence of 1.5% of screen-detected AAA (the lowest reported prevalence in a population of Caucasian origin) and a prevalence of 2.2% in the total population (Wanhainen et al., 2016a). It is estimated that 400-500 deaths annually in men aged ≥65 are AAA rupture related. The number needed to screen and treat was 667 and 1.5 respectively. A significant reduction in AAA specific mortality and a slightly decrease in all-cause mortality has been shown during the screening programme in Sweden (Wanhainen et al., 2016a).

5. The normal structure of the abdominal aortic wall

The wall of aorta consists of tree layers; tunica intima, tunica media and tunica externa (adventitia), **Figure 1**. Tunica intima is separated from blood stream by a single layer of endothelial cells (ECs). The endothelium is an active barrier, providing exchange of substances but also flexibility of the vessel wall by regulation of vessel diameter to forces from the blood stream. Endothelial dysfunction is associated with many CVDs and considered to be involved in development of atherosclerosis (Savoia et al. 2011).

The medial layer of the aortic wall is composed mainly of elastic fibres, smooth muscle cells (SMCs) and collagens. The elastic fibres consist of concentric layers of elastin and SMCs, forming "lamellar units". The number of lamellar units differs in diffrernt segments of aorta (Thompson et al., 2002). Elastic fibres and collagens provide extensibility and tensile strength of the wall, while SMSs produce numerous components of the extracellular matrix (ECM), such as collagens, elastin

and integrins. The tunica media in normal infrarenal aortic wall is suggested to be avascular, making this region prone to development of diseases, including AAA (Thompson et al., 2002).
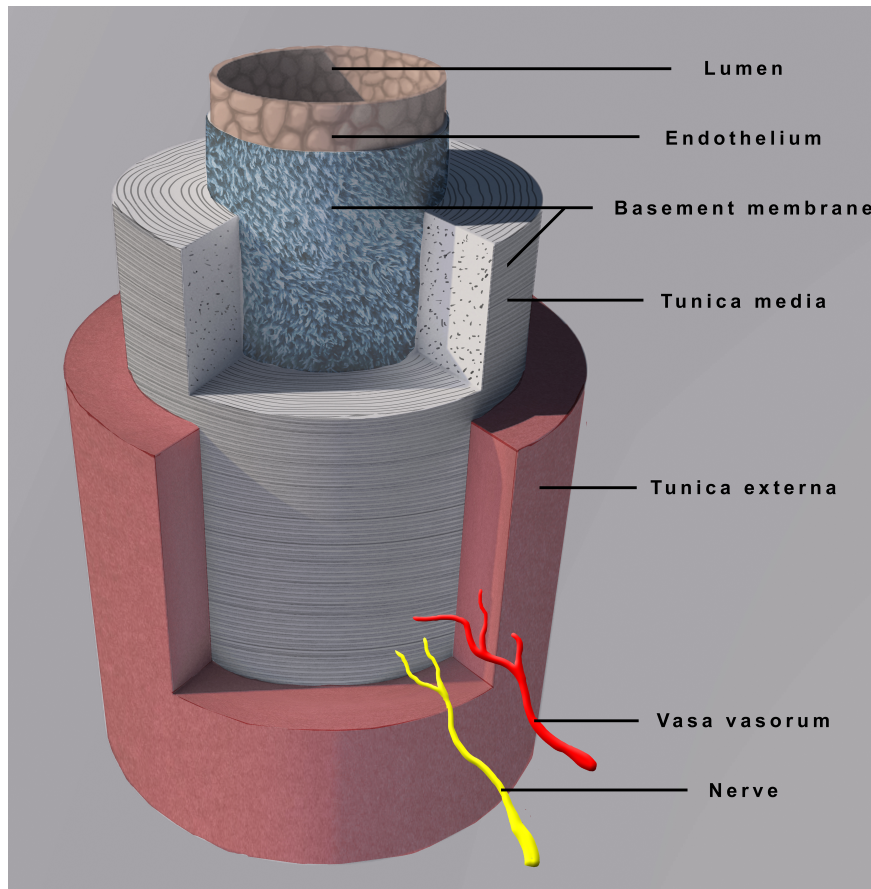
Figure 1. The structures of the abdominal aortic wall.

The outermost adventitial layer of aortic wall consists of a loose connective tissue with fibroblasts, nerve endings and vasa vasorum. The collagen network in the adventitial layer provides tensile strength to the aortic wall and the vasa vasorum promotes nutrition to the vessel wall by delivery of blood and oxygen (Wang et al., 2017). Besides having a structural role, the adventitial layer seems to have a critical role in inflammatory processes in the aortic wall and development of atherosclerosis (Moos et al., 2005).

6. The histological changes in the vessel wall in an AAA

An AAA is characterized by a ubiquitous chronic medial and adventitial inflammation, fragmentation of structural proteins such as elastin and collagens, loss of SMCs and degeneration of ECM. This transmural inflammation recruits many types of inflammatory cells and mediators such as monocytes, macrophages, lymphocytes and cytokines. The action of these leads to increased expression of proteases, which contribute to degeneration and remodelling of the ECM, thus leading to a dilatation of the aortic wall and development of an aneurysm (Dale et al., 2015). Vascular (ECs, SMCs) and inflammatory cells are a major source of these proteases, including matrix metalloproteases (MMPs), cathepsins (Cats) and serine proteases (Qin et al., 2013), which all play an important role in the remodelling of the ECM both in normal conditions, as well as in different diseases such as atherosclerosis and AAA.

7. Proteases in AAA

7.1 Matrix metalloproteases (MMPs)

MMPs belong to a zinc-depended family of proteases, with many members and substrate specifities. MMPs are synthesized as inactive pro-enzymes, and activated extracellularly mostly by other MMPs. MMPs have a key role in turnover of ECM. The activity of MMPs is balanced by activity of their endogenous inhibitors, TIMPs. MMPs have been implicated in AAA pathogenesis by their degradation of elastin and collagen (Hobeika et al., 2007). Among many MMPs, especially MMP-2 and MMP-9 have been extensively studied in AAA, and increased levels of these proteases have been demonstrated in both serum and aneurysm tissue of AAA patients. While MMP-2 is thought to be involved mainly in early stages of AAA development, MMP-9 is considered to have an essential role in expansion and rupture of AAAs. MMP-2 and MMP-9 are produced by many different inflammatory cells, but also by other cell types of the vasculature such as SMCs. Both MMP-2 and MMP-9 are suggested to be important in migration of SMCs by degradation of BMs

and matrices rich in elastin (Thompson et al., 2002). The levels of TIMP-1, an endogenous inhibitor of MMPs, are reduced in AAA reflecting an imbalance in the activity of these proteases in individuals with an AAA (Lindholt et al., 2000), (Petersen et al., 2000).

7.2 Cathepsins (Cats)

Cathepsins are potent lysosomal cysteine proteases, with elastolytic and collagenolytic properties, that are able to degrade all ECM and BM components. Infiltrating macrophages, SMCs and ECs are the source of these proteases.

Decreased levels of their natural extracellular inhibitor, cystatin C has been demonstrated in both plasma and aneurysm tissue and further associated with aortic diameter expansion and rupture of AAA (Abisi et al., 2007). Furthermore, higher levels of cathepsins have been associated with cardiovascular disease, such as hypertension, caused by vascular EC dysfunction. Among the many subtypes of cathepsins, higer levels of Cats S, K and L have been shown in tissue from AAA patients compared with healthy controls and individuals with aortic occlusive disease (Shi et al., 1999), (Maegdefessel et al., 2014), (Abisi et al., 2007).

7.3 Serine proteases

Plasmin, tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (u-PA) and neutrophil elastase are produced by vascular and inflammatory cells such as SMCs, ECs and macrophages. Elevated plasma levels of tPA have been reported in both occlusive and aneurysmal aorta compared to controls with normal aorta (Parry et al., 2009), (Wanhainen et al., 2016b), (Siennicka et al., 2013). Plasmin, an important member in the fibrinolytic system, is regulated by t-PA, u-PA and plasminogen activator inhibitor-1 (PAI-1). Elevated levels of plasmin have been shown in AAA tissue and an association between plasmin-anti-plasmin complex and AAA growth has been reported (Wanhainen et al., 2007), (Parry et al., 2009). Plasmin is moreover involved in degradation of ECM by activation of MMPs (Shimizu et al., 2006), (Wanhainen et al., 2007).

8. The structure of basement membrane

The basement membrane (BM) is a 50-100nm thick structure and a highly specialized ECM. This structure separates the endothelium and epithelium from the stroma, provides a mechanical barrier but also regulates many different cell activities such as adhesion, proliferation, differentiation and migration **(Figure 2**). Furthermore, BMs also surround muscle cells, fat cells and nerve-axons.
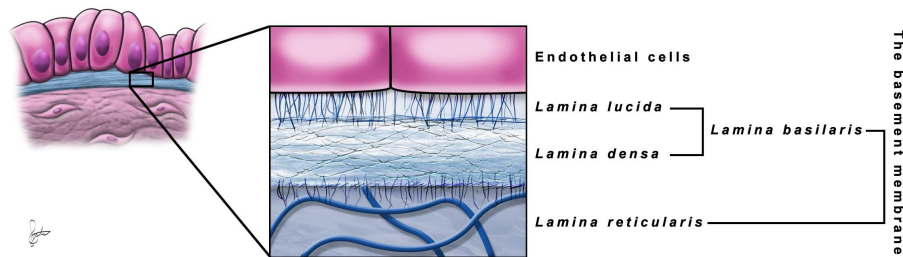
Figure 2: The basement membrane.

The BM consists mainly of type IV collagen, laminin, nidogen, perlecan, and type VIII and XVIII collagens. The constituents of BM are organ-specific, but with diversity of function. Type IV collagen and laminin are capable to "self-assemble" into sheets with a high complexity thus forming two supramolecular networks that provide strength and stability to the BM and the vascular system (Kalluri, 2003).

8.1 Type IV collagen

Type IV collagen is a non-fibrillar collagen and the main protein component of all BMs. This collagen makes up 50% of the protein mass in BMs. There are six genetically distinct type IV collagen α-chains (α1-α6). A single α-chain (monomer) consists of an amino-terminal 7S domain, a middle triple-helical domain, and a carboxy-terminal globular non-collagenous (NC-1) domain. The α-chains form

different protomers, which further assemble into sheet-like structures by interactions between the 7S domain and the NC-1 domain (Hudson et al., 1993) **(Figure 3)**.
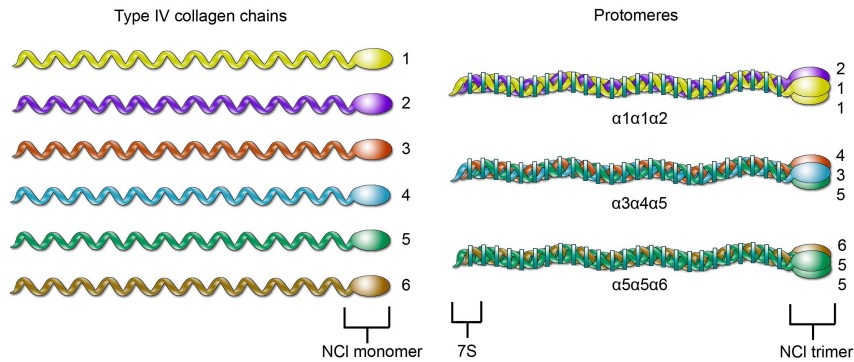
Figure 3: Schematic illustration of type IV collagen.

Among proteases, MMP-2 and MMP-9 are capable of degenerating type IV collagen, and releasing bioactive fragments by proteolytic cleavage of the 7S domain. Type IV collagen has a crucial role in the integrity and stability of vascular BMs, and the mutations in type IV collagen observed in Alports syndrome are associated with essential structural changes in the vascular system (Hudson et al., 1993).

8.2 Type XVIII collagen

Type XVIII collagen, a heparin sulphate proteoglycan, consists of a central interrupted triple helical domain, non-collagenous C-terminal domain (NC1) and a non-collagenous N-terminal domain (N-terminal domain), **(Figure 4)**.
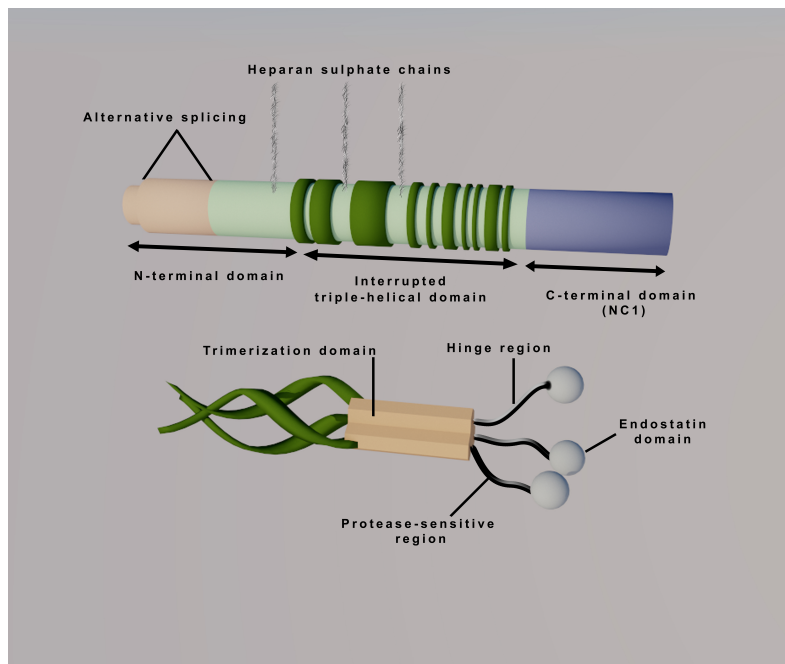
Figure 4: Schematic illustration of type XVIII collagen and the endostatin domain. Modified from the original in Iozzo et al 2005.

There are two isoforms in humans, a short and long isoform, out of which the long isoform is found exclusively in the liver (Iozzo, 2005). Otherwise in all epithelial and endothelial BMs, type XVIII collagen is found as the short isoform (Walia et al., 2015). Beside the tissue distribution the isoforms differ regarding function (Heljasvaara et al., 2017).

Proteolytic cleavage of the NC1 terminal domain, at a "protease-sensitive-site" releases endostatin, a potent anti-angiogenic fragment of 20kDa, into the circulation (Marneros and Olsen, 2005). Endostatin participates in angiogenesis and remodelling of ECM, by affecting cell proliferation, migration, interacting with growth factors, proteases and upregulation of anti-angiogenesis (Heljasvaara et al., 2017). Increased levels of circulating levels of endostatin are associated to many CVDs (Mitsuma et al., 2007). Mutations in type XVIII collagen have been associated with Knobloch syndrome (Kalluri, 2003).

9. Biomarkers associated with an AAA

AAA is a multifactorial disease influenced by different patient-specific risk factors, genetic and environmental factors but also biomechanical properties of the aneurysm, which reflect the large heterogeneity of biomarkers studied in the AAA context. Many markers have been associated with an AAA, its expansion or rupture but few have shown to be clinically useful or having a predictive value.

9.1 Biomarkers associated with inflammation in AAA

Among inflammatory cells and mediators, interest in cytokines has been prominent. Many inflammatory cells, ECs and SMCs produce cytokines, which regulate the activity of proteases and thus affect AAAs in different ways. Increased levels of pro-inflammatory IL-6 and IL-8 in both plasma and tissue have been associated with the size of AAA (Golledge et al., 2008), while INF-γ and TNF-α have been correlated to the expansion and symptoms of an AAA, respectively (Golledge et al., 2008), (Urbonavicius et al., 2008).

Association of the inflammatory marker C-reactive protein (CRP) to aneurysm diameter, but not to expansion, has been demonstrated. Instead CRP is considered to be a risk factor for future cardiovascular events such as myocardial ischemia, stroke and death (Brown and Bittner, 2008).

Both fibrinogen and D-dimer have been associated with AAA size and expansion (Sidloff et al., 2014) and higher concentration of fibrinogen being shown in symtomatic unruptured AAA (Al-Barjas et al., 2006). Further biomarkers linked to inflammatory system, such as proteins of the plasmin-anti-plasmin complex (PAP), t-PU, PAI-1, have been associated with AAA-expansion (Parry et al., 2009), (Davies et al., 2011), (Golledge et al., 2011), (Wanhainen et al., 2016b).

Many of these findings are however from studies with small sample size and

importantly these inflammatory biomarkers are not AAA-specific.

9.2 Biomarkers associated with ECM degeneration in AAA

Elastin derived degeneration products, serum elastin peptides (SEP), have been associated with small AAA diameter in a screening population. Elevated levels were significantly correlated to the diameter of ruptured compared with un-ruptured AAA at follow up, indicating a predictive role for SEP in AAA expansion (Lindholt et al., 1997). Type III collagen is a fibrillar ECM collagen, and as a result of degeneration and increased turnover of this protein, an aminoterminal propeptide of type III collagen (PIIINP) enters the circulation. Elevated serum levels of PIIINP have been associated to both the expansion rate and rupture of AAA (Satta et al., 1997).

Degeneration of structural proteins in the ECM requires protease activity. MMPs, in particular MMP-9 has a key roll in degeneration of ECM components. MMP-9 is secreted from macrophages in the adventitia, a site for inflammatory activity. High levels of MMP-9 have been associated with AAA, and higher MMP-9 expression in male compared to female in experimental AAA formation in rats indicate gender differences (Ramella et al., 2017), (Hobeika et al., 2007), (Ailawadi et al., 2004). Increased levels have been reported in ruptured AAA tissue compare with tissue from asymptomatic AAA patients (Groeneveld et al., 2018), (Petersen et al., 2000).

9.3 Genetic markers in AAA

According epidemiological studies a family history is present in 15-20% of individuals with AAA. The risk is higher among those with a first-degree relative with AAA, and among siblings mostly brothers are affected with an incidence of 9-29% versus 0-11% in sisters (Golledge and Kuivaniemi, 2013), (Linne et al., 2017). In a Swedish twin study, the estimated probability for a monozygotic twin of a person with AAA to have the disease was 24%, a risk 71 times higher than that for a monozygotic twin of an individual without an AAA (Wahlgren and Magnusson, 2011). A meta-analysis of five genome-wide association studies (GWAS) suggests

different etiology between cardiovascular diseases and AAA, despite the large overlapping of pathophysiological factors (Harrison et al., 2018). However, no specific single gene or polymorphism has been identified for AAA disease.

9.4 Biomechanical markers in AAA

Intra-luminal thrombus (ILT) is common in AAA and a considerable factor for aneurysm growth since the thrombus is rich in inflammatory cells and proteases. The volume of ILT has been associated to expansion of AAA in follow up studies of patients using CT-scans. A larger volume of ILT has moreover been reported in ruptured AAA (Siennicka et al., 2013), (Vorp et al., 2001), (Wanhainen et al., 2016b). The reported data about of ILT thickness, volume and its effect on wall stress have been conflicting (Villard and Hultgren, 2018).

Analysis of wall stress by using diffrent imaging methods, including finite element analysis and computer-genereated models, have been of great interest in biomechanical studies of AAA (Stevens et al., 2017). Wall stress is different in different parts of an aneurysm due to the geometry of an AAA. It has been assumed that the site for maximum wall stress is more prone to rupture, and in numerous studies higher peak wall stress (PWS) has been associated with ruptured AAA vs. asymptomatic AAA (Venkatasubramaniam et al., 2004). The higher risk for AAA rupture in women compared with men has been shown to be associated with lower wall strength due to gender differences in biomechanical properties in the aneurysm wall (Villard and Hultgren, 2018), (Gasser et al., 2014)

II. Aims

10.1 Overall aim

The overall aim of this thesis was to study vascular basement membrane (VBM) fragments, type IV and type XVIII collagens, in plasma and their expression in aortic tissue in individuals with an AAA and controls.

10.2 Specific aims

Paper I

To study the plasma levels of type IV and XVIII collagens, in a small set of samples from patients with an AAA.

To study whether there is a difference in plasma levels in patients with an AAA, compared to patients with peripheral artery disease and healthy controls.

Paper II and III

To investigate a possible association between the plasma levels of type IV and XVIII collagens and the aortic diameter in a larger cohort **(paper II)**

To find a possible association between the plasma levels of collagens and the expansion rate of the aortic diameter in patients with AAA **(paper II)**

To characterize the expression pattern of type IV and XVIII collagens in aortic tissue in patients with an AAA, compared with tissue from individuals with a normal aorta **(paper II).**

To study whether there was an association between the plasma levels of VBM collagens and inflammatory markers at baseline and follow up in the same cohort **(paper III)**

Paper IV

To study the alternations of plasma levels of the VBM collagens before and after AAA repair either by OSR or EVAR.

III. Materials and Methods

11.1 Study populations

Paper I

The study was conducted as a prospective pilot study, and included patients admitted to the Department of Surgery at Umeå University Hospital. The participants were ten patients with a known CT-verified AAA ≥50mm, who were referred for elective open surgical repair. Two control groups were recruited with ten individuals in each group. The first group consisted of individuals undergoing elective open surgery by cholecystectomy representing healthy controls (CON), and the other group consisted of individuals with a known peripheral artery disease (PAD), representing patients with general atherosclerosis. To confirm that the patients in the control groups did not have an undiagnosed simultaneous AAA they were examined by an abdominal ultrasound scan.

Blood samples were collected at the time for admission on the day before surgery and stored at −70°C. All clinical characteristics were collected from electronic medical patients charts, and included medical history, medication, laboratory measurements and imaging results.

Paper II

To test the reproducibility of the results in paper I in a larger cohort, but also to analyse a possible association between the VBM collagens and the aortic diameter, a cohort with a total of 642 individuals recruited at Uppsala University Hospital was used.

The participants consisted of individuals from an on-going AAA screening programme of men aged ≥ 65 or patients with an AAA that had been referred to the Vascular Surgery Unit at the Uppsala University Hospital. After applying pre-determined exclusion criteria the final cohort consisted of 615 individuals.

At baseline the cohort of 615 individuals was stratified into three groups based on the initial aortic diameter; normal aorta (NA), sub-aneurysmal aorta (SAA) and AAA. The group with the NA (≤25mm) was used as a control group. Participants with an SAA (26-29 mm) and AAA (≥30mm) were followed up according the surveillance programme. At the time point of follow up a total of 159 individuals had repeated measurements of the aortic diameter by ultrasound and collection of blood samples.

The AAA tissues investigated in the study were obtained from six patients with an asymptomatic AAA who underwent open surgical repair. Their tissues were compared with aortic wall tissue from six organ donors with a normal aorta.

All patient data were analysed based on self-estimated standardized health questionnaire, review of patients electronic medical records and imaging results. The characteristic data of these individuals are presented in the published paper II.

Paper III

In this paper the association of VBM collagens, inflammatory markers and the aortic diameter was studied in the same baseline cohort and follow up cohort that was used in paper II.

Paper IV

Nineteen male patients with an asymptomatic AAA ≥50mm who underwent surgical repair at the Vascular Surgery Unit of the University Hospital of Uppsala were recruited into this pilot study. The study cohort consisted of individuals from an on-going surveillance programme with repeated measurements of aortic diameter by ultrasound until surgery. Preoperative evaluation for the choice of method of surgical repair was based on CT-scan for all patients. Only patients with a standard procedure of either OSR or EVAR for a degenerative AAA with or without iliac artery aneurysm were included. The choice of participants was based on availability of repeated blood samples preoperatively close to the time for

repair and postoperatively at one month and one year after treatment.

11.2 AAA definition and imaging

All individuals in the studies were examined by an ultrasound scan with the purpose to identify an AAA or as a follow up routine according to a surveillance programme. A maximum AP diameter of infrarenal aorta was measured according to the "leading edge to leading edge" (LELE) principle, which means that the aortic diameter is measured between the outer layer of the anterior wall and the inner layer of the posterior wall. LELE is the utilized method at the vascular laboratories at Umeå and Uppsala University Hospitals, and is in accordance with the Swedish AAA screening programme. An AAA was defined as a AP diameter of ≥30 mm.

11.3 Analysis of blood samples

All blood samples from participants were collected into EDTA tubes and plasma was isolated and stored at −70°C until analysis. Enzyme-linked immunosorbent sandwich assays (ELISAs) were used to measure circulating levels of type IV collagen by (EIA) immunoanalysis (Argus Medical, Dublin, Irland) and Collagen XVIII/endostatin by Quantikine Human Endostatin immunoassay (R&D Systems, Minneapolis, MN, USA) **(Papers I, II, III and IV).**

High sensitivity C-reactive protein (hs-CRP) in plasma was also analysed using an ELISA-assay (Cloud Clone Corporation, Houston, Texas, USA). The inflammatory markers, interleukin 1-alpha and beta (IL-1α and IL-1β), interleukin 6 (IL-6), interleukin 8 (IL-8), tumour necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-Y) were analysed using the Milliplex MAP Human Circulating Magnetic Bead Immunoassay (EMD Millipore Corporation, Billerica, MA, USA). This multiplex bead array permits measurement of multiples proteins at the same time. The multiplex data was collected on a Bio-Plex 200 System (Bio-Rad, Hercules, CA, USA) and processed using the Bio-Plex manager v4.1.1 Software (Bio-Rad Laboratories, CA, USA) **(Paper III).**

All samples were run according the manufactures' instruction for use. An intra-assay variation of <15% was accepted otherwise the samples were rerun.

11.4 Analysis of AAA tissue

All tissue samples were embedded in Tissue-Tek OCT™, and immediately frozen in a mixture of isopentane and dry ice, and stored in –70°C until use. Haematoxylin and eosin (H&E) staining for basic histology, and immunofluorescence staining for analysing type I, IV and XVIII collagen/endostatin and CD31 (endothelial cell marker) expression were performed on four micrometer frozen tissue sections. As a negative control, the primary antibodies were omitted and the sections were incubated with secondary antibodies alone. All tissue specimens were analysed with the same light and immunofluorescence microscope and using the same magnification for location, pattern and the intensity of expression of collagens and CD31 and compared to basic histology **(Paper II)**.

11.5 Statistics

Paper I

The data were normally distributed. The differences between the three groups were analysed by one-way analysis of variance (ANOVA) with Bonferroni *post hoc* correction regarding continuous variables, which were presented as mean (SD). Due to the small sample size (<20), for comparison of categorical variables between the groups the Fishers exact test was used

A *p*-value of <0.05 was considered as significant. InStat3 software (GraphPad Inc. La Jolla, CA, USA) was used.

Paper II

Baseline cohort: All cohort characteristics were analysed and prevalence of continuous variables and categorical variables were presented as mean (SD) or

proportion (%), respectively. The differences between the three aortic diameter groups were analysed by ordinal regression analysis with aortic diameter group as the response variable. The correlation between the type IV and XVIII/endostatin collagen levels and aortic diameter were analysed using Persons correlation tests.

Follow up cohort: The expansion rate of aortic diameter for the follow up cohort was estimated as a difference between the last and first aortic aneurysm diameter during the observed time. The cut-off of expansion rate was calculated to 0.2 mm/month (corresponding 75th percentile) and the cohort was divided into two subgroups based on the expansion rate. T-test (for continuous variables) and Fishers exact test (for categorical variables) were used to compare the differences between the expansion groups. A linear regression model was used to analyse the association between the circulating levels of both collagens and the expansion rate.

Tissue analysis: As the data was not normally distributed, analyses of the tissue samples were performed by using the Mann-Whitney U-test.

A p-value of <0.05 was considered significant. SPSS version 23 (IBM Corp, Armork, NY, USA) was used.

Paper III

To compare the differences between the aortic diameter groups in terms of inflammatory markers and other characteristics, ANOVA and Chi2 tests were used for continuous and categorical variables respectively. The association between the aortic diameter groups and inflammatory markers was performed in an ordinal regression analysis adjusted for all risk factors.

A linear regression model was performed to analyse the association between the plasma levels of VBM collagens and inflammatory markers. Paired samples test was used to analyse the alternation in levels of inflammatory markers between the baseline and time point for follow up.

As the expansion subgroups were considered being independent of each other, an independent-samples t-test was performed to compare the differences between the subgroups.

In addition, linear regression model was used for analysis of a possible link between the expansion rate (as a response variable) and all risk factors, VBM collagens and the aortic diameter.

A p-value of <0.05 was considered significant. SPSS version 23 (IBM Corp, Armork, NY, USA) was used.

Paper IV

The patient data were presented as frequency or mean (SD) or (range). As data was not normally distributed the Wilcoxon signed-rank test was performed to compare the VBM collagen levels before and after AAA repair. The differences between the two different surgical interventions were analysed by using Mann-Whitney U-test.

A p-value of <0.05 was considered significant. SPSS version 23 (IBM Corp, Armork, NY, USA) was used.

IV. Results

12.1 Demographic data of AAA and control groups (paper I)

Individuals with an established AAA and the group with PAD were significantly older than the healthy controls (CON). The presence of smoking, hypertension and hyperlipidaemia was higher in the group with an AAA. While these differences were significant between the AAA group and CON group, only smoking was significantly different between the AAA and PAD groups. There was a significant difference regarding hypertension and hyperlipidaemia between the two control groups, but no difference in prevalence of smoking.

12.2 VBM collagens in AAA and control groups (paper I)

The patients with an AAA had significantly higher levels of circulating type IV collagen compared to the healthy controls (CON), but there were no significant differences between the AAA and PAD groups nor the PAD and CON groups. The patients with an AAA also had significantly elevated circulating levels of type XVIII/endostatin collagen in comparison with the CON and PAD groups, but there were no significant differences between the control groups **(Figure 5).**
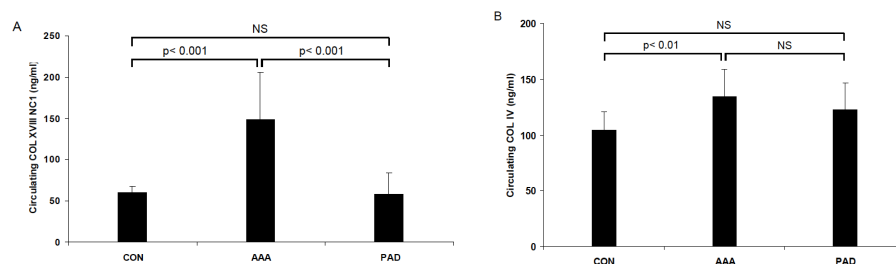
Figure 5: Circulating levels of type XVIII collagen/endostatin (A) and type IV collagen (B) in patientes with an AAA compared to healthy controls (CON) and patients with peripheral artery disease (PAD).

12.3 Demographic data of aortic diameter groups in the baseline cohort (paper II)

In the final study cohort of 615 individuals in paper II, 252 had a normal aortic diameter (NA ≤25mm), 156 a sub-aneurysmal aorta (SAA; 26-29mm) and 207 had an abdominal aortic aneurysm (AAA ≥ 30mm).

The group with an AAA had the highest prevalence of smoking, hypertension, CAD, CVD, COPD, statin and ASA usage. In an adjusted ordinal logistic regression model, with the aortic diameter group as a response variable; age, smoking and hypertension remained significantly associated to the aortic diameter group **(Table 1)**.

Table 1. Baseline characteristics of the three aortic diameter groups and the ordinal logistic regression test results.

Variable	Normal (n=252)	SAA (n=156)	AAA (n=207)	Univariable analysis p-value	Multivariable analysis p-value
Aortic diameter [mm]*	19 (2.1)	27 (1.3)	41 (10.3)		
Age [year]*	66.1 (2.1)	67.1 (3.8)	68.6 (6.0)	<0.001	0.001
Endostatin [ng/ml]*	134 (29)	155 (46)	161 (46)	<0.001	0.001
Type IV collagen [ng/ml]*	105 (42)	124 (46)	127 (47)	<0.001	0.037
BMI [kg/m²]*	26.7 (3.9)	26.4 (3.7)	27.4 (3.7)	0.251	-
1st degree relative with AAA %	25 (10.2)	18 (12.8)	22 (15)	0.158	-
History of smoking %	151 (59.9)	121(77.6)	170 (87.2)	<0.001	<0.001
Hypertension %	19 (7.5)	25 (16)	54 (27.7)	<0.001	0.023
CAD %	13 (5.2)	20 (13.2)	34 (17.4)	<0.001	0.111
CVD %	11 (4.4)	18 (11.5)	40 (20.3)	<0.001	0.054
Renal insufficiency %	1 (0.4)	3 (1.9)	7 (3.6)	0.013	0.844
Diabetes mellitus %	32 (12.9)	20 (12.8)	26 (13.3)	0.914	-
COPD %	11 (4.4)	10 (6.4)	26 (13.3)	0.001	0.230
History of cancer %	34 (13.6)	16 (10.3)	23 (11.7)	0.506	-
Treatment with statin %	56 (22.3)	62 (39.7)	101 (52.1)	<0.001	0.031
Treatment with ASA %	55 (21.6)	64 (41.6)	93 (47.9)	<0.001	0.289

Abbreviations: BMI (Body mass index), CAD (Coronary artery disease), CVD (Cerebral vascular disease), COPD (Chronic obstructive pulmonary disease), ASA (Acetylsalicylic acid). Values are shown in mean (SD) for continuous variables and count (% within aortic diameter groups) for categorical variables. Missing values excluded in percentage calculations.

12.4 VBM collagen levels and the aortic diameter groups (paper II)

A significant difference regarding levels of both collagens was observed between the three aortic diameter groups with highest levels in the AAA group **(Table 2)**. The levels of type IV collagen were 105 ng/ml (SD 42) in the group with NA, 124 ng/ml (SD 46) in the group with SAA and 127ng/ml (SD 47) in the group with AAA. The levels of type XVIII/endostatin collagen were 136 ng/ml (SD 29), 154 ng/ml (SD 45) and 162 ng/ml (SD 46), respectively. Both type IV and XVIII collagen/endostatin showed a weak positive correlation to aortic diameter (r=0.221, p<0.001), (r=0.273, p<0.001), respectively.

In an ordinal regression analysis model with aortic diameter group as the response variable the plasma levels of type IV and type XVIII collagen/endostatin remained significantly associated with the diameter group after adjustment for confounding factors (**Table 2**).

12.5 Demographic data of expansion groups in the follow up cohort (paper II)

Follow up measurements and samples were available from a total of of 159 individuals. Out of these two individuals with NA, 31 with a SAA and 126 with an AAA. The mean follow up time was 35 months (SD 18), and mean expansion rate was estimated to 0.14 mm/month (SD 0.15). Based on the expansion rate (cut-off of 0.2 mm/month), the cohort was divided into a group with slow (0.2mm/month) and fast (≥0.2mm/month) expansion.

The aortic diameter was significantly different between the expansion groups and aortic diameter was significantly associated with the expansion rate when tested in an adjusted linear regression analysis model. There was a significant difference between the groups with slow and fast expansion rates in terms of basic characteristics, with the group with faster expansion rate having a higher

proportion of current smokers but a lower proportion of diabetics. DM had an inverse association with the aortic expansion rate.

12.6 VBM collagen levels in the expansion groups (paper II)

Mean levels of type XVIII collagen/endostatin were higher in the group with a faster expansion rate (173 ng/ml, SD 45) compared with the group with a slower expansion rate (153 ng/ml, SD 41). Plasma levels of type IV collagen were not associated to expansion rate. Despite a significant association between the type XVIII collagen/endostatin levels and expansion rate in a linear regression analysis (p=0.035), the significance was lost when adjusting for confounders.

12.7 Tissue analysis from human aortic wall (paper II)

The expression pattern was altered both VBM collagens in tissue from patients with an AAA (**Figure 6**). As expected the expression of both VBM collagens in aortic tissue from normal aorta were in association with elastic layers of aortic wall and in vascular BM structures. However, tissue from aortic wall in AAA patients revealed a disorganized structure, less abundant expression of both collagens and fragmentation of elastic fibres in the medial layer of the vessel wall.

Interestingly, nodular structures were more frequently observed in AAA tissue compared with normal aorta (p=0.01). To better characterize these nodular structures staining with type I collagen (as a marker for ECM deposition) and CD31 (as a marker of endothelial cells) were performed. The nodular structures had no sign of endothelial cells, while presence of type I collagen as a sign of ECM deposition was demonstrated **(Figure 6)**.
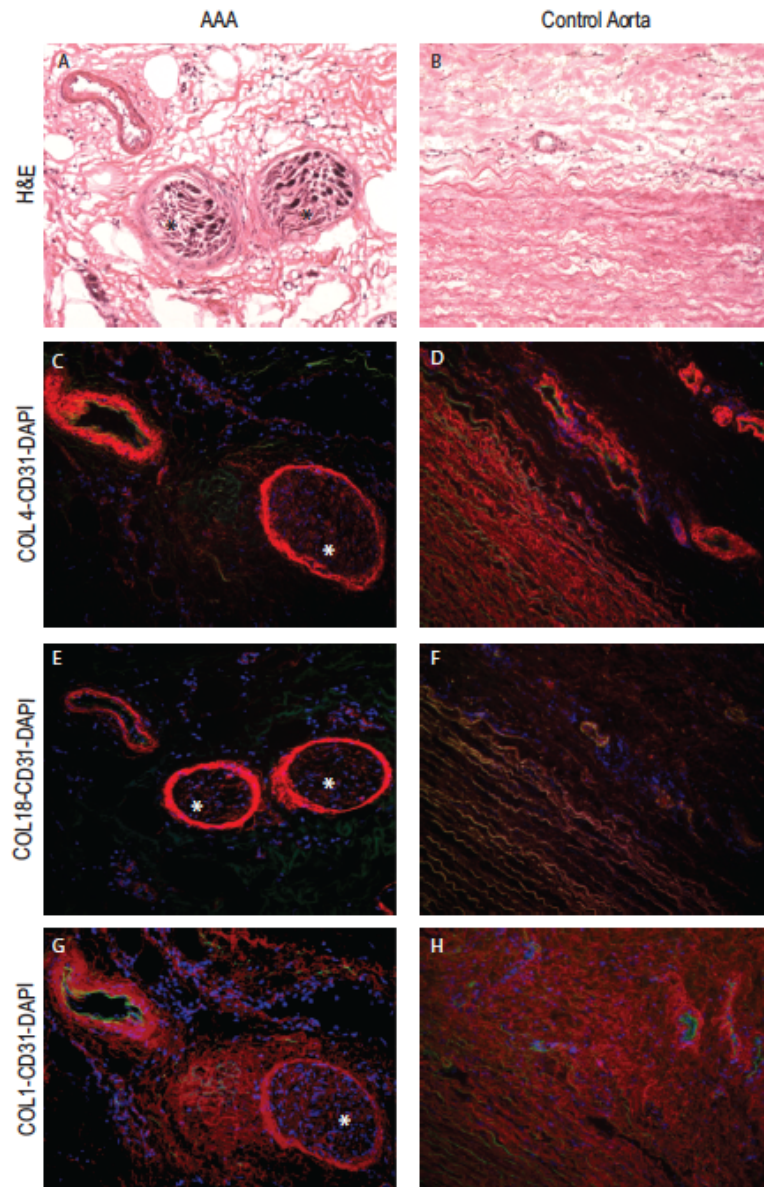
Figure 6: A and B: H&E staining of aortic tissues. Nodular structures are noted (asterisk) in the AAA. C and D: Immunofluorescence (IF) staining of type IV collagen in red, cell nuclei in blue and ECs by CD31 in green. E and F: IF staining of type XVIII collagen/endostatin in red, cell nuclei in blue and ECs in green. A loss of both VBM proteins can be observed in the fragmentation of the elastin layer. An intense staining of VBM collagens is found surrounding and in the nodular structures in AAA (asterisk). G and H: IF staining of type I collagen in red, cell nuclei in blue and ECs in green. No EC staining is observed in the nodular structures (asterisk) when compared to blood vessels (arrows). In addition these structures are rich in type I collagen.

12.8 Risk factors for AAA and inflammatory markers (paper III)

Among the risk factors, age (p=0.005) and CVD (p=0.041) were significantly associated with IL-1β, while history of smoking (p=0.002) and hypertension (p= 0.032) were significantly associated with hs-CRP. No other associations were observed.

12.9 Aortic diameter groups, expansion rate and inflammatory markers (paper III)

In a total of 207 individuals with an AAA (paper II), 157 had a small aneurysm (<50mm) and 50 individuals had a larger aneurysm (≥50mm). There was a significant difference between the aortic diameter groups regarding both VBM collagen levels (both p<0.001). The aortic diameter group with an established AAA had the highest levels of IL-8 and hs-CRP, and larger AAAs had higher levels of IL-8. There was a significant difference between the three aortic diameter groups regarding IL-8 (p<0.001), hs-CRP (p=0.026) and TNF-α (p=0.003). The NA group had the highest levels of TNF-α **(Table 2).**

The association of IL-8, and TNF-α remained significant in an ordinal regression analysis model adjusted for all risk factors, while the association with hs-CRP was lost.

Table 2. Demographic data between different aortic diameter groups at baseline

Variable at baseline#	NA N=252	SAA N=156	AAA N=207	P-value
Age (year), mean (SD)	66 (2)	67 (4)	68 (6)	< 0.001
Diameter (mm), mean (SD)	19.1 (2.1)	26.7 (1.4)	40.9 (10.3)	< 0.001
Col IV collagen (ng/mL), mean (SD)	105 (42)	124 (46)	127 (47)	< 0.001
Col XVIII/endostatin (ng/mL), mean (SD)	134 (29)	155 (46)	162 (46)	< 0.001
History of smoking (%)	159 (59.9)	121 (77.6)	170 (87.2)	< 0.001
First degree relative with AAA (%)	25 (10.2)	18 (12.8)	22 815)	0.442
Hypertension (%)	19 (7.5)	2 (16)	54 (27.7)	< 0.001
CAD (%)	13 (5.2)	20 (13.2)	34 (17.4)	< 0.001
Diabetes (%)	32 (12.9)	20 (12.8)	26 (13.3)	0.963
CVD (%)	11(4.4)	18 (11.5)	40 (20.3)	< 0.001
COPD (%)	11(4.4)	10 (6.4)	26 (13.3)	0.003
Renal insufficiency (%)	1 (0.4)	3 (1.9)	7 (3.6)	0.055
History of cancer (%)	34 (13.6)	16 (10.3)	23 (11.7)	0.605
Treatment with statin (%)	56 (22.3)	62 (39.7)	101 (52.1)	< 0.001
Treatment with ASA (%)	55 (21.6)	64 (41.6)	93 (47.9)	< 0.001
IL-1β*	3.42 (0.55)	3.31 (0.58)	3.35 (0.56)	0.16
IL-6*	3.33 (0.57)	3.35 (0.55)	3.40 (0.56)	0.37
IL-8*	2.90 (0.59	2.90 (0.52)	3.09 (0.61)	< 0.001
TNF-a*	3.60 (0.44	3.30 (0.44	3.47 (0.49)	0.003
IFN-γ*	3.40 (0.49)	3.30 (0.47)	3.34 (0.46)	0.12
hs-CRP (mg/L), mean (SD)	1.28 (1.71)	1.65(2.10)	1.75 (1.98)	0.026

Abbreviations: NA=Normal aorta, SAA=Subaneurysmal aorta, AAA=Abdominal aortic aneurysm,
CAD=coronary artery disease, CVD=cerebral vascular disease, COPD=chronic
obstructive pulmonary disease, ASA=acetylsalicylic acid
Continuous variables were analyzed by one-way ANOVA and categorical variables by Chi2 test
*pg/ml presented as log transformation of measured levels

In addition, a significant difference between the expansion rate groups in levels of IL-1β (p=0.024), IL-6 (p=0.029), type XVIII collagen/endostatin (p=0.013) and the initial aortic diameter (p=0.001) was observed **(Table 3).**

The association of all these, except IL-1β, persisted when tested in a multivariable regression analysis with expansion rate as the dependent variable. Among risk factors, hypertension (p=0.046) was significantly and positively associated with expansion rate.

Table 3: Independent simple t-test between the expansion rate groups (paper III)

Variable	Expansion rate <0.2mm N=120	Expansion rate >0.2mm N=39	p-value
Age (year), mean (SD)	67.7 (5.2)	68.2 (6.2)	0.608
B-Diameter (mm), mean (SD)	33.4 (6.2)	37.7 (7.7)	**0.001**
B-Col IV collagen (ng/mL), mean (SD)	124.0 (46)	134.0 (43)	0.230
B-Col XVIII/endostatin (ng/mL), mean (SD)	153.1 (41.1)	172.7 (45.0)	**0.013**
IL-1β*	3.32(0.54)	3.52(0.43)	**0.024**
IL-6*	3.35(0.56)	3.58(0.57)	**0.029**
IL-8*	3.01(0.60)	3.08(0.63)	0.552
TNF-α*	3.35(0.46)	3.41(0.54)	0.509
INF-γ*	3.50(0.46)	3.55(0.38)	0.488
hs-CRP (mg/L), mean (SD)	1.76(2.23)	1.57(1.63)	0.565

Abberviations: B=Baseline

*pg/mL is presented as log transformation of measured level

## 12.10 VBM collagens, inflammatory markers and risk factors (paper III)

The levels of type IV collagen were significantly associated with the levels of IL-8 in an adjusted linear regression analysis model. Further, a significant association between the type IV collagen levels, hypertension and COPD was found.

The plasma levels of type IV and XVIII/endostatin collagens were significantly associated with plasma levels of both IL-6 and IL-8, but in an adjusted linear regression analysis model these significances were lost. Among risk factors hypertension and renal insufficiency were associated with type XVIII/endostatin collagen.

12.11 VBM collagen levels after AAA repair (paper IV)

In a total of 19 patients with a mean age of 72.6 years (range 64.5-88.6) and AAA diameter of 58 mm (range 50-70 mm), plasma levels of type IV and XVIII collagen/endostatin differed significantly between the time before AAA repair (PreOP) and at two time points (PO-1 and PO-2) after repair (PreOP vs. PO-1, p=0.008 and PreOP vs. PO-2, p=0.012 for type IV collagen and PreOP vs PO-1,

p=0.004 and PreOP vs. PO-2, p=0.038 for type XVIII collagen/endostatin). No significant difference was found between the PO-1 vs. PO-2 values for either of the VBM collagens. There was moreover no significant differences in plasma levels of VBM collagens between the EVAR and OSR groups **(Figure 7).**
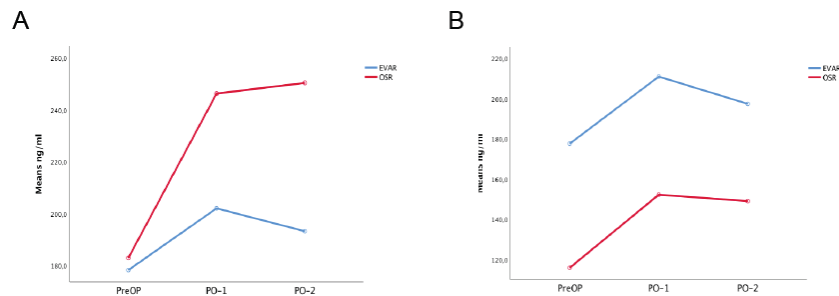
Figure 7: Circulating levels of VBM collagens after AAA repair by endovascular repair (EVAR; in blue) or open surgical repair (OSR; in red). For type IV collagen (A) the levels remain very high after OSR compared to EVAR, even at 1 year after surgery (PO-2). For type XVIII collagen/endostatin (B) the curves of the EVAR and OSR patients mirror each other, although the levels are higher in EVAR.

V. Discussion

Elastin and fibrillar collagens (mainly type I and III collagens) are the most important load bearing structural components of the ECM, providing both compliance and tensile strength to the blood vessel wall. Elastin is not synthesised in adults, and is extremely insoluble with a long half-life of ≈70 years (Cocciolone et al., 2018), while collagen synthesis is constantly on-going with a half-life of weeks and months (Karsdal et al., 2016)

An AAA is a degenerative disease characterized by ECM degradation and loss of structural proteins and cells. The pathological process is mediated by inflammation and protease activity, leading to weakening of the aortic wall with impaired integrity and stability, which contributes to the gradual expansion and eventually rupture of the aneurysm (Thompson et al., 2002). In spite of extensive studies of many ECM components in AAA development and progression, the remodelling and structural changes of the VBM, a highly specialized ECM has not been as well studied in AAA disease.

VBM is a dynamic ultrastructure providing mechanical stability, but it also influences and regulates many different cellular activities (Sund et al., 2004). After proteolysis VBM components are cleaved and fragments become released into circulation and tissue. Many of these are key participants in angiogenic and anti-angiogenic processes (Kalluri, 2003), (Sund et al., 2004). Both type IV and XVIII collagen are required for development and maintenance of BMs. Type XVIII collagen moreover is a hybrid protein with proteoglycan properties, giving a functional diversity to this collagen. Circulating levels of type IV and XVIII collagen fragments have been demonstrated in both physiological and pathophysiological conditions, and have also been related to poorer outcome (Mitsuma et al., 2007). Endostatin, liberated by protease activity on the C-terminal non-collagenous domain of type XVIII collagen has been suggested to use multiple signalling pathways affecting different process such as ECs behaviour, activity of growth factors and proteases (Heljasvaara et al., 2017). This collagen fragment has been

associated with many CVDs such as myocardial infarction, ischemic stroke, and adiposity (de Nooijer et al., 2009), (Mitsuma et al., 2007). The association between type IV collagen and atherosclerotic disease has not been characterized as much as its role in tumour angiogenesis. Degeneration and up-regulation of VBM proteins and enzymes that process this structure had previously been reported in thoracic aortic aneurysms (Cotrufo et al., 2005), but such analysis was lacking in AAA, which thus became the topic of this thesis.

Circulating levels of both collagens were significantly increased in patients with an AAA compared with healthy controls in **paper I**. Interestingly, the VBM collagens differed regarding patients with PAD, since there was no difference in type IV collagen levels between the AAA group and the group with PAD, but a significant difference in circulating type XVIII collagen/endostatin between these groups of patients. The choice of the control groups in this study can be criticized. The group of healthy individuals were not age and/or sex-matched with the AAA and PAD groups, being both younger and mostly female. Moreover, the aortic diameter was not specified in the control groups. The definition of a normal aorta (<30 mm) and an AAA was based on an ultrasound diagnosed AAA with a diameter cut off at 30mm. Finally, a limitation in this study was the small number of individuals. It is therefore difficult to make firm conclusions regarding the impact of risk factors on the VBM collagen levels in plasma. The main purpose of the study was however to investigate the circulating levels of VBM collagens in individuals with an AAA, since such information was lacking in the literature. Despite a small sample size, there was a significantly increased plasma level of both VBM collagens in the AAA group. Differences between the VBM collagen levels in the PAD and AAA groups allow for speculation about different role of these collagens at different stages in AAA, and different manifestations of cardiovascular disease. Recently, an association between type IV collagen and chronic limb ischemia and diabetes mellitus has been elucidated (Hernandez-Aguilera et al., 2018), (Steffensen and Rasmussen, 2018), although the mechanism behind this association is unclear.

The results in paper I gave the incentive for further investigation of VBM collagens

in a substantially larger study cohort. The study cohort used in **paper II** provided the opportunity to investigate the VBM collagens not only in a larger population, but also in relation to different aorta diameters, ranging from normal aorta (NA) to sub-aneurysmal aorta (SAA) and a manifest AAA. Furthermore, some individuals could also be observed over time with repeated analysis and the results could be related to disease progression.

The natural history of an AAA is expansion over time. Similar knowledge of the natural history of SAA has been lacking or is at least very inadequate in literature, since the "narrow window" between when the aortic diameter is considered as SAA or AAA, by using ultrasound measurements, could explain the occurrence of SAA merely as a false negative finding at primary screening. Increasing use of imaging techniques, such as ultrasound, both in AAA screening programmes or when carried out for other reasons has lead to the detection of many small aneurysms and SAAs. It is however increasingly clear that the incidence of later AAAs among the re-screened SAA individuals is not a consequence of false negative findings at the initial screen, and that the individuals with an SAAs have a higher risk to develop a later AAA (Hafez et al., 2008). Others have shown that a significant proportion of individuals with SAA develop an AAA over a time period of 5 years (d'Audiffret et al., 2002), (Svensjo et al., 2014), many with a need of AAA repair in due a reasonable time. With an increasing lifespan among the general population this finding cannot be ignored. Results from studies also make it evident that the occurrence and rate of classical risk factors are similar between the AAA and SAA groups, with higher mortality in both groups related to cardiovascular diseases.

Based on these facts, the finding of highest plasma levels of both VBM collagens in the group with AAA, followed by the group with SAA in **paper II** was not unexpected. The higher rate of cardiovascular diseases in the SAA group was thus also in line with findings in current literature. SAA, also named an "aneurysm in formation" should thus be considered as a precursor of AAA.

In addition to the excessive degeneration of ECM, cell apoptosis and neo-

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angiogenesis are other important features of an AAA (Rodella et al., 2016), (Ramella et al., 2017). Several different types of proteases such as MMPs, Cats and elastases have a pivotal role in AAA pathogenesis. Many experimental and clinical studies have shown increased levels of MMPs (especially MMP-2 and MMP-9), Catthepsins (Cat K, L and S) and elastases with simultaneous decreased activity of their endogenous inhibitors in AAA disease (Liu et al., 2006). The same proteases are highly involved in degradation and remodelling of the VBM and its components, such as type IV and XVIII collagens (Kalluri, 2003), (Walia et al., 2015). Based on these facts it is reasonable to hypothesize that the elevated plasma levels of both type IV collagen and type XVIII collagen/endostatin in patients with an AAA **(paper I, II)** and the significant association with the aortic diameter group **(paper II)** reflect an on-going remodelling of ECM and VBM **(paper I, II)**. In addition, patients with larger aneurysms had higher circulating levels of these VBM collagens **(paper II),** suggesting that larger aneurysms could provide more substrate availability for these proteases.

Moreover, it has been shown in an experimental study on mice that the aorta is a major source of type XVIII collagen/endostatin (Miosge et al., 1999) but the localisation of this collagen had previously not been characterized in detail in the human aorta. Previous data have moreover demonstrated a strong affinity and high association between the type XVIII collagen and all elastin layers in the aortic wall (Miosge et al., 1999). The incresead plasma levels and different expression of non-fibrillar VBM collagens shown in this thesis, combined with deposition of the fibrillar type I collagen in tissue from AAA patients in **paper II** indicate that the aortic aneurysm wall plays an active role in collagen metabolism in individuals with an AAA. The results from experimental studies were confirmed in human tissue in **paper II**, with the finding of strong expression in the normal aortic wall and altered expression of both VBM collagens and defragmentation of elastin layer in patients with an AAA.

Elastin degeneration is suggested to be an early pathophysiological feature in the enlargement of aneurysmatic aorta. Elastin degeneration end products (EDP) have

chemotactic properties, recruiting inflammatory cells such as macrophages, lymphocytes and promoting activity of different proteases in response to several types of cytokines (Kotze et al., 2011). Cytokines thus have a key role in maintenance and progression of inflammation in AAA (Li et al., 2018) **(Figure 8)**.
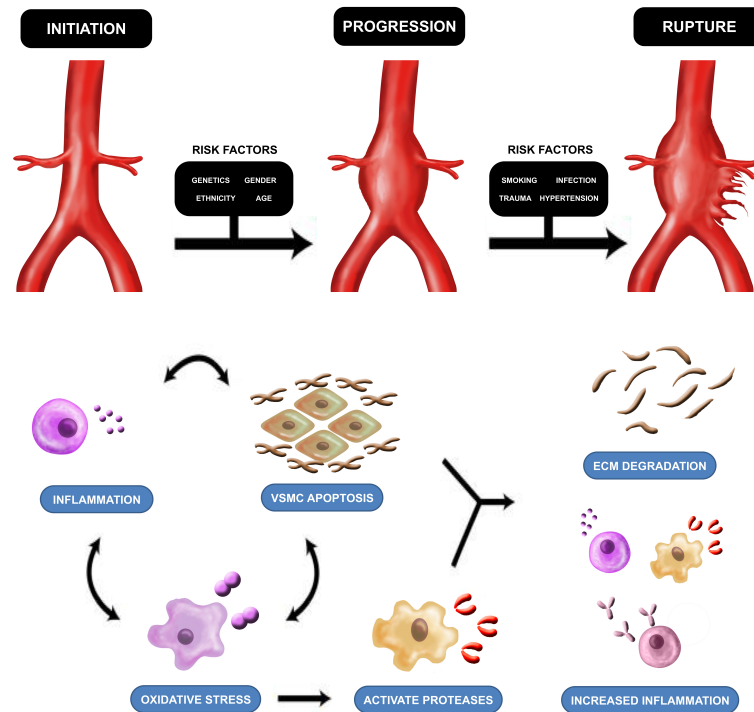
Figure 8. The relationships between risk factors and vascular remodelling in the different stages of AAA pathogenesis. Modified from Kuivaniemi et al., 2015.

The endothelium is involved in AAA pathogenesis by the influence of haemodynamic forces and extrinsic antigens, regulation of expression and activity of both cytokines and proteases (Platt et al., 2007), (Zhou et al., 2014). All this affects inflammation, which in turn is responsible for the destruction of the normal architecture of aortic wall. The association between the inflammatory markers in particular cytokines have been put in attention during last decades as potential therapeutic targets in many diseases including AAA (Juvonen et al., 1997). Increased levels of cytokines have been associated to many CVDs. Among many

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cytokines elevated levels of IL-1β, IL-6, Il-8, TNF-α, IFN-γ and CRP have reported in individuals with an AAA (Shimizu et al., 2006), (Swartbol et al., 2001). The association between the VBM proteins and cytokines in the AAA context had not been previously reported.

IL-8 is a 4-kDa chemokine, which represents a subgroup of the superfamily of cytokines. Chemokines are highly involved in starting the inflammatory reaction in AAA (Li et al., 2018). Pro-inflammatory IL-8 has a chemotactic effect on inflammatory neutrophils and ECs, and its induction of cellular and vascular adhesion molecules seems to have an essential role in pathogenesis of AAA. Previously, elevated concentration and expression of IL-8 has been described in sites of inflammation in AAA tissue when compared with normal aorta. Interestingly, the increased concentration has been shown to be associated with increased intensity of mural inflammation in the adventitial layer and luminal layers of intra-luminal thrombus (ILT), and thus corresponds with the location of neutrophils and macrophages in the ILT. The ILT thus is a major source of IL-8 and has been linked with AAA expansion and rupture (Houard et al., 2009). Additionally, the affinity of IL-8 to ECs has been linked to the promotion of angiogenesis (Middleton et al., 2009). Out of all the tested cytokines, a clear link was found between the levels of VBM collagens and IL-8 **(paper III)**. This association may indicate that remodelling of VBM could be partly mediated by inflammation in the aneurysmal aortic wall. Furthermore, a significant association between plasma levels of IL-8 and AAA diameter was shown, with highest levels in the AAA group and in the largest aneurysms.

The aortic diameter groups differed in levels of hs-CRP with highest values observed in the group with an AAA **(paper III)** as reported previously by others studies (Treska et al., 2011), (Liao et al., 2015). CRP, an acute phase inflammatory marker, is synthesized in liver and regulated by IL-6. Many epidemiological studies have revealed increased levels of CRP in individuals with CVDs, and it is considered as a general marker for cardiovascular disease and a strong predictor for future events in this population (Brown and Bittner, 2008). Evaluation of the prognostic

value of CRP at follow up of patients with peripheral artery disease has linked higher and increasing levels of CRP to an increased risk for all cause mortality (Brown and Bittner, 2008). Thus, the elevated levels of hs-CRP in **paper III** may reflect the severity of disease in our population, as levels rose with increasing aortic diameter, with highest levels in the group with AAA, followed by SAA, two groups that also have high rates of CVD.

In terms of association with expansion rate none was shown to the IL-8 levels. Among all tested inflammatory markers a significant association between IL-1β, IL-6 and expansion rate was found. IL-1β is thought to be essential for AAA development, since elevated circulating levels and increased gene and protein expression have been demonstrated in AAA (Meher and Chen, 2017), (Johnston et al., 2013). Moreover, experimental inhibition of IL-1β attenuates AAA development and vascular inflammation (Johnston et al., 2013). Furthermore, increased levels are mostly associated to early stage of AAA formation prior to manifest aortic dilatation. It perhaps could explain why there were no differences between the three aortic groups. However, the association with IL-1β and expansion rate was lost in an adjusted analysis model, while IL-6 remained significantly associated with expansion rate. Clinical and experimental data suggest an important role of IL-6 by inducing vascular inflammation, and aortic dilation as a response to hypertension and increased mechanical tension (Chae et al., 2001), (Akerman et al., 2018). All of these are potential sources to the protease activation needed for ECM and VBM remodelling and contributes to disease progression. Plasma levels of type XVIII collagen collagen/endostatin was also associated with expansion rate in an adjusted analysis model. The subgroup with faster expansion rate had significantly higher levels of IL-6, IL-1β and type XVIII collagen/endostatin **(Paper II, III).**

Smoking and hypertension belong to known risk factors and are associated with the expansion rate of an AAA. It has been reported that current smoking increases AAA expansion with 0.5 mm/year and 10 mmHg increased diastolic blood pressure causes increased expansion rate with 0.20 mm/year (Bhak et al., 2015).

Smoking cessation and blood pressure control are thus recommended therapy for individuals with an AAA (Sweeting et al., 2012). The results in this thesis were in line with prior reports, with a larger proportion of smokers among patients with an AAA and among those with a faster AAA expansion rate; further hypertension was associated with expansion rate **(Paper II, III).**

In contrary, there was an inverse relationship between diabetes mellitus (DM) and the aortic diameter group and expansion rate, with highest DM prevalence in the group with slower expansion rate compared with faster AAA expansion group (17.5% vs 5.1%) **(Paper II, III).** This inverse association is paradoxical. It has been shown that diabetics have lower occurrence, slower growth and lower AAA-related mortality, and DM is thus thought to be a protector against AAA. Hyperglycaemia and advanced glycation end products (AGEs) stimulate inflammation and increase protease activity, while histologically DM is characterized with decreased MMP activity, increased collagen deposition, thickening of BMs with increasing stiffness of the aortic wall. These conflicting features have put DM medication with metformin in focus. According to experimental studies metformin reduces inflammation by glycemic and lipid control, and reduces activity of pro-inflammatory cytokines thus exerting a protective role against AAA (Patel et al., 2018). The exact mechanism of this protective role of DM remains unclear. However, the present findings regarding DM prevalence and growth rate were in accordance with current literature.

Plasma levels of TNF-α were also inversely associated with AAA, with highest levels in the group with normal aorta. The implication of TNF-α in AAA has been demonstrated by showing slightly higher levels in AAA in comparison with healthy controls and those with coronary heart disease (CHD), but with no differences in levels between the control groups (Juvonen et al., 1997). Others have shown conflicting results, with lower TNF-α levels in tissue from AAA compared with AOD (Davis et al., 2001). TNF-α has been assigned a role in up-regulation of MMP activity, and the inhibitory effect of statins may partly be mediated via this pro-inflammatory cytokine (Ramella et al., 2017). Probably due its properties as an

inflammatory acute phase reactant, TNF-α has been associated with symptomatic AAA (Flondell-Site et al., 2009). The lowest levels of TNF-α in the AAA group in the **paper III** could reflect the composition of our aortic diameter groups, namely individuals with small asymptomatic AAAs with a high frequency of statin users. Cytokines act differently depending on the context. The heterogeneity of individuals with AAA in general and the variety of analysis methods and control groups may explain some of the different outcomes regarding cytokines in literature.

Chronic inflammation with degradation of extracellular matrix (ECM) is an AAA hallmark (Dale et al., 2015), (Li et al., 2018). The inflammation process is complex and advanced with a variety of cellular and molecular components and pathways. Whether inflammation is associated with enhanced remodelling of the VBM and thus increases the turnover of the VBM collagens, such as type IV and XVIII collagens, in AAA is unknown but it is a possible assumption based on the results in this thesis.

Biomarkers of chronic inflammation and ECM degeneration have been excessive studied as potential prognostic and/or therapeutic predictors in AAA. The effect of AAA repair on the levels of these markers might give a good insight into understanding biological activity in aneurysms, but also provide strategical treatment options in patients with an AAA.

To investigate whether exclusion and repair of AAA would affect the plasma levels of the VBM-collagens seamed to be a natural next step after the findings in paper I, II and III in this thesis. Our preliminary results in **paper IV** show overall marked differences in plasma levels of both collagens postoperatively. Significantly increased levels of both VBM collagens were found one month after repair in all patients. This finding could be assumed due to increased inflammatory response and turnover of ECM and VBM components, in particular collagens, caused by surgical trauma in these patients. Indeed, increased levels of both type IV collagen and type XVIII collagen/endostatin after injury, including surgical trauma, has been described (Fujikawa et al., 1984), (Akerfeldt et al., 2014). Although

decreasing levels of both VBM collagens at one year after AAA repair was shown, compared to the levels at one month postoperatively, the levels remained significantly higher at this point compared with the preoperative baseline levels, which is an interesting finding.

EVAR is the dominating surgical treatment for AAA, due to advantages in terms of outcomes regarding morbidity and mortality. A comparison between the EVAR and OSR groups showed more marked increases in type XVIII collagen/endostatin levels, and less increases in type IV collagen levels within the EVAR group both at short- and long-term follow-up. However, these differences in mean levels of the two VBM collagens were not statistically significant in this small pilot study.

Data from experimental studies suggest changes in BM components with formation of a "scar" and increased assembly and deposition of type IV collagen has been demonstrated in repaired BM after injury (Ramos-Lewis et al., 2018). Thus the increased VBM collagen levels in **paper IV** could be due to remodelling of the VBM after surgical treatment of AAA. In general, the small number of individuals and variability of plasma levels of both collagens in **paper IV** should be considered as a strong limiting factor to draw any firm conclusions. Moreover, fewer individuals in OSR group made it difficult to confirm a possible difference between the levels of VBM collagens in the EVAR and OSR groups depending on the surgical intervention.

So far the observed findings in this thesis indicate that patients with an AAA have increased levels of VBM collagens, with higher levels in larger aneurysms and those with faster expansion. Moreover, the inflammatory activity in AAA patients is associated with VBM collagen levels and the aortic diameter, suggesting inflammation as a potential driver of VBM remodelling and aneurysm growth. Additionally, the findings of altered expression of both collagens in individuals with an AAA and changed circulating levels of both collagens after surgical repair make these biological markers of VBM remodelling an interesting field for further studies.

VI. Conclusions

Paper I

Circulating levels of type IV and XVIII collagen fragments were significantly increased in patients with an AAA .

There was no significant difference in circulating levels of type IV collagen levels between the patients with an AAA and PAD, while circulating levels of type XVIII collagen/endostatin differed significantly between the CON and PAD groups.

The was no difference in circulating levels of both collagens between the CON and PAD groups.

Paper II

The increased circulating levels of both collagens in patients with an AAA were confirmed in a larger cohort.

Circulating levels of type IV and XVIII collagen/endostatin fragments were significantly associated with aortic diameter with highest levels in AAA patients.

Plasma levels of type IV collagen were not associated with expansion rate and the significant association between the type XVIII collagen/endostatin and expansion rate was lost after adjusting for initial aortic diameter.

The location of type XVIII collagen in aortic wall in association to the elastin layer could be confirmed also in human aorta.

The expression of both collagens was altered in AAA compared with normal aortic tissue. The AAA wall was disorganized with fragmentation of all elastic fibres and less abundant type IV and XVIII collagen expression was shown.

Paper III

The circulating levels of VBM collagens were associated with the levels of several inflammatory markers at baseline. Additionally, plasma levels of inflammatory marekers were associated with the baseline aortic diameter and the expansion rate.

Paper IV

Circulation levels of type IV and XVIII/endostatin were altered after repair of an AAA in comparison with the baseline levels before repair and remain high after one year of follow up.

VI. Future perspectives

Recent experimental and clinical studies show evidence of an important role of endothelial cells (ECs) in AAA pathology. ECs involvement is mediated by recruitment of inflammatory cells such as macrophages, and secretion of cytokines and proteases into the medial layer (Ramella et al., 2017). Increased EC expression of cathepsins has been demonstrated in aortic wall lesions in AAA (Lohoefer et al., 2012) and experimental studies have demonstrated that mechanical stretch enhances MMP expression and activity by oxidative stress, another hallmark in vascular remodelling (Grote et al., 2003). EC dysfunction is associated with many cardiovascular diseases (Siasos et al., 2015) and recently some of endothelial dysfunction-biomarkers such as ILT, fibrinogen and D-dimer have been linked with AAA progression and expansion (Siennicka et al., 2013) In addition, endothelial dysfunction has been associated with many risk factors like aging, smoking and hypertension, all also risk factors involved in AAA development.

These aspects make endothelium a highly interesting structure for future AAA research. Furthermore, the direct exposure of the endothelium to blood flow and cells could provide new therapeutic targets in AAA. Thus, constant exposure of vessels to mechanical stretch and ECs to shear stress regulate expression and activity of proteases, which are highly involved in remodelling and degradation of ECM and VBM components such type IV and XVIII collagen/endostatin. Results from this thesis suggest that release of VBM collagens could partly be mediated by inflammation. The trigger mechanism of this inflammation is however not clear.

The role of mechanical forces in remodelling of VBM and in particular leading to changes in type IV and XVIII collagen/endostatin expression and activity could be further investigated by model systems using experimentally induced AAA, which would provide a better mechanistic insight into changes and remodelling of sub-endothelial VBM and its components during different stages of an AAA development and expansion.

As mentioned previously elastin has been assigned to be responsible for compliance, while type I and type III collagens for tensile strength of vessel wall. Degradation of medial elastin layer and these fibrillar collagens in adventitia is one of the most histological features in AAA.

Findings in paper II regarding frequent presence of "nodular structures" in tissue from AAA patients in comparison with normal aortic tissues were interesting, as such findings has not been well noticed in prior histological descriptions in AAA tissue. Further staining by CD31 and type I collagen were performed to improve assessment of the nodular structures and in purpose to exclude any confusion with occluded vasa vasorum. As expected the elastin layer in our AAA tissues were disorganized and fragmented. There were no sign of occluded vasa vasorum but increased of type I collagen, and some other ECM components. Further investigation to characterize contents of these nodular structures is planned. It would also be interesting to study in more detail the differences in risk factor profiles in and between the patients with an AAA as such may have influenced the findings.

Acknowledgments

If we knew what it was we were doing, it would not be called research, would it?

Albert Einstein

Regardless what we research, a good research team will inspire, provide an open and pleasant environment for critical review and promote development of the co-workers and of the project towards goal and new challenges.

During this time of research I learned that results are achieved through collaboration with different actors in many aspects.

There are many I would like to thank:

My supervisor professor **Malin Sund,** with great knowledge, interest and commitment in clinical and research area. Your clear and sharp vision with an always gently constructive leadership has helped me to develop in many ways. Your never-ending energy and enthusiasm is impressive. I am so grateful for your many insightful and supporting discussions. This is not the end!

I look forward to our future projects and the opportunity to learn more from you.

Professor **Anders Wanhainen**, my co-supervisor, a leading force in aneurysm epidemiology and with long experience of vascular surgery. When I met him for the first time at the Department of Surgery in Örnsköldsvik in 1998, I had no idea how important he would be in my future work. Anders has helped me throughout this project with his brilliance, friendly advices and constructive criticism. It has been a great honour and pleasure to have you as a co-tutor.

Professor **Martin Björck,** co-author and great inspiration source with clear vision in clinical and scientific work. I am thankful for your advices in this project from the start.

Funding

References

1998. Mortality results for randomised controlled trial of early elective surgery or ultrasonographic surveillance for small abdominal aortic aneurysms. The UK Small Aneurysm Trial Participants. *Lancet,* 352**,** 1649-55.

ABISI, S., BURNAND, K. G., WALTHAM, M., HUMPHRIES, J., TAYLOR, P. R. & SMITH, A. 2007. Cysteine protease activity in the wall of abdominal aortic aneurysms. *J Vasc Surg,* 46**,** 1260-6.

AHMED, R., GHOORAH, K. & KUNADIAN, V. 2016. Abdominal Aortic Aneurysms and Risk Factors for Adverse Events. *Cardiol Rev,* 24**,** 88-93.

AILAWADI, G., ELIASON, J. L., ROELOFS, K. J., SINHA, I., HANNAWA, K. K., KALDJIAN, E. P., LU, G., HENKE, P. K., STANLEY, J. C., WEISS, S. J., THOMPSON, R. W. & UPCHURCH, G. R., JR. 2004. Gender differences in experimental aortic aneurysm formation. *Arterioscler Thromb Vasc Biol,* 24**,** 2116-22.

AKERFELDT, T., GUNNINGBERG, L., SWENNE, C. L., RONQUIST, G. & LARSSON, A. 2014. Elective orthopedic and cardiopulmonary bypass surgery causes a reduction in serum endostatin levels. *Eur J Med Res,* 19**,** 61.

AKERMAN, A. W., STROUD, R. E., BARRS, R. W., GRESPIN, R. T., MCDONALD, L. T., LARUE, R. A. C., MUKHERJEE, R., IKONOMIDIS, J. S., JONES, J. A. & RUDDY, J. M. 2018. Elevated Wall Tension Initiates Interleukin-6 Expression and Abdominal Aortic Dilation. *Ann Vasc Surg,* 46**,** 193-204.

AL-BARJAS, H. S., ARIENS, R., GRANT, P. & SCOTT, J. A. 2006. Raised plasma fibrinogen concentration in patients with abdominal aortic aneurysm. *Angiology,* 57**,** 607-14.

ALUND, M., MANI, K. & WANHAINEN, A. 2008. Selective screening for abdominal aortic aneurysm among patients referred to the vascular laboratory. *Eur J Vasc Endovasc Surg,* 35**,** 669-74.

ANJUM, A. & POWELL, J. T. 2012. Is the incidence of abdominal aortic aneurysm declining in the 21st century? Mortality and hospital admissions for England & Wales and Scotland. *Eur J Vasc Endovasc Surg,* 43**,** 161-6.

ASHTON, H. A., BUXTON, M. J., DAY, N. E., KIM, L. G., MARTEAU, T. M., SCOTT, R. A., THOMPSON, S. G., WALKER, N. M. & MULTICENTRE ANEURYSM SCREENING STUDY, G. 2002. The Multicentre Aneurysm Screening Study (MASS) into the effect of abdominal aortic aneurysm screening on mortality in men: a randomised controlled trial. *Lancet,* 360**,** 1531-9.

BASNYAT, P. S., AIONO, S., WARSI, A. A., MAGEE, T. R., GALLAND, R. B. & LEWIS, M. H. 2003. Natural history of the ectatic aorta. *Cardiovasc Surg,* 11**,** 273-6.

BAXTER, B. T., TERRIN, M. C. & DALMAN, R. L. 2008. Medical management of small

abdominal aortic aneurysms. *Circulation,* 117**,** 1883-9.

BECQUEMIN, J. P., PILLET, J. C., LESCALIE, F., SAPOVAL, M., GOUEFFIC, Y., LERMUSIAUX, P., STEINMETZ, E., MARZELLE, J. & TRIALISTS, A. C. E. 2011. A randomized controlled trial of endovascular aneurysm repair versus open surgery for abdominal aortic aneurysms in low- to moderate-risk patients. *J Vasc Surg,* 53**,** 1167-1173 e1.

BHAK, R. H., WININGER, M., JOHNSON, G. R., LEDERLE, F. A., MESSINA, L. M., BALLARD, D. J., WILSON, S. E., ANEURYSM, D. & MANAGEMENT STUDY, G. 2015. Factors associated with small abdominal aortic aneurysm expansion rate. *JAMA Surg,* 150**,** 44-50.

BRADY, A. R., THOMPSON, S. G., FOWKES, F. G., GREENHALGH, R. M., POWELL, J. T. & PARTICIPANTS, U. K. S. A. T. 2004. Abdominal aortic aneurysm expansion: risk factors and time intervals for surveillance. *Circulation,* 110**,** 16-21.

BROWN, L. C. & POWELL, J. T. 1999. Risk factors for aneurysm rupture in patients kept under ultrasound surveillance. UK Small Aneurysm Trial Participants. *Ann Surg,* 230**,** 289-96; discussion 296-7.

BROWN, P. M., SOBOLEV, B. & ZELT, D. T. 2003a. Selective management of abdominal aortic aneurysms smaller than 5.0 cm in a prospective sizing program with gender-specific analysis. *J Vasc Surg,* 38**,** 762-5.

BROWN, P. M., ZELT, D. T. & SOBOLEV, B. 2003b. The risk of rupture in untreated aneurysms: the impact of size, gender, and expansion rate. *J Vasc Surg,* 37**,** 280-4.

BROWN, T. M. & BITTNER, V. 2008. Biomarkers of atherosclerosis: clinical applications. *Curr Cardiol Rep,* 10**,** 497-504.

CHAE, C. U., LEE, R. T., RIFAI, N. & RIDKER, P. M. 2001. Blood pressure and inflammation in apparently healthy men. *Hypertension,* 38**,** 399-403.

CHAIKOF, E. L., DALMAN, R. L., ESKANDARI, M. K., JACKSON, B. M., LEE, W. A., MANSOUR, M. A., MASTRACCI, T. M., MELL, M., MURAD, M. H., NGUYEN, L. L., ODERICH, G. S., PATEL, M. S., SCHERMERHORN, M. L. & STARNES, B. W. 2018. The Society for Vascular Surgery practice guidelines on the care of patients with an abdominal aortic aneurysm. *J Vasc Surg,* 67**,** 2-77 e2.

CHOKE, E., LEE, K., MCCARTHY, M., NASIM, A., NAYLOR, A. R., BOWN, M. & SAYERS, R. 2012a. Risk models for mortality following elective open and endovascular abdominal aortic aneurysm repair: a single institution experience. *Eur J Vasc Endovasc Surg,* 44**,** 549-54.

CHOKE, E., VIJAYNAGAR, B., THOMPSON, J., NASIM, A., BOWN, M. J. & SAYERS, R. D. 2012b. Changing epidemiology of abdominal aortic aneurysms in England and Wales: older and more benign? *Circulation,* 125**,** 1617-25.

CHUN, K. C., TENG, K. Y., CHAVEZ, L. A., VAN SPYK, E. N., SAMADZADEH, K. M., CARSON,

J. G. & LEE, E. S. 2014. Risk factors associated with the diagnosis of abdominal aortic aneurysm in patients screened at a regional Veterans Affairs health care system. *Ann Vasc Surg,* 28**,** 87-92.

COCCIOLONE, A. J., HAWES, J. Z., STAICULESCU, M. C., JOHNSON, E. O., MURSHED, M. & WAGENSEIL, J. E. 2018. Elastin, arterial mechanics, and cardiovascular disease. *Am J Physiol Heart Circ Physiol,* 315**,** H189-H205.

COLLABORATORS, R., BOWN, M. J., SWEETING, M. J., BROWN, L. C., POWELL, J. T. & THOMPSON, S. G. 2013. Surveillance intervals for small abdominal aortic aneurysms: a meta-analysis. *JAMA,* 309**,** 806-13.

COLLIN, J. 1988. Screening for aortic aneurysm. *Lancet,* 2**,** 1431-2.

COTRUFO, M., DE SANTO, L., DELLA CORTE, A., DI MEGLIO, F., GUERRA, G., QUARTO, C., VITALE, S., CASTALDO, C. & MONTAGNANI, S. 2005. Basal lamina structural alterations in human asymmetric aneurismatic aorta. *Eur J Histochem,* 49**,** 363-70.

D'AUDIFFRET, A., SANTILLI, S., TRETINYAK, A. & ROETHLE, S. 2002. Fate of the ectatic infrarenal aorta: expansion rates and outcomes. *Ann Vasc Surg,* 16**,** 534-6.

DALE, M. A., RUHLMAN, M. K. & BAXTER, B. T. 2015. Inflammatory cell phenotypes in AAAs: their role and potential as targets for therapy. *Arterioscler Thromb Vasc Biol,* 35**,** 1746-55.

DAVIES, R. S., ABDELHAMID, M., WALL, M. L., VOHRA, R. K., BRADBURY, A. W. & ADAM, D. J. 2011. Coagulation, fibrinolysis, and platelet activation in patients undergoing open and endovascular repair of abdominal aortic aneurysm. *J Vasc Surg,* 54**,** 865-78.

DAVIS, V. A., PERSIDSKAIA, R. N., BACA-REGEN, L. M., FIOTTI, N., HALLORAN, B. G. & BAXTER, B. T. 2001. Cytokine pattern in aneurysmal and occlusive disease of the aorta. *J Surg Res,* 101**,** 152-6.

DE NOOIJER, R., BOT, I., VON DER THUSEN, J. H., LEEUWENBURGH, M. A., OVERKLEEFT, H. S., KRAAIJEVELD, A. O., DORLAND, R., VAN SANTBRINK, P. J., VAN HEININGEN, S. H., WESTRA, M. M., KOVANEN, P. T., JUKEMA, J. W., VAN DER WALL, E. E., VAN BERKEL, T. J., SHI, G. P. & BIESSEN, E. A. 2009. Leukocyte cathepsin S is a potent regulator of both cell and matrix turnover in advanced atherosclerosis. *Arterioscler Thromb Vasc Biol,* 29**,** 188-94.

DUBOST, C., ALLARY, M. & OECONOMOS, N. 1952. 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,* 64**,** 405-8.

EICKHOFF, J. H. 1993. Incidence of diagnosis, operation and death from abdominal aortic aneurysms in Danish hospitals:

results from a nation-wide survey, 1977-1990. *Eur J Surg,* 159**,** 619-23.

FINK, H. A., LEDERLE, F. A., ROTH, C. S., BOWLES, C. A., NELSON, D. B. & HAAS, M. A. 2000. The accuracy of physical examination to detect abdominal aortic aneurysm. *Arch Intern Med,* 160**,** 833-6.

FLEMING, C., WHITLOCK, E. P., BEIL, T. L. & LEDERLE, F. A. 2005. Screening for abdominal aortic aneurysm: a best-evidence systematic review for the U.S. Preventive Services Task Force. *Ann Intern Med,* 142**,** 203-11.

FLONDELL-SITE, D., LINDBLAD, B., KOLBEL, T. & GOTTSATER, A. 2009. Cytokines and systemic biomarkers are related to the size of abdominal aortic aneurysms. *Cytokine,* 46**,** 211-5.

FUJIKAWA, L. S., FOSTER, C. S., GIPSON, I. K. & COLVIN, R. B. 1984. Basement membrane components in healing rabbit corneal epithelial wounds: immunofluorescence and ultrastructural studies. *J Cell Biol,* 98**,** 128-38.

GASSER, T. C., NCHIMI, A., SWEDENBORG, J., ROY, J., SAKALIHASAN, N., BOCKLER, D. & HYHLIK-DURR, A. 2014. 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,* 47**,** 288-95.

GIANFAGNA, F., VERONESI, G., BERTU, L., TOZZI, M., TARALLO, A., FERRARIO, M. M., CASTELLI, P.

& RO, C. A. V. P. I. 2016. Prevalence of abdominal aortic aneurysms and its relation with cardiovascular risk stratification: protocol of the Risk of Cardiovascular diseases and abdominal aortic Aneurysm in Varese (RoCAV) population based study. *BMC Cardiovasc Disord,* 16**,** 243.

GOLLEDGE, J. 2018. Abdominal aortic aneurysm: update on pathogenesis and medical treatments. *Nat Rev Cardiol.*

GOLLEDGE, J. & KUIVANIEMI, H. 2013. Genetics of abdominal aortic aneurysm. *Curr Opin Cardiol,* 28**,** 290-6.

GOLLEDGE, J., MULLER, R., CLANCY, P., MCCANN, M. & NORMAN, P. E. 2011. Evaluation of the diagnostic and prognostic value of plasma D-dimer for abdominal aortic aneurysm. *Eur Heart J,* 32**,** 354-64.

GOLLEDGE, J., TSAO, P. S., DALMAN, R. L. & NORMAN, P. E. 2008. Circulating markers of abdominal aortic aneurysm presence and progression. *Circulation,* 118**,** 2382-92.

GROENEVELD, M. E., MEEKEL, J. P., RUBINSTEIN, S. M., MERKESTEIN, L. R., TANGELDER, G. J., WISSELINK, W., TRUIJERS, M. & YEUNG, K. K. 2018. Systematic Review of Circulating, Biomechanical, and Genetic Markers for the Prediction of Abdominal Aortic Aneurysm Growth and Rupture. *J Am Heart Assoc,* 7.

GROTE, K., FLACH, I., LUCHTEFELD, M., AKIN, E., HOLLAND, S. M., DREXLER, H. & SCHIEFFER, B. 2003. Mechanical stretch enhances mRNA expression

and proenzyme release of matrix metalloproteinase-2 (MMP-2) via NAD(P)H oxidase-derived reactive oxygen species. *Circ Res,* 92**,** e80-6.

HAFEZ, H., DRUCE, P. S. & ASHTON, H. A. 2008. Abdominal aortic aneurysm development in men following a "normal" aortic ultrasound scan. *Eur J Vasc Endovasc Surg,* 36**,** 553-8.

HARRISON, S. C., HOLMES, M. V., BURGESS, S., ASSELBERGS, F. W., JONES, G. T., BAAS, A. F., VAN 'T HOF, F. N., DE BAKKER, P. I. W., BLANKENSTEIJN, J. D., POWELL, J. T., SARATZIS, A., DE BORST, G. J., SWERDLOW, D. I., VAN DER GRAAF, Y., VAN RIJ, A. M., CAREY, D. J., ELMORE, J. R., TROMP, G., KUIVANIEMI, H., SAYERS, R. D., SAMANI, N. J., BOWN, M. J. & HUMPHRIES, S. E. 2018. Genetic Association of Lipids and Lipid Drug Targets With Abdominal Aortic Aneurysm: A Meta-analysis. *JAMA Cardiol,* 3**,** 26-33.

HEIKKINEN, M., SALENIUS, J. P. & AUVINEN, O. 2002. Ruptured abdominal aortic aneurysm in a well-defined geographic area. *J Vasc Surg,* 36**,** 291-6.

HELJASVAARA, R., AIKIO, M., RUOTSALAINEN, H. & PIHLAJANIEMI, T. 2017. Collagen XVIII in tissue homeostasis and dysregulation - Lessons learned from model organisms and human patients. *Matrix Biol,* 57-58**,** 55-75.

HERNANDEZ-AGUILERA, A., NIELSEN, S. H., BONACHE, C., FERNANDEZ-ARROYO, S., MARTIN-PAREDERO, V., FIBLA, M., KARSDAL, M. A., GENOVESE, F., MENENDEZ, J.

A., CAMPS, J. & JOVEN, J. 2018. Assessment of extracellular matrix-related biomarkers in patients with lower extremity artery disease. *J Vasc Surg,* 68**,** 1135-1142 e6.

HERNESNIEMI, J. A., VANNI, V. & HAKALA, T. 2015. The prevalence of abdominal aortic aneurysm is consistently high among patients with coronary artery disease. *J Vasc Surg,* 62**,** 232-240 e3.

HOBEIKA, M. J., THOMPSON, R. W., MUHS, B. E., BROOKS, P. C. & GAGNE, P. J. 2007. Matrix metalloproteinases in peripheral vascular disease. *J Vasc Surg,* 45**,** 849-57.

HOUARD, X., TOUAT, Z., OLLIVIER, V., LOUEDEC, L., PHILIPPE, M., SEBBAG, U., MEILHAC, O., ROSSIGNOL, P. & MICHEL, J. B. 2009. Mediators of neutrophil recruitment in human abdominal aortic aneurysms. *Cardiovasc Res,* 82**,** 532-41.

HUDSON, B. G., REEDERS, S. T. & TRYGGVASON, K. 1993. Type IV collagen: structure, gene organization, and role in human diseases. Molecular basis of Goodpasture and Alport syndromes and diffuse leiomyomatosis. *J Biol Chem,* 268**,** 26033-6.

IOZZO, R. V. 2005. Basement membrane proteoglycans: from cellar to ceiling. *Nat Rev Mol Cell Biol,* 6**,** 646-56.

JOHNSTON, K. W., RUTHERFORD, R. B., TILSON, M. D., SHAH, D. M., HOLLIER, L. & STANLEY, J. C. 1991. 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,* 13**,** 452-8.

JOHNSTON, W. F., SALMON, M., SU, G., LU, G., STONE, M. L., ZHAO, Y., OWENS, G. K., UPCHURCH, G. R., JR. & AILAWADI, G. 2013. Genetic and pharmacologic disruption of interleukin-1beta signaling inhibits experimental aortic aneurysm formation. *Arterioscler Thromb Vasc Biol,* 33**,** 294-304.

JUVONEN, J., SURCEL, H. M., SATTA, J., TEPPO, A. M., BLOIGU, A., SYRJALA, H., AIRAKSINEN, J., LEINONEN, M., SAIKKU, P. & JUVONEN, T. 1997. Elevated circulating levels of inflammatory cytokines in patients with abdominal aortic aneurysm. *Arterioscler Thromb Vasc Biol,* 17**,** 2843-7.

KALLURI, R. 2003. Basement membranes: structure, assembly and role in tumour angiogenesis. *Nat Rev Cancer,* 3**,** 422-33.

KARSDAL, M. A., GENOVESE, F., MADSEN, E. A., MANON-JENSEN, T. & SCHUPPAN, D. 2016. Collagen and tissue turnover as a function of age: Implications for fibrosis. *J Hepatol,* 64**,** 103-9.

KOTZE, C. W., GROVES, A. M., MENEZES, L. J., HARVEY, R., ENDOZO, R., KAYANI, I. A., ELL, P. J. & YUSUF, S. W. 2011. What is the relationship between (1)(8)F-FDG aortic aneurysm uptake on PET/CT and future growth rate? *Eur J Nucl Med Mol Imaging,* 38**,** 1493-9.

KUROSAWA, K., MATSUMURA, J. S. & YAMANOUCHI, D. 2013. Current Status of Medical Treatment for Abdominal Aortic Aneurysm. *Circ J.*

KURVERS, H., VEITH, F. J., LIPSITZ, E. C., OHKI, T., GARGIULO, N. J., CAYNE, N. S., SUGGS, W. D., TIMARAN, C. H., KWON, G. Y., RHEE, S. J. & SANTIAGO, C. 2004. Discontinuous, staccato growth of abdominal aortic aneurysms. *J Am Coll Surg,* 199**,** 709-15.

LEDERLE, F. A. 2011. The rise and fall of abdominal aortic aneurysm. *Circulation,* 124**,** 1097-9.

LEDERLE, F. A., JOHNSON, G. R., WILSON, S. E., BALLARD, D. J., JORDAN, W. D., JR., BLEBEA, J., LITTOOY, F. N., FREISCHLAG, J. A., BANDYK, D., RAPP, J. H., SALAM, A. A. & VETERANS AFFAIRS COOPERATIVE STUDY, I. 2002a. Rupture rate of large abdominal aortic aneurysms in patients refusing or unfit for elective repair. *JAMA,* 287**,** 2968-72.

LEDERLE, F. A., JOHNSON, G. R., WILSON, S. E., CHUTE, E. P., HYE, R. J., MAKAROUN, M. S., BARONE, G. W., BANDYK, D., MONETA, G. L. & MAKHOUL, R. G. 2000. The aneurysm detection and management study screening program: validation cohort and final results. Aneurysm Detection and Management Veterans Affairs Cooperative Study Investigators. *Arch Intern Med,* 160**,** 1425-30.

LEDERLE, F. A., JOHNSON, G. R., WILSON, S. E., CHUTE, E. P.,

LITTOOY, F. N., BANDYK, D., KRUPSKI, W. C., BARONE, G. W., ACHER, C. W. & BALLARD, D. J. 1997a. Prevalence and associations of abdominal aortic aneurysm detected through screening. Aneurysm Detection and Management (ADAM) Veterans Affairs Cooperative Study Group. *Ann Intern Med,* 126**,** 441-9.

LEDERLE, F. A., JOHNSON, G. R., WILSON, S. E., GORDON, I. L., CHUTE, E. P., LITTOOY, F. N., KRUPSKI, W. C., BANDYK, D., BARONE, G. W., GRAHAM, L. M., HYE, R. J. & REINKE, D. B. 1997b. Relationship of age, gender, race, and body size to infrarenal aortic diameter. The Aneurysm Detection and Management (ADAM) Veterans Affairs Cooperative Study Investigators. *J Vasc Surg,* 26**,** 595-601.

LEDERLE, F. A., KANE, R. L., MACDONALD, R. & WILT, T. J. 2007. Systematic review: repair of unruptured abdominal aortic aneurysm. *Ann Intern Med,* 146**,** 735-41.

LEDERLE, F. A., WILSON, S. E., JOHNSON, G. R., REINKE, D. B., LITTOOY, F. N., ACHER, C. W., BALLARD, D. J., MESSINA, L. M., GORDON, I. L., CHUTE, E. P., KRUPSKI, W. C., BUSUTTIL, S. J., BARONE, G. W., SPARKS, S., GRAHAM, L. M., RAPP, J. H., MAKAROUN, M. S., MONETA, G. L., CAMBRIA, R. A., MAKHOUL, R. G., ETON, D., ANSEL, H. J., FREISCHLAG, J. A., BANDYK, D., ANEURYSM, D. & MANAGEMENT VETERANS AFFAIRS COOPERATIVE STUDY, G. 2002b. Immediate repair compared with surveillance of small abdominal aortic aneurysms. *N Engl J Med,* 346**,** 1437-44.

LI, H., BAI, S., AO, Q., WANG, X., TIAN, X., LI, X., TONG, H., HOU, W. & FAN, J. 2018. Modulation of Immune-Inflammatory Responses in Abdominal Aortic Aneurysm: Emerging Molecular Targets. *J Immunol Res,* 2018**,** 7213760.

LIAO, M., LIU, C. L., LV, B. J., ZHANG, J. Y., CHENG, L., CHENG, X., LINDHOLT, J. S., RASMUSSEN, L. M. & SHI, G. P. 2015. Plasma cytokine levels and risks of abdominal aortic aneurysms: A population-based prospective cohort study. *Ann Med,* 47**,** 245-52.

LINDHOLT, J. S., HEICKENDORFF, L., HENNEBERG, E. W. & FASTING, H. 1997. Serum-elastin-peptides as a predictor of expansion of small abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg,* 14**,** 12-6.

LINDHOLT, J. S., JUUL, S., FASTING, H. & HENNEBERG, E. W. 2002. Hospital costs and benefits of screening for abdominal aortic aneurysms. Results from a randomised population screening trial. *Eur J Vasc Endovasc Surg,* 23**,** 55-60.

LINDHOLT, J. S., JUUL, S., FASTING, H. & HENNEBERG, E. W. 2005. Screening for abdominal aortic aneurysms: single centre randomised controlled trial. *BMJ,* 330**,** 750.

LINDHOLT, J. S., JUUL, S., HENNEBERG, E. W. & FASTING, H. 1998. Is screening for abdominal aortic aneurysm acceptable to the population? Selection and

recruitment to hospital-based mass screening for abdominal aortic aneurysm. *J Public Health Med,* 20**,** 211-7.

LINDHOLT, J. S., VAMMEN, S., FASTING, H., HENNEBERG, E. W. & HEICKENDORFF, L. 2000. The plasma level of matrix metalloproteinase 9 may predict the natural history of small abdominal aortic aneurysms. A preliminary study. *Eur J Vasc Endovasc Surg,* 20**,** 281-5.

LINNE, A., FORSBERG, J., LEANDER, K. & HULTGREN, R. 2017. Screening of siblings to patients with abdominal aortic aneurysms in Sweden. *Scand Cardiovasc J,* 51**,** 167-171.

LIU, J., SUKHOVA, G. K., YANG, J. T., SUN, J., MA, L., REN, A., XU, W. H., FU, H., DOLGANOV, G. M., HU, C., LIBBY, P. & SHI, G. P. 2006. Cathepsin L expression and regulation in human abdominal aortic aneurysm, atherosclerosis, and vascular cells. *Atherosclerosis,* 184**,** 302-11.

LOHOEFER, F., REEPS, C., LIPP, C., RUDELIUS, M., ZIMMERMANN, A., OCKERT, S., ECKSTEIN, H. H. & PELISEK, J. 2012. Histopathological analysis of cellular localization of cathepsins in abdominal aortic aneurysm wall. *Int J Exp Pathol,* 93**,** 252-8.

MAEGDEFESSEL, L., DALMAN, R. L. & TSAO, P. S. 2014. Pathogenesis of abdominal aortic aneurysms: microRNAs, proteases, genetic associations. *Annu Rev Med,* 65**,** 49-62.

MARNEROS, A. G. & OLSEN, B. R. 2005. Physiological role of collagen XVIII and endostatin. *Faseb J,* 19**,** 716-28.

MCGREGOR, J. C., POLLOCK, J. G. & ANTON, H. C. 1975. Ultrasonography and possible ruptured abdominal aortic aneurysms. *Br Med J,* 3**,** 78-9.

MEHER, A. K. & CHEN, Y. C. 2017. Analysis of volatile compounds by open-air ionization mass spectrometry. *Anal Chim Acta,* 966**,** 41-46.

MICHEL, J. B., MARTIN-VENTURA, J. L., EGIDO, J., SAKALIHASAN, N., TRESKA, V., LINDHOLT, J., ALLAIRE, E., THORSTEINSDOTTIR, U., COCKERILL, G., SWEDENBORG, J. & CONSORTIUM, F. E. 2011. Novel aspects of the pathogenesis of aneurysms of the abdominal aorta in humans. *Cardiovasc Res,* 90**,** 18-27.

MIDDLETON, R. K., BOWN, M. J., LLOYD, G. M., JONES, J. L., LONDON, N. J. & SAYERS, R. D. 2009. Characterisation of Interleukin-8 and monocyte chemoattractant protein-1 expression within the abdominal aortic aneurysm and their association with mural inflammation. *Eur J Vasc Endovasc Surg,* 37**,** 46-55.

MIOSGE, N., SASAKI, T. & TIMPL, R. 1999. Angiogenesis inhibitor endostatin is a distinct component of elastic fibers in vessel walls. *Faseb J,* 13**,** 1743-50.

MITSUMA, W., KODAMA, M., HANAWA, H., ITO, M., RAMADAN, M. M., HIRONO, S., OBATA, H., OKADA, S., SANADA, F., YANAGAWA, T., KASHIMURA, T., FUSE, K., TANABE, N. &

AIZAWA, Y. 2007. Serum endostatin in the coronary circulation of patients with coronary heart disease and its relation to coronary collateral formation. *Am J Cardiol,* 99**,** 494-8.

MOFIDI, R., GOLDIE, V. J., KELMAN, J., DAWSON, A. R., MURIE, J. A. & CHALMERS, R. T. 2007. Influence of sex on expansion rate of abdominal aortic aneurysms. *Br J Surg,* 94**,** 310-4.

MOOS, M. P., JOHN, N., GRABNER, R., NOSSMANN, S., GUNTHER, B., VOLLANDT, R., FUNK, C. D., KAISER, B. & HABENICHT, A. J. 2005. The lamina adventitia is the major site of immune cell accumulation in standard chow-fed apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol,* 25**,** 2386-91.

PARRY, D. J., AL-BARJAS, H. S., CHAPPELL, L., RASHID, T., ARIENS, R. A. & SCOTT, D. J. 2009. Haemostatic and fibrinolytic factors in men with a small abdominal aortic aneurysm. *Br J Surg,* 96**,** 870-7.

PATEL, K., ZAFAR, M. A., ZIGANSHIN, B. A. & ELEFTERIADES, J. A. 2018. Diabetes Mellitus: Is It Protective against Aneurysm? A Narrative Review. *Cardiology,* 141**,** 107-122.

PEDERSEN, O. M., ASLAKSEN, A. & VIK-MO, H. 1993. Ultrasound measurement of the luminal diameter of the abdominal aorta and iliac arteries in patients without vascular disease. *J Vasc Surg,* 17**,** 596-601.

PETERSEN, E., GINEITIS, A., WAGBERG, F. & ANGQUIST, K. A. 2000. Activity of matrix metalloproteinase-2 and -9 in abdominal aortic aneurysms. Relation to size and rupture. *Eur J Vasc Endovasc Surg,* 20**,** 457-61.

PLATT, M. O., ANKENY, R. F., SHI, G. P., WEISS, D., VEGA, J. D., TAYLOR, W. R. & JO, H. 2007. Expression of cathepsin K is regulated by shear stress in cultured endothelial cells and is increased in endothelium in human atherosclerosis. *Am J Physiol Heart Circ Physiol,* 292**,** H1479-86.

POWELL, J. T. & BRADY, A. R. 2004. Detection, management, and prospects for the medical treatment of small abdominal aortic aneurysms. *Arterioscler Thromb Vasc Biol,* 24**,** 241-5.

POWELL, J. T., GOTENSPARRE, S. M., SWEETING, M. J., BROWN, L. C., FOWKES, F. G. & THOMPSON, S. G. 2011. Rupture rates of small abdominal aortic aneurysms: a systematic review of the literature. *Eur J Vasc Endovasc Surg,* 41**,** 2-10.

QIN, Y., CAO, X., YANG, Y. & SHI, G. P. 2013. Cysteine protease cathepsins and matrix metalloproteinases in the development of abdominal aortic aneurysms. *Future Cardiol,* 9**,** 89-103.

RAMELLA, M., BOCCAFOSCHI, F., BELLOFATTO, K., MD, A. F., FUSARO, L., BOLDORINI, R., CASELLA, F., PORTA, C., SETTEMBRINI, P. & CANNAS, M. 2017. Endothelial MMP-9 drives the inflammatory response in abdominal aortic aneurysm (AAA). *Am J Transl Res,* 9**,** 5485-5495.

RAMOS-LEWIS, W., LAFEVER, K. S. & PAGE-MCCAW, A. 2018. A scar-like lesion is apparent in basement membrane after wound repair in vivo. *Matrix Biol,* 74**,** 101-120.

RODELLA, L. F., REZZANI, R., BONOMINI, F., PERONI, M., COCCHI, M. A., HIRTLER, L. & BONARDELLI, S. 2016. Abdominal aortic aneurysm and histological, clinical, radiological correlation. *Acta Histochem,* 118**,** 256-62.

SANTILLI, S. M., LITTOOY, F. N., CAMBRIA, R. A., RAPP, J. H., TRETINYAK, A. S., D'AUDIFFRET, A. C., KUSKOWSKI, M. A., ROETHLE, S. T., TOMCZAK, C. M. & KRUPSKI, W. C. 2002. Expansion rates and outcomes for the 3.0-cm to the 3.9-cm infrarenal abdominal aortic aneurysm. *J Vasc Surg,* 35**,** 666-71.

SATTA, J., HAUKIPURO, K., KAIRALUOMA, M. I. & JUVONEN, T. 1997. Aminoterminal propeptide of type III procollagen in the follow-up of patients with abdominal aortic aneurysms. *J Vasc Surg,* 25**,** 909-15.

SAVOIA, C., SADA, L., ZEZZA, L., PUCCI, L., LAURI, F. M., BEFANI, A., ALONZO, A. & VOLPE, M. Vascular inflammation and endothelial dysfunction in experimental hypertension. *Int J Hypertens,* 2011**,** 281240.

SCOTT, R. A., WILSON, N. M., ASHTON, H. A. & KAY, D. N. 1995. Influence of screening on the incidence of ruptured abdominal aortic aneurysm: 5-year results of a randomized controlled study. *Br J Surg,* 82**,** 1066-70.

SHI, G. P., SUKHOVA, G. K., GRUBB, A., DUCHARME, A., RHODE, L. H., LEE, R. T., RIDKER, P. M., LIBBY, P. & CHAPMAN, H. A. 1999. Cystatin C deficiency in human atherosclerosis and aortic aneurysms. *J Clin Invest,* 104**,** 1191-7.

SHIMIZU, K., MITCHELL, R. N. & LIBBY, P. 2006. Inflammation and cellular immune responses in abdominal aortic aneurysms. *Arterioscler Thromb Vasc Biol,* 26**,** 987-94.

SIASOS, G., GOULIOPOULOS, N., MOSCHOS, M. M., OIKONOMOU, E., KOLLIA, C., KONSOLA, T., ATHANASIOU, D., SIASOU, G., MOUROUZIS, K., ZISIMOS, K., PAPAVASSILIOU, A. G., STEFANADIS, C. & TOUSOULIS, D. 2015. Role of endothelial dysfunction and arterial stiffness in the development of diabetic retinopathy. *Diabetes Care,* 38**,** e9-e10.

SIDLOFF, D. A., STATHER, P. W., CHOKE, E., BOWN, M. J. & SAYERS, R. D. 2014. A systematic review and meta-analysis of the association between markers of hemostasis and abdominal aortic aneurysm presence and size. *J Vasc Surg,* 59**,** 528-535 e4.

SIENNICKA, A., DROZDZYNSKA, M., CHELSTOWSKI, K., CNOTLIWY, M. & JASTRZEBSKA, M. 2013. Haemostatic factors and intraluminal thrombus thickness in abdominal aortic aneurysm. Is secondary fibrinolysis relevant? *J Physiol Pharmacol,* 64**,** 321-30.

STATHER, P. W., SAYERS, R. D., CHEAH, A., WILD, J. B., BOWN, M. J. & CHOKE, E. 2012. Outcomes of endovascular aneurysm repair in patients with hostile neck anatomy. *Eur J Vasc Endovasc Surg,* 44**,** 556-61.

STEFFENSEN, L. B. & RASMUSSEN, L. M. 2018. A role for collagen type IV in cardiovascular disease? *Am J Physiol Heart Circ Physiol,* 315**,** H610-H625.

STERPETTI, A. V., SCHULTZ, R. D., FELDHAUS, R. J., CHENG, S. E. & PEETZ, D. J., JR. 1987. Factors influencing enlargement rate of small abdominal aortic aneurysms. *J Surg Res,* 43**,** 211-9.

STEVENS, R. R. F., GRYTSAN, A., BIASETTI, J., ROY, J., LINDQUIST LILJEQVIST, M. & GASSER, T. C. 2017. Biomechanical changes during abdominal aortic aneurysm growth. *PLoS One,* 12**,** e0187421.

SUND, M., XIE, L. & KALLURI, R. 2004. The contribution of vascular basement membranes and extracellular matrix to the mechanics of tumor angiogenesis. *APMIS,* 112**,** 450-62.

SVENSJO, S., BJORCK, M., GURTELSCHMID, M., DJAVANI GIDLUND, K., HELLBERG, A. & WANHAINEN, A. 2011. Low prevalence of abdominal aortic aneurysm among 65-year-old Swedish men indicates a change in the epidemiology of the disease. *Circulation,* 124**,** 1118-23.

SVENSJO, S., BJORCK, M. & WANHAINEN, A. 2014. Update on screening for abdominal aortic aneurysm: a topical review. *Eur J Vasc Endovasc Surg,* 48**,** 659-67.

SWARTBOL, P., TRUEDSSON, L. & NORGREN, L. 2001. The inflammatory response and its consequence for the clinical outcome following aortic aneurysm repair. *Eur J Vasc Endovasc Surg,* 21**,** 393-400.

SWEETING, M. J., THOMPSON, S. G., BROWN, L. C., POWELL, J. T. & COLLABORATORS, R. 2012. Meta-analysis of individual patient data to examine factors affecting growth and rupture of small abdominal aortic aneurysms. *Br J Surg,* 99**,** 655-65.

THOMPSON, R. W., GERAGHTY, P. J. & LEE, J. K. 2002. Abdominal aortic aneurysms: basic mechanisms and clinical implications. *Curr Probl Surg,* 39**,** 110-230.

THOMPSON, S., KIM, L. & SCOTT, A. 2005. Screening for abdominal aortic aneurysm: screening reduces deaths related to aneurysm. *Bmj,* 330**,** 601; author reply 601-2.

TRESKA, V., TOPOLCAN, O., KOCOVA, J., MOLACEK, J., HOUDEK, K., TONAR, Z., VRZALOVA, J., TRESKOVA, I., KRIZKOVA, V. & BOUDOVA, L. 2011. [Plasmatic levels of proinflammatory cytokines in abdominal aortic aneurysms]. *Rozhl Chir,* 90**,** 37-41.

ULLERY, B. W., HALLETT, R. L. & FLEISCHMANN, D. 2018. Epidemiology and contemporary management of abdominal aortic aneurysms. *Abdom Radiol (NY),* 43**,** 1032-1043.

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UNITED KINGDOM SMALL ANEURYSM TRIAL, P., POWELL, J. T., BRADY, A. R., BROWN, L. C., FOWKES, F. G., GREENHALGH, R. M., RUCKLEY, C. V. & THOMPSON, S. G. 2002. Long-term outcomes of immediate repair compared with surveillance of small abdominal aortic aneurysms. *N Engl J Med,* 346**,** 1445-52.

URBONAVICIUS, S., URBONAVICIENE, G., HONORE, B., HENNEBERG, E. W., VORUM, H. & LINDHOLT, J. S. 2008. Potential circulating biomarkers for abdominal aortic aneurysm expansion and rupture--a systematic review. *Eur J Vasc Endovasc Surg,* 36**,** 273-80; discussion 281-2.

VEGA DE CENIGA, M., GOMEZ, R., ESTALLO, L., RODRIGUEZ, L., BAQUER, M. & BARBA, A. 2006. Growth rate and associated factors in small abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg,* 31**,** 231-6.

VENKATASUBRAMANIAM, A. K., FAGAN, M. J., MEHTA, T., MYLANKAL, K. J., RAY, B., KUHAN, G., CHETTER, I. C. & MCCOLLUM, P. T. 2004. A comparative study of aortic wall stress using finite element analysis for ruptured and non-ruptured abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg,* 28**,** 168-76.

VILLARD, C. & HULTGREN, R. 2018. Abdominal aortic aneurysm: Sex differences. *Maturitas,* 109**,** 63-69.

VOLODOS, N. L., SHEKHANIN, V. E., KARPOVICH, I. P., TROIAN, V. I. & GUR'EV IU, A. 1986. [A self-fixing synthetic blood vessel endoprosthesis]. *Vestn Khir Im I I Grek,* 137**,** 123-5.

VORP, D. A., LEE, P. C., WANG, D. H., MAKAROUN, M. S., NEMOTO, E. M., OGAWA, S. & WEBSTER, M. W. 2001. Association of intraluminal thrombus in abdominal aortic aneurysm with local hypoxia and wall weakening. *J Vasc Surg,* 34**,** 291-9.

WAHLGREN, C. M. & MAGNUSSON, P. K. 2011. Genetic influences on peripheral arterial disease in a twin population. *Arterioscler Thromb Vasc Biol,* 31**,** 678-82.

WALIA, A., YANG, J. F., HUANG, Y. H., ROSENBLATT, M. I., CHANG, J. H. & AZAR, D. T. 2015. Endostatin's emerging roles in angiogenesis, lymphangiogenesis, disease, and clinical applications. *Biochim Biophys Acta,* 1850**,** 2422-38.

WANG, D., WANG, Z., ZHANG, L. & WANG, Y. 2017. Roles of Cells from the Arterial Vessel Wall in Atherosclerosis. *Mediators Inflamm,* 2017**,** 8135934.

WANHAINEN, A. 2008. How to define an abdominal aortic aneurysm--influence on epidemiology and clinical practice. *Scand J Surg,* 97**,** 105-9; discussion 109.

WANHAINEN, A., BJORCK, M., BOMAN, K., RUTEGARD, J. & BERGQVIST, D. 2001. Influence of diagnostic criteria on the prevalence of abdominal aortic aneurysm. *J Vasc Surg,* 34**,** 229-35.

WANHAINEN, A., HULTGREN, R., LINNE, A., HOLST, J., GOTTSATER, A., LANGENSKIOLD, M.,

SMIDFELT, K., BJORCK, M., SVENSJO, S. & SWEDISH ANEURYSM SCREENING STUDY, G. 2016a. Outcome of the Swedish Nationwide Abdominal Aortic Aneurysm Screening Program. *Circulation,* 134**,** 1141-1148.

WANHAINEN, A., MANI, K. & GOLLEDGE, J. 2016b. Surrogate Markers of Abdominal Aortic Aneurysm Progression. *Arterioscler Thromb Vasc Biol,* 36**,** 236-44.

WANHAINEN, A., NILSSON, T. K., BERGQVIST, D., BOMAN, K. & BJORCK, M. 2007. Elevated tissue plasminogen activator in patients with screening-detected abdominal aortic aneurysm. *J Vasc Surg,* 45**,** 1109-13.

WILD, J. B., STATHER, P. W., BIANCARI, F., CHOKE, E. C., EARNSHAW, J. J., GRANT, S. W., HAFEZ, H., HOLDSWORTH, R., JUVONEN, T., LINDHOLT, J., MCCOLLUM, C., PARVIN, S., SAYERS, R. D. & BOWN, M. J. 2013. A multicentre observational study of the outcomes of screening detected sub-aneurysmal aortic dilatation. *Eur J Vasc Endovasc Surg,* 45**,** 128-34.

ZHOU, J., LI, Y. S. & CHIEN, S. 2014. Shear stress-initiated signaling and its regulation of endothelial function. *Arterioscler Thromb Vasc Biol,* 34**,** 2191-8.