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ATHEROSCLEROTIC TRAITS OF INTRACRANIAL ANEURYSM PATIENTS

Focus on aortic calcification

Ville Rantasalo

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SARJA – SER. D OSA – TOM. 1803 | MEDICA – ODONTOLOGICA | TURKU 2024

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To my family and Venla

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ABSTRACT

With a 3% prevalence in the general population, intracranial aneurysms (IA) are not rare. Asymptomatic and unruptured aneurysms are harmless, but growth is associated with rupture risk. Other characteristics, such as their location, morphology and certain risk factors can also increase their rupture risk, and small aneurysms can also sometimes rupture. Inflammation which drives their formation might also cause aneurysm to rupture. Aneurysms prone to rupture should be identified more efficiently, as an aneurysmal subarachnoid haemorrhage (aSAH) can be fatal or cause permanent disability. Although the pathophysiology of IA formation is yet incompletely understood, inflammation, endothelial dysfunction and loss of structural integrity have been found to be important pathophysiological issues.

Atherosclerosis is the common and well-established aetiology for the majority of cardiovascular diseases (CVD). Inflammation, lipid-accumulation, endothelial dysfunction and alterations in artery intima and media are all hallmarks of it's pathophysiology. Atherosclerotic CVD is a widespread health issue associated with varying risk factors and comorbidities, and patients with IAs are one of the most recently found subpopulations to carry an excess burden of atherosclerotic CVDs.

The first study (I) of this dissertation investigates aortic calcification as a marker of atherosclerosis in IA patients and shows that aortic calcification is greater among IA patients than among matched controls. The second study (II) establishes the potential of aortic calcification as a prognostic factor in atherosclerotic population. The third study (III) investigates the association between the classical ankle-brachial index (ABI) and IAs and shows that IAs are clearly more prevalent among patients with low or borderline ABI values than among those with normal ABI values. The fourth study (IV) explores the soluble inflammatory profile of aortic atherosclerosis.

KEYWORDS: Intracranial Aneurysms, atherosclerosis, aortic calcification index, ankle-brachial index

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TIIVISTELMÄ

Aivovaltimoiden aneurysmat (IA) eivät ole harvinaisia (3% prevalenssi) väestötasolla. Oireettomat ja pienet aneurysmat ovat harmittomia, mutta aneurysman repeämän riski kasvaa koon kasvun myötä. Repeämisherkät aneurysmat olisikin syytä tunnistaa nykyistä tehokkaammin, sillä aneurysman repeämisestä johtuva lukinkalvoalainen verenvuoto voi olla kohtalokas tai johtaa pysyvään vammaan. Aneurysmien patofysiologia tunnetaan vielä melko huonosti, mutta endoteelin toimintahäiriö, matala-asteinen tulehdustila suonen seinämässä ja rakenteellisen yhtenäisyyden rikkoontuminen ovat tunnettuja tekijöitä aneurysmien synnyn taustalla.

Ateroskleroosi on hyvin tunnettu ja yleinen sydän- ja verisuonisairauksien aiheuttaja. Suonen seinämän inflammaatio, lipidien kertyminen ja endoteelin toimintahäiriö ovat ateroskleroosin patofysiologian tunnusmerkkejä. Ateroskleroottiset sydän- ja verisuonisairaudet ovat laajalti tunnistettu terveysongelma joka liittyy useisiin riskitekijöihin ja liitännäissairauksiin. Aivovaltimon aneurysmaa sairastavat potilaat ovat potilasryhmä jolla on melko vastikään todettu olevan verrokkiväestöä korkeampi riski sydän- ja verisuonisairauksiin.

Väitöskirjan ensimmäinen osatyö (I) tutkii aortan kalkkisuuden yhteyttä aivovaltimoaneurysmiin. Toinen osatyö (II) pyrkii osoittamaan aortan kalkkisuusasteen käyttökelpoisuuden riskinarviotyökaluna. Kolmas osatyö (III) selvittää nilkka-olkavarsipainesuhteen yhteyttä aivovaltimoaneurysmiin. Neljäs osatyö (IV) esittelee aortan kalsifikaation yhteydessä esiintyviä tulehdusvälittäjäaineita.

Osatyö I näyttää, että IA potilailla on verrokkeja kalkkisempi aortta. Osatyö II osoittaa, että aortan kalsifikaatioindeksi soveltuu riskinarviotyökaluksi. Osatyö III osoittaa, että IA potilailla on matalampi nilkka-olkavarsipainesuhde kuin verrokkiväestöllä. Osatyö IV esittelee aortan kalkkisuuteen liittyvää inflammatorista profiilia.

AVAINSANAT: aivovaltimoaneurysma, ateroskleroosi, aortan kalsifikaatioindeksi, nilkka-olkavarsipaineindeksi

Table of Contents

Abbreviations

AAA = Abdominal aortic aneurysm
ABI = Ankle-brachial Index
ACA = Anterior cerebral artery
ACI = Aortic calcification index
aSAH = Aneurysmal subarachnoid haemorrhage
AUC = Area under curve
ApoB = Apolipoprotein B
CABG = Coronary artery bypass grafting surgery
CAD = Coronary artery disease
CART = Classification and regression tree analysis
CI = Confidence interval
CLTI = Critical limb-threatening ischemia
CTA = Computed Tomography Angiography
CTACK = Cutaneous T cell-attracting chemokine
CVD = Cardiovascular disease
DSA = Digital subtraction sngiography
EC = Endothelial cell
ECM = Extracellular matrix
FDA = U.S. Food and Drug Administration
GWAS = Genome-wide association study
HDL = High-density lipoprotein
HU = Hounsfield unit
HR = Hazard ratio
IA = Intracranial aneurysm
IC = Intermittent claudication
ICA = Internal carotid Artery
ICC = Interclass correlation
IL-x = Interleukin x where x is a corresponding molecule
IMT = Intima-media thickness
INFg = Interferon gamma
IQR = Interquartile range

LDL = Low-density lipoprotein
MACE = Major adverse cardiovascular event
MALE = Major adverse leg event
MC = Mast cell
MCA = Middle cerebral artery
MHC = Major histocompability complex
MCP-1 = Monocyte chemoattractant protein 1
MIG = Monokine induced by interferon gamma
MIP = Macrophage inflammatory protein
MMP x = Matrix metalloproteinase x where x is corresponding molecule
MRA = Magnetic resonance angiography
MRI = Magnetic resonance imaging
M1 = Type 1 macrophage
M2 = Type 2 macrophage
NOS = Nitric oxide synthase
OR = Odds ratio
oxLDL = oxidized LDL
PACS = Picture archiving and communication system
PAD = Peripheral artery disease
PCA = Posterior cerebral artery
PCR = Polymerase chain reaction
PCSK9 = Proprotein convertase subtilisin/kexin type 9
PURE ASO = name of PAD patient's cohort in TUH
RIA = Ruptured intracranial aneurysm
ROC = Receiver operating characteristic (curve)
SD = Standard deviation
TBI = Toe-brachial Index
TGFb = Transforming growth factor beta
Th 1 = T-helper 1 cell
TNFa = Tumor necrosis factor alfa
TUH= Turku university hospital
UIA = Unruptured intracranial aneurysm
VCAM = Vascular cell adhesion molecule
VSMC = Vascular smooth muscle cell
WSS = Wall shear stress

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Rantasalo V, Gunn J, Kiviniemi T, Hirvonen J, Saarenpää I, Kivelev J, Rahi M, Lassila E, Rinne J and Laukka D. Intracranial aneurysm is predicted by abdominal aortic calcification index: A retrospective case-control study. *Atherosclerosis,* 2021; 334:30–8.

II Rantasalo V, Laukka D, Nikulainen V, Jalkanen J, Gunn J and Hakovirta HH. Aortic calcification index predicts mortality and cardiovascular events in operatively treated patients with peripheral artery disease: A prospective PURE ASO cohort follow-up study. *Journal of Vascular Surgery,* 2022; 76:1657-1666.

III Laukka D, Kangas E, Kuusela A, Hirvonen J, Rissanen T, Rahi M, Kivelev J, Rantasalo V, Venermo M, Rinne J and Hakovirta H. Low and Borderline Ankle–Brachial Index Is Associated With Intracranial Aneurysms: A Retrospective Cohort Study. *Neurosurgery*, 2024; 94;1282-1290.

IV Rantasalo V, Laukka D, Nikulainen V, Jalkanen J, Gunn J, Kiviniemi T and Hakovirta H. Association between aortic calcification index and cytokine levels in patients with peripheral artery disease (Manuscript).

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1 Introduction

The pathophysiology of vascular diseases has been under vigorous investigation during last decades. After atherosclerosis was established as the common denominator of multiple vascular diseases, its cellular inflammatory mechanism was revealed and current research now focuses on the triggers of artery wall inflammation which drives atherosclerosis (Wolf and Ley 2019). Anti-inflammatory agents have also taken a huge leap forward and anti-inflammatory therapies have been investigated in the search for a cure for the disease. The scope of this dissertation is the idiopathic aneurysms in the intracranial arteries and does not cover aneurysms related to known, specific genetic syndromes such as the Ehlers-Danlos, Marfan, Loeys-Dietz syndromes.

Research on intracranial aneurysms (IA) has been more difficult, as aneurysms are more rare, often asymptomatic and biologically less accessible for researchers. Their formation seems to be more ambiguous and complex than atherosclerosis. Still, recent advances in vascular science suggest that the pathophysiology of the formation of atherosclerosis and IA have common risk factors and characteristics (Frösen et al. 2019; Libby et al. 2019).

It has been postulated that IA and atherosclerotic diseases can co-exist, and clinical endpoints of both have been found in conjunction. For example, aSAH patients have been reported to have greater mortality and morbidity due atherosclerotic cardiovascular diseases (CVD) (Huhtakangas et al. 2015). CVDs are more common among patients with IA, and vice versa (Cho et al. 2019). Studies of aneurysms' pathophysiology and cellular inflammatory mechanisms have shown that they have some similar inflammatory mechanisms to those of atherosclerosis (Frösen et al. 2019; Libby et al. 2019). However, evidence of joint disease progression is lacking in terms of their co-existence and cellular mechanisms. Currently no biomarkers or specific clinical tools exist for detecting aneurysms in populations presenting with atherosclerotic manifestations, and aneurysm patients' risk of other CVDs has not been evaluated consistently. Screening recommendations for IAs are currently based on family history, and not on risk factors or comorbidities (Etminan et al. 2022). Therefore, this dissertation focuses on the common characteristics of IA and atherosclerosis by showing how atherosclerotic disease burden is associated with aneurysms in the intracranial arteries.

2 Review of the Literature

2.1 Prevalence and incidence of intracranial aneurysms

2.1.1 Unruptured intracranial aneurysms

IAs are pathological dilatations of the intracranial arteries. They are most commonly seen in the circle of Willis, where four main arteries entering the cranium anastomose together and form the base for intracranial circulation. Arterial bifurcations are common sites for IAs. IAs are usually asymptomatic until they rupture – after which they pose immediate mortal danger in the form of subarachnoid haemorrhage (aSAH). Most aneurysms do not rupture during a person's lifetime. Rupture risk evaluation warrants further research, as current risk evaluation tools are imperfect. Recent data suggest that as few as 0.3% of IAs rupture during a lifetime (Hackenberg, Hänggi, and Etminan 2018). As well as the risk of rupture, large UIAs might cause symptoms due to their size and their effect on adjacent structures (Hackenberg, Hänggi, and Etminan 2018; de Oliveira et al. 2009).

IAs are present in about 3% of the middle-age and past middle age population, and the risk increases with age, as IAs most often develop gradually throughout a person's lifetime as discussed in detail in the following chapters (Vlak et al. 2011). Family history is also a significant factor related to IA risk. Furthermore, the rate of incidentally detected aneurysms in elderly patients has risen in recent years, probably due to the increased application of cranial imaging. IAs are categorized by their size, location and number – a little over 30% of patients have more than one IA (multiple IA). A recent study on multiple IAs concluded that multiple IAs are also associated with classic IA risk factors. In addition, IA location affects the risk of rupture (Dinger et al. 2022; Etminan et al. 2022; Rousseau et al. 2021; Laukka et al. 2024).

2.1.2 Ruptured intracranial aneurysms

IA rupture causes aSAH, which is a medical emergency with potentially devastating outcomes. Relatively high mortality rates and considerable morbidity even after technically successful invasive endovascular or surgical treatment is a major issue in

this population of patients. The incidence of such an event is about 10/100,000, with geographical and demographical variance (Etminan, Dörfler, and Steinmetz 2020; Rinkel and Algra 2011; Etminan et al. 2019). As only a few of all IAs rupture, identifying rupture-prone aneurysms is paramount. However, invasive treatment of unruptured aneurysms carries a considerable risk of severe adverse effects (Hackenberg, Hänggi, and Etminan 2018; Ihn et al. 2018; Naggara et al. 2012; Kotowski et al. 2013).

Only one third of patients with an aSAH return to work and 40% die within one year of the incident. After the rupture, blood in subarachnoidal space together with multiple other factors, causes vasospasm and delayed cerebral ischemia. This leads to a difficult medical emergency that requires complex, urgent, intensive, and usually multiple medical and surgical interventions. Adverse effects caused by the invasive treatment of IAs might also cause devastating long-term effects. (Dodd et al. 2021).

2.2 Pathophysiology of intracranial aneurysms

2.2.1 Modifiable and non-modifiable risk factors

Age and sex are non-modifiable risk factors for IA presence. Women more often develop IAs, and age increases the risk for developing IA. Other non-modifiable risk factors are family history, polycystic kidney disease and genetic syndromes that are linked with IAs.

The differences between IA prevalence among women and men is not prevalent in every data presented on the subject, but meta-analysis from 2011 concluded that female sex is associated with a higher risk of IA, even after adjustment for age and comorbidities.

Geographical region might also affect IA prevalence. Previous studies have suggested that aSAH incidence is greater in Finland and Japan than in other regions, whereas UIA prevalence seems to be similar globally. Most recent studies have reported lower aSAH incidence in Finland than reported earlier, comparable to globally reported incidence numbers. (Jalava et al. 2017). While geographical region of origin of the patient is usually considered as a non-modifiable risk factor, it is not known how the risk of IA changes with migration. Overall risk of CVD is associated with geographical region, but the risk of CVD is altered with migration presumably due to the change in environmental factors (Bhatnagar 2017).

Modifiable risk factors are smoking, hypertension and alcohol consumption. Smoking is strongly associated with the development of IAs, and combined with hypertension, the risk might be even greater. The dangerous potential of smoking is highlighted by the results of a study published by Can et al. in 2017. The study found that smoking increases the risk of IA rupture, and that even though the risk decreased

after cessation, it does not reach the level of non-smokers (Vlak et al. 2013a; Can et al. 2017). The effect of hypertension and smoking on IA rupture is presented in a meta-analysis by Etminan et al. (2019). The study shows the decreasing trend in RIA incidence between 1980 and 2014 when global incidence of aSAH declined from 10.2/100,000 person years to 6.1, in conjunction with overall decreasing blood pressure and reduced prevalence of smoking (Etminan et al. 2019).

Hypertension is a classic risk factor for general CVD but also a risk factor for IAs. Epidemiologically not all CVD types over-represented in IA patients. For example, the prevalence of coronary artery disease (CAD) has been reported to be lower among IA patients, especially in ruptured intracranial aneurysm (RIA) patients (Kang et al. 2015). Hypercholesterolemia is a risk factor for CVD, and alongside with hypertension and smoking it could also be a credible risk factor for IA, but hypercholesterolemia has been associated with a reduced risk of IA (Vlak et al. 2013a; Kang et al. 2015).

Some preliminary results suggest that gut microbiota might affect IA formation. For example, mice with triggered IA formation experienced a substantial reduction in IAs when their gut microbiota was deleted with antibiotics. In human, gut microbiota is different among patients with IAs compared to those without IAs, and gut microbiota seems to be different among patients with RIAs than with UIAs. These results suggest that different gut microbiota affects the pathophysiology of IA formation (Kawabata et al. 2022; Shikata et al. 2019; Ma et al. 2023). Gut microbiota has been under vigorous investigation in recent years in relation various diseases and health issues including CVD (Witkowski, Weeks, and Hazen 2020).

Furthermore, dental bacteria have been detected in IA tissue samples, suggesting that oral infections, which are common, might be associated with IA (M. J. Pyysalo et al. 2013). Gingival bleeding and severe periodontitis are associated with RIA and UIA, and parodontitis is more common among IA patients than general population (M. J. Pyysalo et al. 2018; Hallikainen, Lindgren, et al. 2020).

In addition to the above, alcohol consumption is a risk factor as heavy alcohol consumption is strongly associated with IA rupture and IA-related mortality (Juvela and Lehto 2015). Hypertension and smoking are also risk factors for IA rupture (Karhunen et al. 2021).

Finally, physical activity is known to reduce the risk of cardiovascular diseases. It has been feared that strenuous physical exercise might increase the risk of aSAH, but moderate physical activity is associated with a reduced risk of IAs (Lindbohm et al. 2019).

2.2.2 Genetic factors affecting the formation of intracranial aneurysms

IA development is multifactorial, and the non-syndromic IAs considered in this dissertation have complex genetic backgrounds. Genetic background is a notable underlying risk factor for IA, even though no novel monogenic or single-mutation genetic faults causing IAs have been demonstrated besides syndromes listed in Table 1. It has been estimated that up to 40% of IAs are hereditary to some degree, and screening is recommended for individuals with two or more first degree relatives with RIA or UIA for their pronounced IA risk. The risk of an IA increases in accordance with the number of affected family members. Nevertheless, genetic factors are not a guarantee of heritability (Bakker and Ruigrok 2021; Thompson et al. 2015).

Table 1. Monogenic disorders and genetics related to Ias. Reproduced with the permission of Wolters Kluwer Health, Inc. from (Bakker and Ruigrok 2021).

DISEASE	GENES IMPLICATED	EVIDENCE OF IA PREDISPOSITION
AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE	PKD1, PKD2	10% of patients have UIA
TYPE IV EHLERS-DANLOS SYNDROME (VASCULAR SUBTYPE)	COL3A1	In 8 of 59 (14%) patients screened UIA were found. Patients compared with controls (both groups n=13883) more likely to have aSAH and hemorrhagic stroke (0.3% vs 0.2%) and UIA (0.2% vs 0.1%)
MARFAN SYNDROME	FBN1	In 12 of 99 (12%) patients screened UIA were found. Patients more often admitted because of an IA than controls (n=9000, 0.4% vs 0.09%, P<0.01).
LOEYS DIETZ SYNDROME	TGFBR1, TGFBR2, TGFB2, TGFB3, SMAD2, SMAD3	In 7 of 25 (28%) patients screened UIA were found.16 Cerebral hemorrhage (aSAH and intracerebral hemorrhage) in 2 of 90 (7%) patients.
MICROCEPHALIC/MAJEWSKI'S OSTEODYSPLASTIC PRIMORDIAL DWARFISM, TYPE II	PCNT	UIA in up to 50% of patients

Monogenic disorders (Table 1) cause only a small proportion of the IAs, and other mutations and genotypes associated with the risk of IA are being established.

Genome-wide association studies (GWAS) have revealed multiple gene loci that are associated with IA risk, although these genetic loci are not causative by themselves. Rather, they complement the overall risk profile associated with IAs. Some of the recently reported 17 IA-associated genetic loci overlap with the genetic factors associated with clinical risk factors of smoking and hypertension. Although these genetic loci are not considered causative agents, they serve as additional risk factors. Table 2 and Figure 1 (Bakker et al. 2020; Bakker and Ruigrok 2021).

Autosomal polycystic kidney disease, a genetic disorder caused by mutations in genes encoding proteins polycystin 1 and 2, could also be regarded as non-modifiable genetic risk factor. Up to 10% of these patients present with IA (Vlak et al. 2011).

Table 2. Common genetic variants associated with Ias. Modified from (Bakker and Ruigrok 2021).

COMMON GENETIC VARIANTS	2Q33.1, 3P14.2, 4Q31.22, 5Q31.1, 6Q16.1, 7P21.1, 8Q11.23, 9P21.3, 10Q23.33, 10Q24.33, 11P15.5, 12P12.2, 12Q21.33, 12Q22, 13Q13.1, 15Q25.1, 16Q23.1, 18Q11.2, 20P11.23, 22Q12.2
LOW-FREQUENCY GENETIC VARIANT LOCI	2q23.3, 5q31.3, 6q24.2.
RARE GENETIC VARIANTS, GENETIC LOCI	1p34.3-36.13, 2p13, 11q24-25, 12p12.3, 13q14.12-21.1, 14q22-31, 17cen, 19q13.11-13.3, Xp22.
MENDELIAN RISK GENES	LOXL2 (chr8), NFX1 (chr9), ARHGEF17 (chr11), ADAMTS15 (chr11), THSD1 (chr13), RNF213 (chr17), ANGPTL6 (chr19), PCNT (chr21)

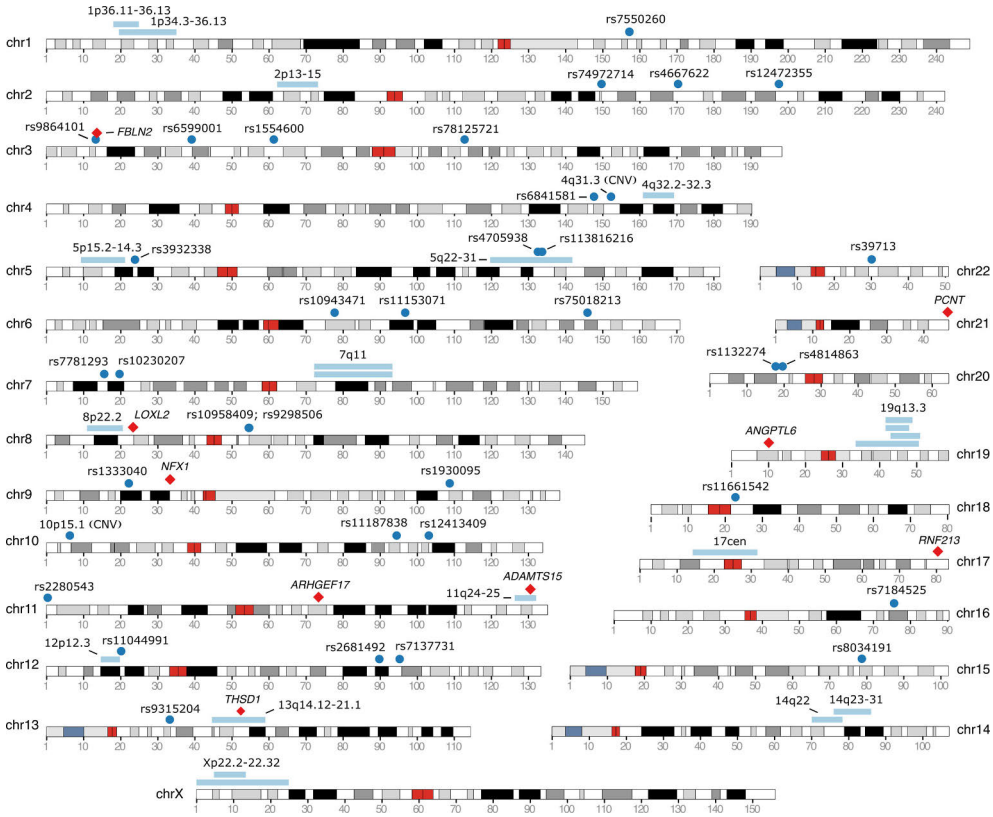

Figure 1. Graphical overview of all loci associated with increased risk of IAs (Bakker and Ruigrok 2021).

2.2.3 Inflammation in intracranial aneurysms

Abnormal hemodynamic stress is an important factor in the initiation of IA formation. Blood flow applies a force called wall shear stress (WSS) to the artery wall. In the bifurcations and other curvatures of the vasculature, turbulent blood flow alters WSS, and IA can form in these sites as shown in Figure 2 (Frösen et al. 2019; Cebral et al. 2017; Sheinberg et al. 2019).

IA pathophysiology is characterised by endothelial dysfunction, disruption of the elastic lamina, extracellular matrix (ECM) remodelling, prominent inflammation, and changes in the function and amount of vascular wall smooth muscle cells (VSMCs). The insult of the endothelial cell (EC) layer is believed to be one of the initiators of IA formation. EC junctions are disrupted, their ability to keep vascular tension via nitric oxide synthase (NOS) is impaired, and the pro-inflammatory transcription factor nuclear factor kappa-beta (NF-kB) is activated. The endothelium begins to express chemotactic cytokines vascular cell adhesion molecule (VCAM) and monocyte chemoattractant protein 1 (MCP-1) thus allowing inflammatory cells

to influx into the vessels medium layer (Tulamo et al., 2018; Cebral et al., 2017; Sheinberg et al., 2019).

Mast cells (MC) are also involved in IA pathophysiology. Presence of MCs in IA wall is associated with erosion of ECs and neovessel formation. Especially EC loss (endothelial erosion or "de-endothelization" has been associated with IA rupture (Ollikainen et al. 2014; Frösen et al. 2004).

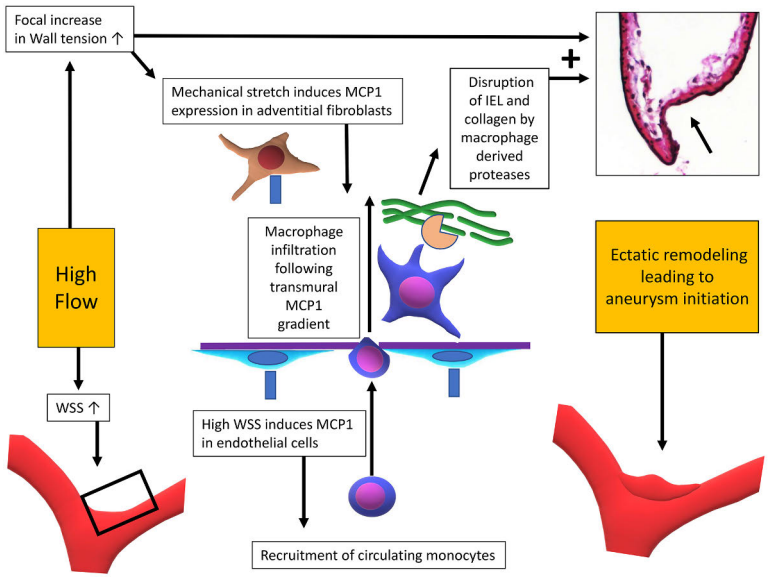

Figure 2. Wall shear stress (WSS) and IA formation. Reproduced with permission of publisher Journal of Neurosurgery from (Frösen et al. 2019).

The upregulated cytokines and inflammatory cells involved in the inflammatory process in IA formation include tumor necrosis factor alfa (TNFa) (secreted by macrophages, neutrophils, lymphocytes and potentially by VSMCs), MCP-1 (macrophages, VSMCs), macrophage inflammatory protein 1 (MIP-1) (neutrophils), interleukin 1b (IL-1b) (neutrophils, VSMCs), interferon gamma (IFNg) (lymphocytes) and IL-6 (lymphocytes). The inflammation induced by the cytokines and hemodynamic circumstances results in the degradation of ECM and cell death (apoptosis) as well as a phenotype switch in VSMCs that can no longer maintain vascular tension as their contractile function weakens and synthetizing and inflammatory functions increase. At the molecular level, collagen is re-arranged and re-synthesized, but it is altered and weaker than it originally was. ECM degradation is primarily achieved by Matrix metalloproteinases (MMP) 2 and 9, which are secreted by neutrophils, macrophages and VSMCs. Cellular and molecular changes manifest in aneurysm formation and growth; the vessel wall weakens and bulges,

and can eventually rupture. These mechanisms are shown in Figures 3 and 4 (Frösen et al. 2019; Caird et al. 2006; Hosaka and Hoh 2014; Monsour et al. 2022; Starke, Chalouhi, et al. 2014).

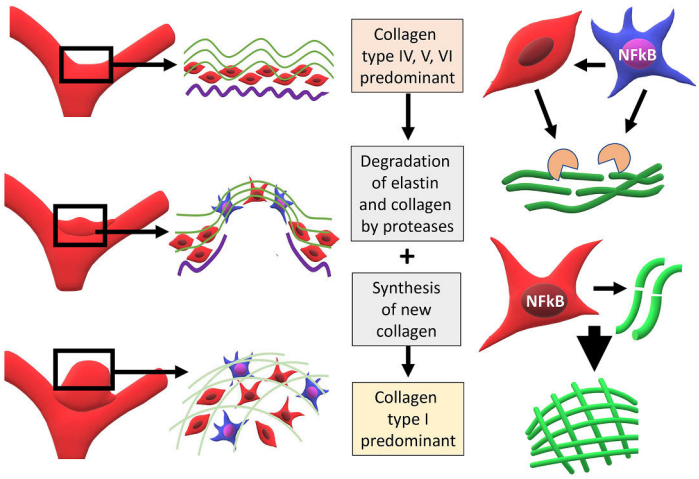

Figure 3. Intracranial aneurysms formation and growth and vessel wall remodeling. Reproduced with permission of publisher Journal of Neurosurgery from (Frösen et al. 2019). NFkB = nuclear factor kappa B.

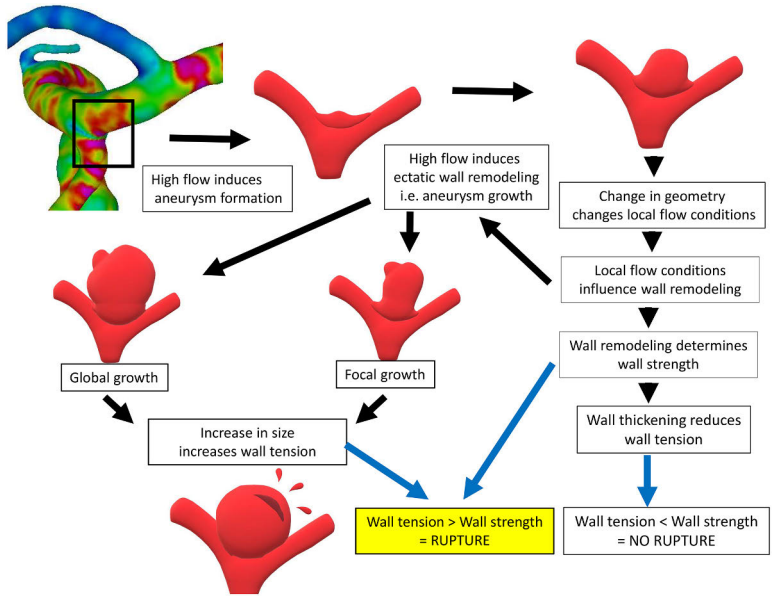

Figure 4. Development of IAs in bifurcations due alterations in blood flow. Reproduced with permission of publisher Journal of Neurosurgery from (Frösen et al. 2019).

VSMCs are the main cellular component maintaining artery wall integrity. The inflammatory cascade also affects them. In IAs, the VSMCs undergo a phenotype switch, in which they lose their contractile functions and they become synthetising and acquire macrophage-like pro-inflammatory characteristics. Apoptosis and the proliferation of VSMCs promotes this shift. The secretory function of VSMCs changes from sustainable to destructive, as they secrete ECM-degrading MMPs and pro-inflammatory cytokines in conjunction with macrophages (Frösen et al. 2019; Signorelli et al. 2018; Shao et al. 2017; Etminan and Rinkel 2016; Tulamo et al. 2018; Z. Wang et al. 2023).

Polarization of macrophages towards either pro-inflammatory M1 type or anti-inflammatory M2 type has been discussed in the context of IAs, and it may play a substantial role in the inflammatory cascade. Macrophages secrete pro-inflammatory cytokines, promote the apoptosis of the VSMCs and the phenotype switch, and promote cells of adaptive immunity. The neutrophils present in IAs also secrete cytokines and promote apoptosis. The lymphocytes phenotype also seems to be pro-inflammatory with Th1 cells predominance. Infiltration of inflammatory cells in vessel wall is shown in Figure 5 (Frösen et al. 2019; Signorelli et al. 2018; Shao et al. 2017; Etminan and Rinkel 2016; Tulamo et al. 2018)

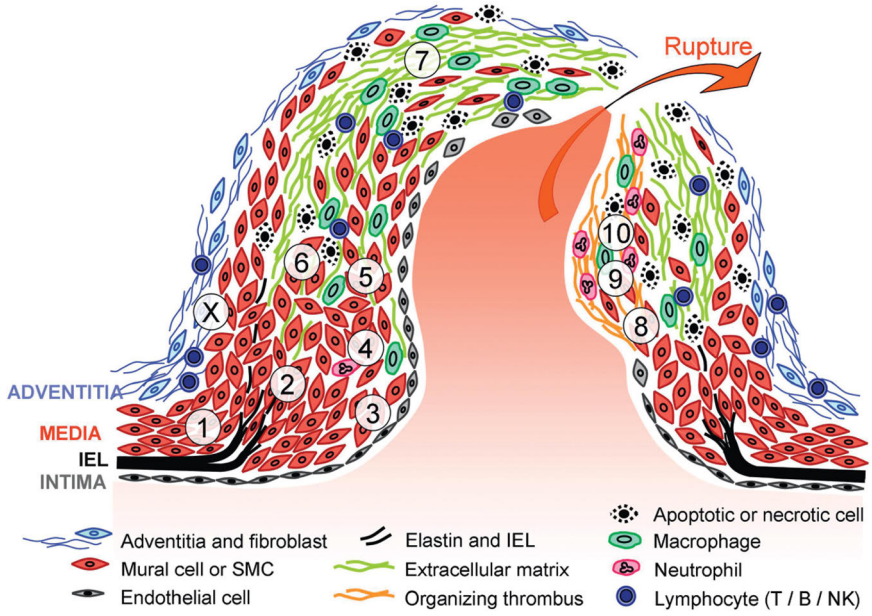

Figure 5. IA vessel wall degeneration and inflammatory cells. Reproduced with the permission of BMJ Publishing Group Ltd from (Tulamo et al. 2018). Numbers represent the hypothetical order of cellular and molecular level changes that take place in artery wall during aneurysm formation. Note the disruption of endothelial cell layer.

2.2.4 Atherosclerotic calcification in intracranial aneurysms

IA formation is driven by inflammation, and wall of IAs are weakened by structural remodeling. In addition to this, calcification, which often occurs in IAs, seems to affect the structural integrity of the artery wall (Gade et al. 2019; Rahmani et al. 2022). Different patterns of calcification have been attributed to distinct clinical outcomes such as rupture. A recent study explored calcification on a microscopic level and concluded that a most calcification associated with IA rupture seems to be of non-atherosclerotic origin (Gade et al. 2019; Rahmani et al. 2022).

The potential triggers of calcification in IAs are atherosclerosis and the non-atherosclerotic calcification of the medial layer of the artery (aneurysm), the cause of which is still poorly understood. In both instances, it is the VSMC phenotype switch in response to the inflammatory setting that seems to play a role in IA calcification. In cases of atherosclerotic calcification in IAs, lipid accumulation has been found (Frösen et al. 2014; Rahmani et al. 2022; Gade et al. 2019).

Lipids are paramount in atherosclerosis, and can also be seen in atherosclerotic lesions in IAs. Pools of lipids have been histologically identified in IAs in the presence of calcification, suggesting that lipids might be associated with IA formation or presence. VSMC-derived foam cells loaded with lipids have also been identified in IAs, and lipoproteins associated with atherosclerosis have also been detected in IA immunohistochemistry (Gade et al. 2019; Libby et al. 2019; Ollikainen et al. 2016; Frösen et al. 2014; Tulamo et al. 2010).

2.3 Management of patients with intracranial aneurysms

Preventive invasive treatment of UIAs is recommended for when the risk of aneurysm rupture exceeds the risk of morbidity in the selected treatment modality. The invasive treatment options for UIAs are endovascular procedures or open surgery. Endovascular interventions include intra-aneurysmal devices and stenting, and open surgery most often refers to surgical clipping of the aneurysm (Steiner et al. 2013).

Conservative treatment of IAs means repeated imaging surveillance, optimal medical therapy directed at modifiable risk factors and reassessment of the patient's individual risk of UIA rupture. This includes weighing the procedural risk against the risk of overall mortality and morbidity for other causes. Similar methods are applied to patients who have multiple UIAs or who have survived RIA (Hoh et al. 2023).

The current European Stroke Organizations guidelines on UIA treatment recommend optimal medical therapy for established modifiable risk factors (hypertension, smoking, alcohol consumption), based on their impact on IAs.

However, the recommendation does not specifically address the concomitant cardiovascular risk, even though these risk factors overlap. The need for treatment for dyslipidaemia and antithrombotic medications are waived in the context of IAs (Etminan et al. 2022).

The need for follow-up imaging is also assessed in cases of UIA patients. The factors considered include aneurysm size, the lifetime risk of rupture and individual risks related to possible invasive treatments. The aim of follow-up imaging is to detect aneurysms that become very large or grow rapidly, as this indicates a risk of rupture, and thus to detect rupture-prone IAs that should undergo preventive occlusion (Etminan et al. 2022).

The frequency and duration of radiological monitoring in follow-up are generally advised on the basis of an individual aneurysm- and patient-related risk assessment. Recommended imaging modalities for follow-up are magnetic resonance angiography (MRA) or computed tomography angiography (CTA) (Etminan et al. 2022).

The next step in imaging surveillance might involve the visualisation of inflammation to further estimate rupture risk. Aneurysmal wall enhancement found in imaging seems to be associated with inflammatory histology and rupture risk, but no definitive thresholds or unified classification systems exist, as data on them are still too scarce (Samaniego, Roa, and Hasan 2019).

2.4 Atherosclerotic disease

2.4.1 Clinical significance of atherosclerosis

Atherosclerosis is a systemic inflammatory vascular disease characterised by lipid accumulation in the subendothelial layer of the artery wall, and ccompanied by dysregulated chronic inflammation. Inflammation and lipids build atherosclerotic plaques in artery walls, narrowing the lumen. This phenomenon becomes clinically evident when blood flow (and therefore, oxygen supply) to the tissues downstream of the plaque is compromised. This can happen either rapidly, when a plaque ruptures, leading to acute ischemia, or slowly, when plaques grow over time, resulting in chronic ischemia. Atherosclerosis can affect multiple systems, and the clinical sequelae depend on the degree of blood supply deficit and affected organ. Figure 6 (Libby et al. 2019).

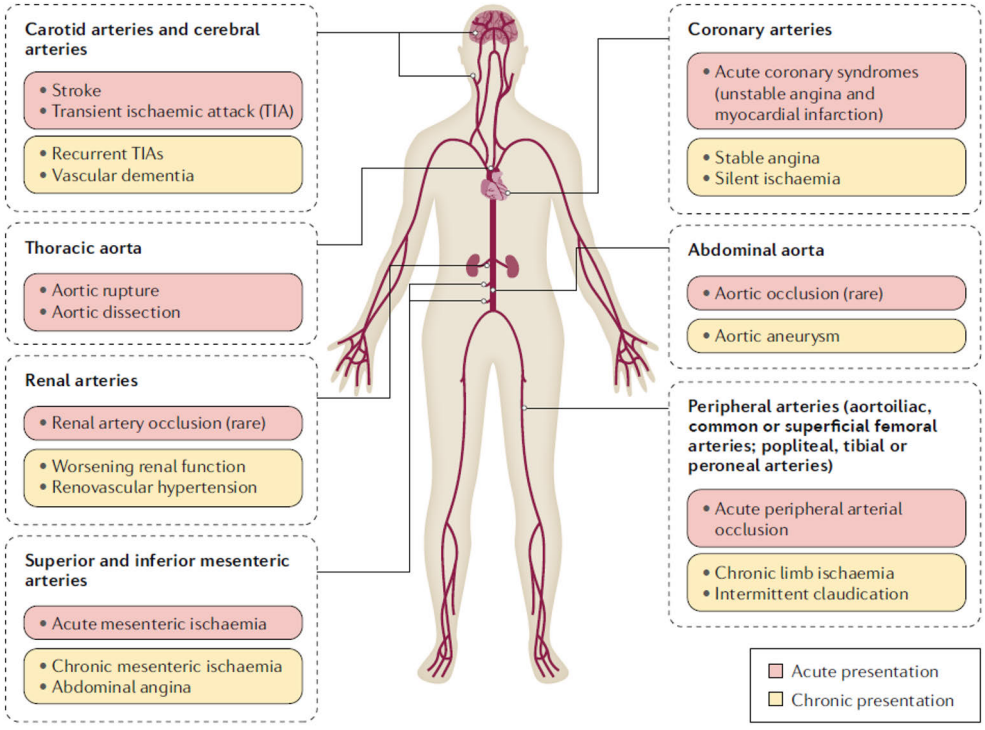

Figure 6. Sites of atherosclerosis in arterial treen and clinical sequelae of atherosclerosis. Reproduced with the permission from Springer Nature from (Libby et al. 2019).

The development of atherosclerosis is influenced by multiple risk factors and protective factors, including genetic predispositions, the presence or absence of

modifiable and non-modifiable risk factors, pharmacotherapies, and lifestyle interventions aiming for risk reduction. In addition to these factors, some novel risk factors for atherosclerosis are also emerging: increasing obesity, rising psychosocial stress and socioeconomic disadvantage in populations (Libby et al. 2019; Libby 2021).

Combined CVDs are the leading cause of death worldwide. The emergence of CVD as one of the leading causes of death globally undoubtedly demonstrates how CVD risk factors have become increasingly prevalent in recent decades, not only in the western world, but also in countries that have only just gained wealth and its consequence, sedentary lifestyle. (Libby 2021; Townsend et al. 2022; Vaduganathan et al. 2022).

In Europe, although the overall prevalence of CVD morbidity is up to 1000-1300/100,000, modern therapies have improved the situation. For example, the mortality after acute coronary artery disease event has been in decline in 2003 – 2018. This reduced mortality allows patients to survive, and their cardiovascular disease remain to be treated. Such a phenomenon might overestimate the prevalence of CVD and render a larger proportion of the population prone to subsequent complications of atherosclerotic disease (Townsend et al. 2022; Thrane et al. 2023).

The increase in CVD burden causes increased direct and undirect costs. It has been estimated that in Finland, 10% (2 billion euros) of healthcare as was due cardiovascular diseases in 2015. In the USA over 300 billion dollars are spent in healthcare services for cardiovascular diseases, and costs are rising. (Wilkins et al. 2017; Birger et al. 2021). CVDs are expensive worldwide, and especially people with low socioeconomic status are in pronounced risk for atherosclerotic CVD. This comparison highlights the abhorrent inequality that is present in cardiovascular morbidity. Overall, global atherosclerotic burden is visualized in Figure 7 (Henderson et al. 2022; Vaduganathan et al. 2022).

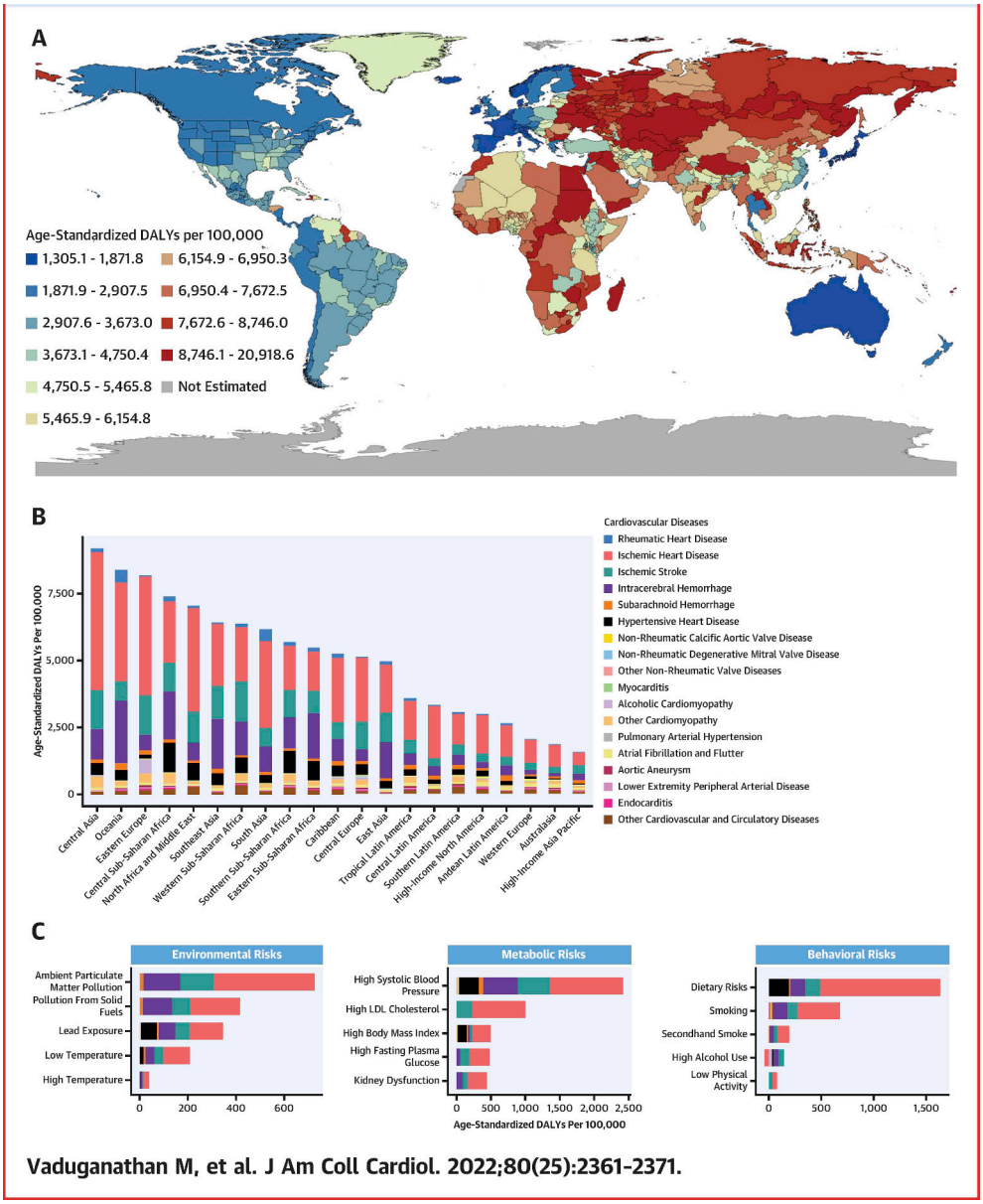

Figure 7. Global burden of CVDs and risks. Modified and Reprinted with permsission of American College of Cardiology from (Vaduganathan et al. 2022). DALY = Disease-associated Life Year.

The clinical diseases caused by atherosclerosis (Figure 6) are CAD (heart attack, myocardial infarct, congestive heart failure), extra- and intracranial artery atherosclerosis (stroke, dementia, cognitive decrement), visceral artery atherosclerosis (impaired renal function, mesenteric ischemia) and peripheral artery

disease (PAD) (claudication, chronic limb-threatening ischemia, amputation, Figure 8). Each disease is related to factors presented in this chapter, and these factors might have different proportional influence in the atherosclerosis of different vascular beds. This dissertation discusses atherosclerosis in general, and does not refer to any particular vascular bed disease, even though minor distinctions may exist at the molecular and histological level of atherosclerosis in different vascular beds (Teixeira et al. 2022).

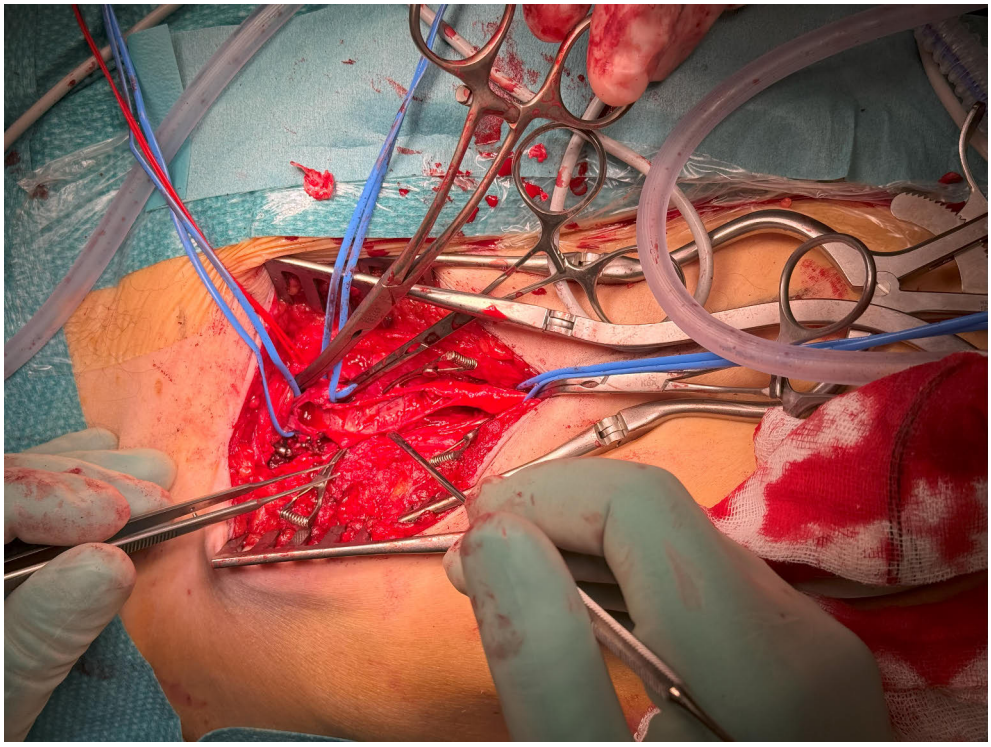

Figure 8. Femoral endarterectomy. Ville Rantasalo and Kimmo Korhonen perform an arteriotomy of the femoral artery during femoral endarterectomy performed due to severe atherosclerosis. (Copyright Ville Rantasalo)

2.4.2 Modifiable and non-modifiable risk factors

The prevalence of atherosclerosis and the incidence of atherosclerotic CVD are affected by a variety of epidemiological risk factors, which can be classified as comorbid diseases and behavioural factors. The established modifiable risk factors for atherosclerosis include high blood cholesterol levels, hypertension, smoking, diabetes mellitus, chronic kidney disease and poor dental health. (Lockhart et al. 2012). As discussed in following chapters, these overlap with IA risk factors. For

example, poor dental health is associated with IAs also (Hallikainen, Lindgren, et al. 2020).

It is well known that smoking causes atherosclerosis, because it damages the endothelium and endothelium-derived nitric oxide cascade, subsequently promoting inflammation (Higashi 2023b). Smoking promotes atherosclerosis in a dose-dependent manner and cessation is beneficial, in the long run as it takes years to decades for smoking-caused risk to diminish (Ding et al. 2019).

The risk of hypertension for atherosclerosis has been known for decades. The mechanisms of its effect on atheroma development are not completely understood, but its effects are presumably mediated by the inflammatory cascade and the way in which this affects the cells that accumulate and act when atherosclerosis develops (Björkegren and Lusis 2022). Lowering blood pressure is an effective way to reduce the risk of CVD that is attributable to high blood pressure (Shin et al. 2013; Law, Morris, and Wald 2009; Alexander 1995).

As lipid accumulation in the vessel wall is a key component of the disease process, it is plausible that high levels of serum lipids are causative in nature and associated with atherosclerosis development. Higher levels are associated with an increased risk for cardiovascular events. Reducing lipid levels also lowers the risk (Ference et al. 2017; Di Giovanni et al. 2023; Baigent et al. 2010).

Age, sex and genetic factors are non-modifiable risk factors for atherosclerosis. Males are at a pronounced risk of atherosclerotic diseases in the age group corresponding to pre-menopausal women, but a few years after menopause, females' risk of CVD becomes similar to that of men of the same age. The incidence of CVD grows with age. This might be because of certain mechanisms of atherosclerosis develop during aging. In detail, macrophages and inflammatory signalling related to them changes with age, and senescent ECs, VSMCs and macrophage's function differ from original. Clonal changes in haematopoiesis also occurs with age and associates to atherosclerosis (Björkegren and Lusis 2022).

Genetic factors can be either risk factors or protective factors in disease and in health, and atherosclerosis is no exception. A family history of CVDs is associated with a pronounced risk of CVD, when related demographic factors are taken into account, and the risk is most likely mediated by polygenic factors in the absence of known genetic syndromes. There is a vast collection of genes associated with CVD. However, usually each gene has only small contribution (Aragam and Natarajan 2020; Björkegren and Lusis 2022).

It has been established that alcohol consumption has a dose-dependent effect on atherosclerosis, with the exception of the lower end of the spectrum of alcohol exposure: some studies suggest that totally abstinent people might be at a slightly higher risk of atherosclerosis than those with moderate consumption (Riccardi et al. 2022).

The association between poor dental health and atherosclerotic CVD is supported by multiple studies. Epidemiological evidence shows an increased risk of cardiovascular events. Currently it is believed that periodontal disease enhances or even triggers a systemic pro-inflammatory state, which is required for atherosclerosis to develop. The next chapter elaborates on this. In a Danish study, Hansen et al. showed that patients with periodontal disease suffered from an increased burden of atherosclerotic CVD. They also found that cardiovascular risk factors were more common among patients with periodontal disease, and the ability of periodontal diseases to increase the risk of CVD was significant even after adjustment for risk factors. One of these risk factors was low socioeconomic status, which is known to be associated with CVD (Carrizales-Sepúlveda et al. 2018; Hansen et al. 2016; Henderson et al. 2022).

Diabetes is an undeniable risk factor for atherosclerosis. The mechanisms are likely to be numerous, but endothelial dysfunction and accelerated vascular calcification are the most robust ones associated with atherosclerotic disease. Prompt treatment of diabetic hyperglycaemia and of classic risk factors reduces the risk of vascular complications in diabetic individuals (La Sala, Prattichizzo, and Ceriello 2019; Yahagi et al. 2017; Kelsey et al. 2022). Chronic kidney disease, especially in its advanced stage, poses a risk of atherosclerosis. This risk is accelerated when patients receive haemodialysis treatment (Valdivielso et al. 2019).

Among the most novel research interests in CVD epidemiology is gut microbiota, which has been linked to the development of atherosclerosis (Lindskog Jonsson and Bäckhed 2017).

2.4.3 Pathophysiology

2.4.3.1 Lipids

The first step in the pathophysiology of atherosclerosis is the accumulation of lipid particles in the artery wall. Translocation of lipids through the endothelium seems to be associated with endothelial dysfunction, which is present in atherosclerosis and in IAs. Apolipoprotein B (ApoB) carries cholesterol and triglycerides in the blood and in atherosclerosis it gets trapped in the artery wall. Released from ApoB, cholesterol undergoes phagocytosis by macrophages and macrophage-like cells, as shown in Figure 9 (Ference, Kastelein, and Catapano 2020; Higashi 2023a; Sheinberg et al. 2019). Extensive evidence shows that lipids are a part of disease mechanisms in atherosclerosis, and that lowering serum lipid levels is an effective way to slow down the atherosclerotic process, as well as decreasing the rate of endpoints that would occur in affected vascular bed (Mach et al. 2020).

ApoB-based lipoproteins are the most potent causative factors of atherosclerosis. The great majority of studies and the literature discuss low-density lipoprotein cholesterol, (LDL or LDL-C), which is the most important circulating lipid, as it is the most abundant lipoprotein particle that contains ApoB (Libby et al. 2019).

Recently it has been proposed that that in addition to LDL cholesterol, the measurement of ApoB lipoprotein as the core component of metabolite could enhance accuracy in certain scenarios. Still, LDL is the mainstay of lipid-related atherosclerotic risk, and its causal and cumulative relation to atherosclerotic CVDs has been firmly proven (Mach et al. 2020; Halasz and Piepoli 2021; Quispe et al. 2021; Ference, Kastelein, and Catapano 2020).

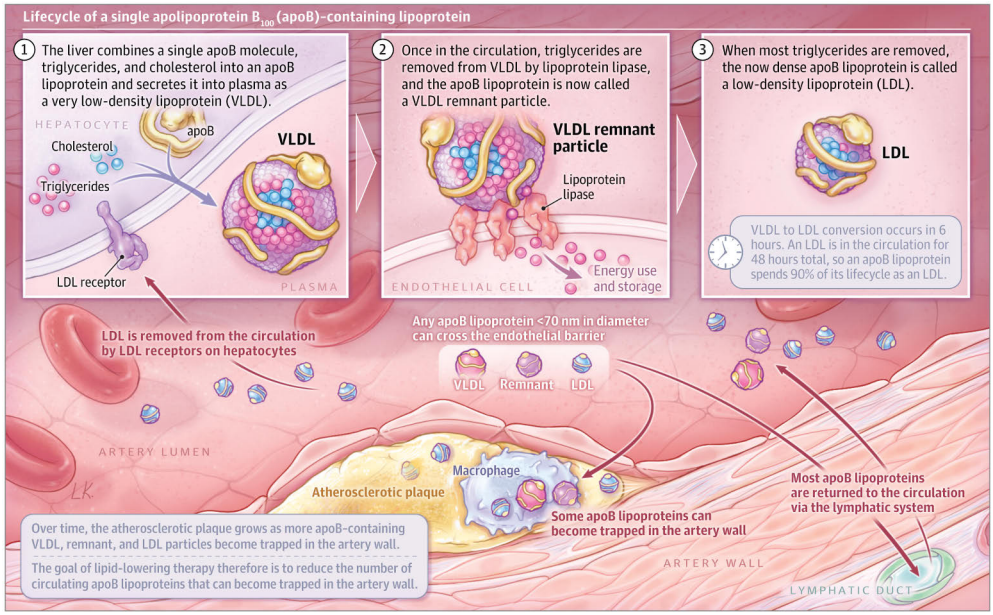

Figure 9. Apolipoprotein B100 molecule and LDL in the development of atherosclerosis. Reproduced with the permission of American Medical Association from (Ference, Kastelein, and Catapano 2020).

2.4.3.2 Inflammation

Inflammation is the key driving mechanism of atherosclerosis. The cells responsible of atherosclerotic inflammation disease are arteries ECs, VSMCs and cells of the immune system, monocytes and macrophages, neutrophils and lymphocytes and MCs. Macrophages play a special role in atherosclerosis. They devour cholesterol-carrying LDL by phagocytosis and become foam cells and form the core of the atherosclerotic plaque.

In atherosclerosis, VSMCs switch phenotype: they become pro-inflammatory, macrophage-like cells that actively promote inflammation and they lose their contractile function. As macrophage-like, they may ingest lipids and become foam cells. The interplay between inflammatory cells and cytokines is maladaptive. Furthermore, genetic factors can alter inflammatory responses. ECs dysfunction causes them to fail in maintaining vascular wall homeostasis, allowing inflammatory events. Finally, MCs are also present in atherosclerotic plaques, and possibly MCs participate in multiple phases of the atherogenesis. They reside adjacent to ECs and interact with them and secrete inflammatory mediators affecting multiple cell types. Mechanism is illustrated in Figure 10 (Wolf and Ley 2019; Björkegren and Lusis 2022; Bennett, Sinha, and Owens 2016; Kovanen and Bot 2017).

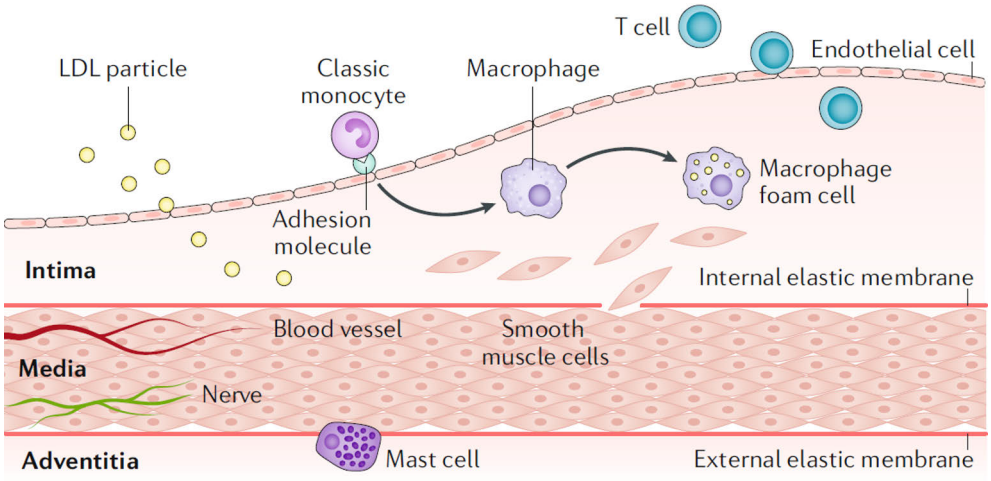

Figure 10. Initiation and progression of atherosclerosis. Reproduced with the permission of Springer Nature from (Libby et al. 2019)

The initial step of atherosclerosis is the accumulation of oxidised LDL in the subendothelial space of the artery wall. This happens when there is a functional defect in endothelium. The accumulation of lipids attracts monocytes and macrophages that take up lipids (LDL cholesterol, oxLDL). Monocytes and macrophages use scavenger receptors to take up these lipids. In atherosclerosis macrophages (foam cells) are activated by oxLDL. Oxidized LDL accumulates in these cells up to the point at which lipids form droplets in the macrophages cytoplasm. When this happens, macrophage becomes a foam cell. Foam cells are the core and bulk of the atherosclerotic plaque. Lipid-rich foam cells activate pro-

inflammatory responses and act as a progenitor of atherosclerotic inflammation. (Gisterå and Hansson 2017; Wolf and Ley 2019).

Macrophages are generally categorised as type M1 and M2. M1 are inflammatory and in atherosclerosis they are atherogenic promoting atherosclerotic plaque development and prominently present. M2 are anti-inflammatory and in this context, they are atheroprotective, trying to prevent atherosclerotic plaque formation. M1 are present more often than M2 and they secrete pro-inflammatory cytokines. The atheroprotective role of M2 is mediated by their ability to secrete anti-inflammatory cytokines and participate in lipid metabolism in the vessel wall in a manner that reduces the lipid-associated burden (Bartlett et al. 2019; Wolf and Ley 2019; Maguire, Pearce, and Xiao 2019; Eshghjoo et al. 2022; Gisterå and Hansson 2017). Macrophages promote and secrete MMPs. MMPs are molecules that degrade the artery wall´s ECM and remodel the artery wall. MMPs are also secreted by inflammatory cells other than macrophages and many of them participate in inflammatory signalling (M. Wang et al. 2015; Galis and Jaikirshan 2002).

Adaptive immunity is also involved in atherosclerosis' low-grade inflammation. T-lymphocytes are the main player of adaptive immunity in atherosclerosis. CD4-positive T helper (Th) 1 cells are the most abundant subset of T cells in atherosclerosis, and they drive atherosclerosis. A vast selection of T cells are present in atherosclerosis – some are regulatory and atheroprotective, others are atherogenic and pro-inflammatory. Most are CD4 positive, but CD8 positive T cells are also present in atherosclerosis. The complex interplay mediated by the cytokine dialogue is being investigated and adaptive immunity in atherosclerosis might reveal therapeutic targets in the future.

Atherosclerosis presents with mature T cells. Mature T cells have been introduced to their specific antigen by antigen presenting cells using major histocompatibility complex (MHC) molecules. In this process, T cells adapt to launch their immune response only in response to specific antigen, i.e. naive T cells become mature T cells. Mature T cells in atherosclerosis are activated by autoantigens oxLDL and apoB and some heat-shock proteins. In addition, certain alleles of MHC are more capable of creating atherogenic mature T cells than other MHC alleles (Saigusa, Winkels, and Ley 2020; Björkegren and Lusis 2022; Lee, Bartlett, and Dwivedi 2020; Hinkley et al. 2023; Roy et al. 2022).

B cells also participate in atherosclerosis, and their function via antibodies may be either atheroprotective or atherogenetic, depending on their subset. The role of B cells in atherosclerosis has not yet been comprehensively clarified (Srikakulapu and McNamara 2017). The immune cells of innate and adaptive immunity mediate their effects via the various cytokines that they secrete, and like the cells that secrete them, cytokines can also be categorised by their effect as pro- or anti-atherosclerotic, as illustrated in Figure 11 (Tousoulis et al. 2016).

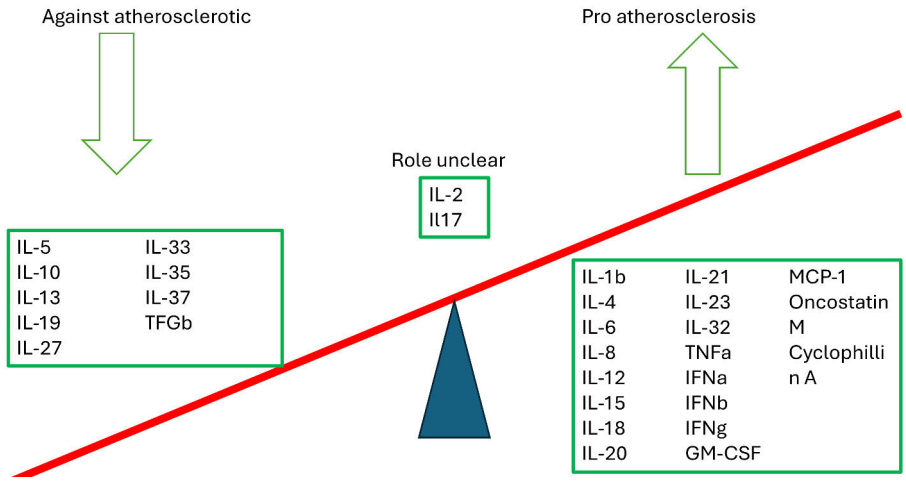

Figure 11. Data adapted from (Tousoulis et al. 2016). Pro- and antiatherosclerotic cytokines.

The current understanding is that ECs are the tissue that are first affected in atherosclerosis development. Inflammation of the artery wall takes place where ECs barrier breaks. This is often encountered in areas of vasculature where local flow-conditions cause blood flow to interrupt from laminar to interrupted or turbulent, although atherosclerosis is not restricted to these locations exclusively. The dysfunction of ECs in atherosclerosis is mediated by the bioavailability of nitric oxide to the adjacent VSMCs and by their ability to attract inflammatory cells by expressing chemoattractants. ECs also play a role in inflammatory cascade in atherosclerosis (Gimbrone and García-Cardeña 2016).

VSMCs are an important structural component of the artery wall. In atherosclerosis, their function is impaired. Some of VSMC dedifferentiate and eventually become macrophage-like cells that do not possess contractile capabilities, but are inflammatory and contribute to the destructive process in the necrotic core of the atherosclerotic plaque. The susceptibility VSMCs to participate in atherosclerosis might vary depending on the vascular bed, and unchanged, still differentiated VSMCs can protect from plaque rupture and its sequelae by stabilising the fibrous cap. VSMCs that have switched phenotype participate in inflammation and have a capability to become foam cells. Overall, VSMCs in atherosclerosis undergo a variety of changes and are involved in multiple processes in atherosclerosis. (Zhang et al. 2022; Maguire, Pearce, and Xiao 2019; Bennett, Sinha, and Owens 2016).

MCs are inflammatory cells also involved in multiple phases of atherosclerosis. MCs function by releasing inflammatory mediators when stimulated, and in atherosclerosis they reside in the intimal layer and in the adventitia of the artery wall. Their multiple functions are present in intitation, progression and late stages of atherosclerosis. Most important mediators secreted by MCs seem to be proteases, for

example tryptase, histamine and chymase, although they secrete inflammatory cytokines as well. For example, MCs activate MMPs and are associated with endothelial erosion. Number of MCs and their activity seem to be correlated with clinical atherosclerotic diseases and atherosclerotic changes in artery wall (Kovanen and Bot 2017; Mäyränpää et al. 2006).

2.4.4 Management of atherosclerosis

Currently, atherosclerosis cannot be reversed. However, its course of progression can be altered through risk prevention and specific therapies directed towards slowing down the disease. The mainstay of risk prevention and subsequently the mainstay of the treatment are lifestyle modifications (low-fat diet or avoidance of high-fat diet combined with exercise) and optimal medical therapy.

Present-day pharmacotherapy for atherosclerosis has evolved enormously since lovastatin was approved by the U.S. Food and Drug Administration (FDA) in 1987. In modern day practice, statins have been found not only to lower blood cholesterol but also to suppress inflammation and stabilise the atherosclerotic plaque. A variety of statins are available and as they are well-tolerated substances, they are likely to maintain their status in the treatment of hypercholesterolemia and atherosclerosis (Grundy et al. 2019).

In addition to statins, other novel pharmacotherapies for hypercholesterolemia and atherosclerosis are ezetimibe and proprotein convertase subtilisin/kexin 9 -inhibitors (PCSK9). Ezetimibe enhances the effectiveness of statins by interrupting cholesterol absorption and PCSK9 inhibitors enhance LDL uptake from circulation by increasing the expression of the LDL receptors in hepatocytes. This reduces the level of LDL cholesterol. Both ezetimibe and PCSK9 inhibitors have been found to be effective among high-risk patients for who statins alone do not sufficiently reduce the risks (Khan et al. 2022).

The CANTOS study, which followed a population of CAD patients for up to five years found that the interleukin 1b-antagoist Canakinumab statistically significantly reduced the risk of cardiovascular events. Although the CANTOS study presented an experimental view to the treatment of atherosclerosis, its inflammation-reduction methods are warranted, given that these methods would provide acceptable risk-benefit profile and proven clinical effectiveness. Current atherosclerosis treatment options do not include anti-inflammatory pharmacotherapy (Ridker et al. 2017).

Pharmacotherapies and invasive treatment modalities are secondarily to the preventive methods applied by the individual themself. Preventive methods can take place before consultation with medical professionals, and education of CVD prevention can be offered publicly. These methods include physical exercise, that is both low-intensity high-durability continual exercise and higher intensity muscle strength training. The encouragement of non-smoking is of the essence in the

population-based prevention of CVD and atherosclerosis. As obesity has become a worldwide health issue and is strongly related to atherosclerotic CVD, it should also be a target for preventive measures. Dietary awareness is a third mainstay in this matter. The consensus is that these methods should be applied throughout the population and life span (Raitakari, Pahkala, and Magnussen 2022).

Smoking cessation reduces the risks of cardiovascular diseases, although it may not entirely diminish the risk. Consultation for smoking cessation should be a part of the treatment plan for individuals who smokes and have been diagnosed with atherosclerosis. (Ding et al. 2019; Raitakari, Pahkala, and Magnussen 2022)

Optimal medical therapy and optimal conservative treatment may fail, and in this case, revascularisation should be considered. Modern practice uses both open surgery and endovascular procedures to restore the blood supply past the artery that is occluded or critically narrowed by atherosclerosis (Neumann et al. 2019; Bonati et al. 2021; Huber et al. 2021; Conte, Bradbury, Kolh, White, Dick, Fitridge, Mills, Ricco, Suresh, and Murad 2019).

2.5 Ankle-brachial index and the burden of atherosclerotic disease

2.5.1 Ankle-brachial index

The Ankle brachial index (ABI) is a well-recognised tool for diagnosing PAD. Widely accepted reference values with more than adequate clinical counterparts make the ABI a reliable biomarker for clinical practice and scientific research (Aboyans et al. 2018). The ABI has been found to be a significant prognostic factor regarding CVD endpoints, as shown in Figure 12. Low ABI values are strongly associated with reduced survival and event rates attributable to CVD. This is plausible as PAD is widely associated with other vascular bed atherosclerosis and on the other hand, as atherosclerosis is a systemic inflammatory disease (Aboyans et al. 2018; Criqui et al. 2021).

In more detail, in PAD, low ABI values are indicative of the disease classification. In claudication, ABI values are usually higher than in critical limb-threatening ischemia (CLTI). An ABI value less than 0.5 usually indicates a severe disease and decreasing ABI values are associated with claudication progressing to critical ischemia (Rymer et al. 2020). Values less than 0.3 are generally considered to represent CLTI. High ABI values are usually regarded as “false-positive”; mediasclerosis stiffened arteries yields high values that do not reflect healthy arteries. Low ABI values are associated with diminished walking ability and lower quality of life (Criqui et al. 2021; Aboyans et al. 2018). ABI is also linked to wound healing – low ABI values, for example less than 0.5, might be associated with reduced wound healing potential. Wound healing potential estimation must be considered when the patient has diabetes, as this is a

confounding factor that affects ABI values. It is a risk factor for PAD but might cause abnormally false-high ABI values (Chuter et al. 2023).

Patients presenting with atherosclerosis in the cerebral arteries and low ABI values are at higher risk of CAD. This emphasises the robustness of the ABI as a biomarker; low values in cohorts investigated for atherosclerotic manifestations other than PAD can be categorised for risk of atherosclerosis in other vascular beds (Roh et al. 2020). Cerebral circulation atherosclerosis (extra- and intracranial) is also associated with low ABI. Cerebrovascular disease is caused by extracranial or intracranial arteries disease, which is usually caused by atherosclerosis. Extracranially, the affected carotid arteries are especially significant and the degree of their stenosis in digital subtraction angiography (DSA) or computed tomography angiography (CTA) or intima-media thickness (IMT) in ultrasound (US) is considered in disease classification. Imaging findings and IMT, together with ABI values, are associated with intracranial artery atherosclerosis, and IMT and ABI values are correlated in a cross-sectional setting (Zwartbol et al. 2019; Vila et al. 2023). Chang et al. (2023) reported that intracranial large artery atherosclerosis in particular is affected by low ABI values, but Shaik et al. found in their imaging findings that small and large artery atherosclerosis were associated with low ABI values, and that some clinical markers of attenuated cognitive ability were associated with lower ABI values (Chang et al. 2023; Shaik et al. 2017).

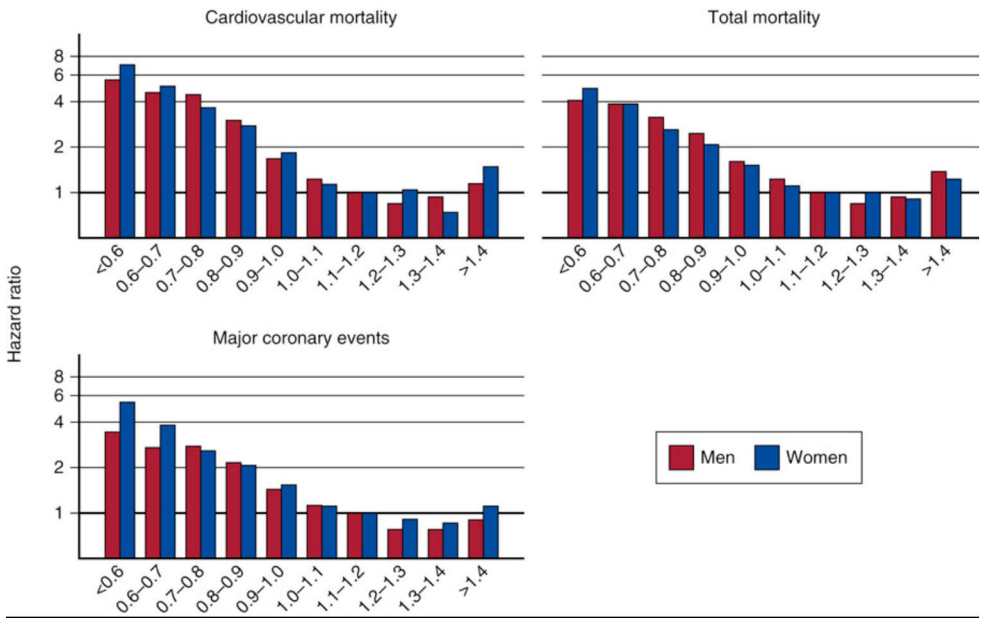

Figure 12. J-shaped association of ABI and cardiovascular events and mortality. Low values and abnormally high values are associated with events and mortality. Reproduced from Rutherford's Vascular Surgery and Endovascular Therapy, 2-Volume Set, ISBN: 9780323427913, 2018, Sidawy et al with the permission of Elsevier Ltd.

CAD is also strongly connected to lower ABI values. For example, the ARIC study, a large follow-up cohort study with a vast number of patients, established a clear inverse correlation between ABI values and CAD events. ABI values lower than the threshold value of 0.9-0.8 were associated with increased rates of CAD events, and as a continuous variable, lower ABI values also increased the risk of CAD events by 20-30% in different subpopulations and models (Weatherley et al. 2007). A meta-analysis of nine studies of patients with CAD has similar results: abnormal ABI values increased the odds of major adverse cardiovascular events (MACE) and mortality, with an emphasis on low ABI values. Mechanistic evidence also shows that coronary artery calcium scores are higher at the high and low ends of ABI categories (Liu et al. 2020; Allison et al. 2006).

Abnormally high and low ABI values are related to poor CVD outcomes, and the risk factors for CVD are also more closely associated with abnormally high and low ABI values than with normal ABI values. There is a U-shaped correlation between ABI values and CVD endpoints, and ABI values and CVD risk factors (Resnick et al. 2004). The ABI has been suggested as an addition for cardiovascular risk estimation calculations, because it has shown the ability to predict CVD even after adjustment for multiple risk factors, ethnicity and sex (Fowkes et al. 2008; Criqui et al. 2010).

2.5.2 ABI and aneurysms

There are only scarce data on ABI and aneurysms. In cases of IAs, this connection has not been explored thoroughly. One earlier study with a small sample found no association between low ABI values and IAs, but it did find that pulse-wave velocity was higher among patients with IA as a marker of arterial stiffness, which is indicative of present atherosclerotic vascular disease (Matsukawa et al. 2014).

The connection between abdominal aortic aneurysms (AAA) and atherosclerosis has vast support. Epidemiologically, AAA is associated with PAD and with low ABI values. The ARIC study revealed that both symptomatic and asymptomatic PAD are associated with AAA incidence. In fact, the presence of asymptomatic PAD defined by an ABI value of <0.9, increased the odds of AAA incidence even more than symptomatic PAD. This gives merit to the ABI as a predictive biomarker of AAA (Hicks, Al-Qunaibet, et al. 2021). A population with claudication, the mildest clinical presentation of PAD, also had higher AAA prevalence than general population (Giugliano et al. 2012).

It seems that no comprehensive studies have evaluated the association between ABI values and thoracic aortic aneurysms. Furthermore, studies of ABI in other vascular beds aneurysms are also lacking. This might be due to the rarity of aneurysms in visceral arteries and other sites.

2.6 Aortic calcification and the burden of atherosclerotic disease

Aortic calcification is a radiological marker of atherosclerosis in the aorta. It is often seen in imaging performed for variety of different indications, but there are only a few studies stating the clinical relevance of the aortic calcification. However, greater aortic calcification is associated with the burden of atherosclerotic CVD and the adverse events caused by them. Most studies have found that aortic calcification proportionally increases the risk of cardiovascular events (Gonçalves et al. 2012; Zettervall et al. 2018; Wilson et al. 2001; Tullos et al. 2013; Criqui et al. 2014).

Aortic calcification has shown similar potential to predict CVD to coronary artery calcium scores, and aortic calcification correlates with coronary artery calcifications. Calcification in other vascular beds (coronary, carotid and lower limb arteries) is also analogously associated with the atherosclerotic CVD burden measured by aortic calcification the different vascular beds atherosclerotic lesions are plausibly associated with each other (Oishi et al. 2020; Hata et al. 2022; Bytyçi et al. 2021; Tullos et al. 2013; Criqui et al. 2014; Takayama et al. 2016; Tsushima et al. 2008; Zheng et al. 1997; Vila et al. 2023; Allison et al. 2006).

This phenomenon is not new in epidemiological studies on this subject, but aortic calcification has gained little credit in risk prediction, as measurement methods vary, and study populations have often been heterogenous or vastly different and in different settings. Therefore, although the increasing trend of CVD manifestations has been established, no clear cutoff values or thresholds have been suggested for the Aortic Calcification Index (ACI). The ACI has also shown predictive value in a prospective setting. Figures 13 and 14 illustrate the results of meta-analysis which included studies using similar quantitative aortic calcification measurement methods (Gonçalves et al. 2012; Allison et al. 2012; Zettervall et al. 2018; Wilson et al. 2001; Oishi et al. 2020; Hata et al. 2022; Bytyçi et al. 2021; Tullos et al. 2013; Criqui et al. 2014; Takayama et al. 2016; Tsushima et al. 2008; Zheng et al. 1997; Vila et al. 2023; Allison et al. 2006).

The ACI summarises atherosclerotic burden regardless of recorded risk factors, can be easily utilised and is relatively free of bias. The measurement of ACI value is a simple method and manual measurement avoids the risk of inconsistency which might arise when using automated or semi-automated methods. Moreover, manual ACI value measurements require only minimal resources, can be taken by a researcher and require no additional software or hardware. A variety of quantitative methods exist for measuring aortic calcification, for example plain radiographs showing lumbar spine and simultaneously abdominal aortas calcifications. However, many studies have shown that regardless of the method used, abdominal aortic calcification seems to be proportionally associated with CVD and their events. Furthermore, some studies on this subject have examined patients with chronic

kidney disease and haemodialysis who present accelerated aortic calcification, and have also show parallel results (Takayama et al. 2016; Nitta et al. 2004; Lewis et al. 2018; Yamamoto et al. 2016; Tatami et al. 2015).

Aortic calcification can be compared with ABI. Increased aortic calcification and abnormally low or high ABI values are indicative of atherosclerotic disease, and they are correlated with each other. Both are also associated with increased healthcare costs (Tullos et al. 2013; Schousboe et al. 2020).

Multilevel atherosclerosis is associated with clinical manifestations of diseases of different vascular beds. Earlier it has been reported that PAD lesions on different levels correlate with carotid and intracranial arteries' atherosclerotic lesions, and that certain cytokine levels are higher among patients with more severe PAD. Furthermore, peripheral arteries calcification is associated with greater ischemia classification among PAD patients. Associations of calcification or atherosclerotic CVD in different arterial beds are summarised in Table 3 (Jalkanen et al. 2015; Virtanen et al. 2020; Zettervall et al. 2018; Allison et al. 2012).

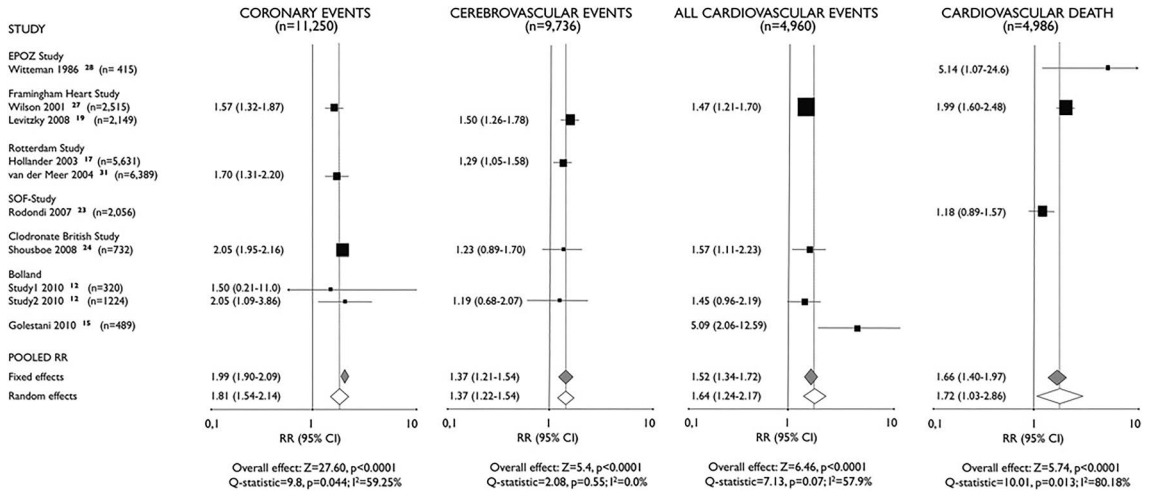

Figure 13. Forest plot showing meta-analysis results (RRs) of cardiovascular end points according to aortic calcification. Reproduced with the permission of BMJ Publishing Group Ltd. (Gonçalves et al. 2012).

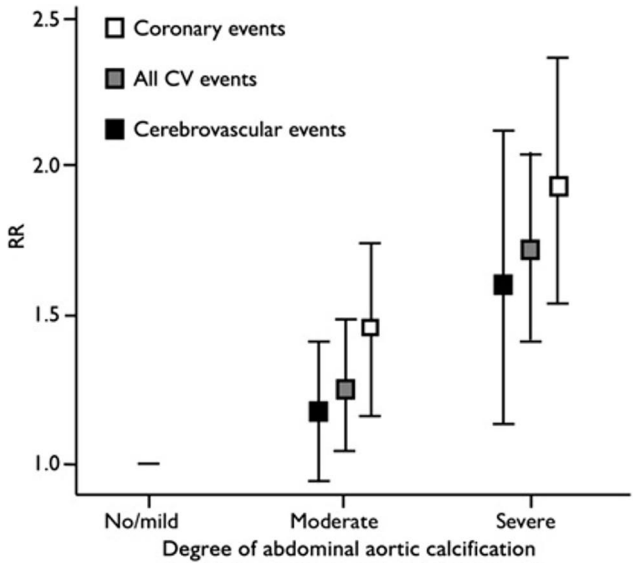

Figure 14. Second and third tertiles of aortic calcification are associated with increased risk of coronary and cerebrovascular events. Reproduced with permission of BMJ Publishing Group Ltd. from the journal Heart (Gonçalves et al. 2012).

Table 3. Illustration of correlations between different vascular beds atherosclerosis.

	ABDOMINAL AORTIC CALCIFICATION	**CORONARY ARTERY CALCIFICATION / CAD**	**CAROTID INTIMA-MEDIA THICKNESS / CEREBROVASCULAR DISEASE**	**ANKLE-BRACHIAL INDEX**
ABDOMINAL AORTIC CALCIFICATION	NA	✓ Takayama et al. 2016 Hata et al. 2022 Tullos et al. 2013	✓ Tsushima et al. 2008	✓ Tullos et al. 2013
CORONARY ARTERY CALCIFICATION / CAD		NA	✓/? Bytyçi et al. 2021	✓ Tullos et al. 2013 Allison et al. 2006
CAROTID INTIMA-MEDIA THICKNESS / CEREBROVASCULAR DISEASE			NA	(✓) Zheng et al. 1997 Vila et al. 2023
ANKLE-BRACHIAL INDEX				NA

2.7 IA and atherosclerosis

2.7.1 Clinical similarities and common risk factors

Atherosclerotic diseases co-exist with IAs on an epidemiological level. The prevalence of CVD is higher among UIA patients than with general population: there seems to be excess mortality among IA patients which is related to CVD rather than IA itself (L. Pyysalo et al. 2013; Huhtakangas et al. 2015; Uehara, Tabuchi, and Mori 1998). In contrast, an abnormally high IA prevalence has been established in patients categorised as atherosclerotic CVD patients. For example, patients with increased coronary artery calcification have a higher prevalence of IA. Coronary artery calcification in turn is associated with aortic calcification (Hata et al. 2022; Cho et al. 2019).

IAs share clinical characteristics with (other) CVD. Atherosclerotic CVD are associated with poor dental health, as are IAs. Recent studies have found that DNA of odontogenic bacteria in IA samples using polymerase chain reaction (PCR). It is speculated that dental bacteria can participate in the inflammatory process that is required for IA formation. The same periodontal bacteria seem to be associated with atherosclerosis. (M. J. Pyysalo et al. 2013; M. J. Pyysalo et al. 2016; Hallikainen, Keränen, et al. 2020; Carrizales-Sepúlveda et al. 2018).

Dental health is also a trending topic in cardiovascular risk epidemiology, like gut microbiota. It is convenient that gut microbiota has been examined in relation to IAs and atherosclerosis, and that the preliminary results are the same – changes in microbiota seem to affect the risk of the disease (Witkowski, Weeks, and Hazen 2020; Shikata et al. 2019).

The common risk factors of IAs and classical CVD are hypertension, smoking, alcohol consumption and age. While men are generally in higher risk for atherosclerosis, women exposed to smoking exhibit a high IA prevalence up to 19% (Libby et al. 2019; Uehara, Tabuchi, and Mori 1998; Ogilvy et al. 2020; Thompson et al. 2015).

There seems to a paucity of studies directly comparing atherosclerotic diseases and their progression rates to IAt pathophysiology or the common modalities of their pathophysiology.

2.7.2 Pathophysiological similarities

The earlier chapters of this dissertation elaborated on inflammation as a driver of IA formation and atherosclerosis. The inflammatory processes in IAs and atherosclerosis have some similarities. Endothelial dysfunction initiates both pathogeneses. In IAs, changes in local hemodynamics (abnormally low or high WSS) presumably alter the ability of ECs to maintain vascular tone, mainly by

altering nitric oxide synthesis (eNOS, endothelial nitric oxide synthase) (Sheinberg et al. 2019). A similar phenomenon has been observed in atherosclerosis. The curves and bifurcations of the arterial tree most commonly provide these sites, and WSS together with mechanical wall stress is associated with progression of atherosclerosis (Frösen et al. 2019; Gimbrone and García-Cardeña 2016).

In atherosclerosis, the availability of endothelium-produced nitric oxide is reduced in conjunction with excess reactive oxygen species (Higashi et al. 2009). In both, adhesion molecules VCAM and MCP-1 are at play when monocytes and macrophages are first recruited into the process (Moriya 2019; Frösen et al. 2019). Tumour necrosis factor alfa (TNFa) is associated with the inflammation of both IAs and atherosclerosis. TNFa is upregulated in various cells (VSMC, T cells, macrophages) and causes the formation and growth of IA by enhancing the inflammation and remodelling the vessel wall. In atherosclerosis, TNFa promotes inflammation and apoptosis and enhances plaque formation. (Starke, Raper, et al. 2014; Passos et al. 2020).

Cytokines that promote inflammation in atherosclerosis are TNFa (secreted by macrophages, induces VSMCs changes), IL-6 (secreted by macrophages, fibroblasts, VSMCs, T and B lymphocytes, induces T cell differentiation and induces VSMC changes), IL-1b (role still unclear), IL-18 (secreted by macrophages, induces calcification, induces VSMC changes), IFNg (secreted by T cells, role unclear), IL-17A (secreted by T cells, role unclear), transforming growth factor beta (TGFb) (secreted by macrophages, might play protective (reactive) role) and myeloperoxidase (secreted by neutrophils, stimulate macrophages). The molecules that remodel the artery wall in atherosclerosis are MMPs 2, 3, 7, 8, 9, 13 and 19 and cathepsin (Passos et al. 2020; M. Wang et al. 2015).

The cytokines that promote inflammation in IAs are TNFa (secreted by macrophages, neutrophils and Th1 cells, promotes endothelial dysfunction, promotes macrophage infiltration, inflammatory cytokines, VSMC change, and upregulates adhesion molecules), IL-1b (secreted by macrophages), IL-6 (secreted by macrophages and Th1 cells), stromal cell derived factor-1 alpha (SDF-1a) (secreted by macrophages, chemoattractant), TGFb (secreted by M2 macrophages and Treg cells, role unclear), MIP-1a (secreted by neutrophils, amplifies inflammatory cell recruitment), myeloperoxidase (secreted by neutrophils, artery wall remodeling, ROS functions), IFNg (secreted by Th1 cells). Molecules that remodel the artery wall in IAs are MMPs 1, 2 and 9 (secreted by macrophages and VSMCs) (Passos et al. 2020; Tulamo et al. 2018; Signorelli et al. 2018).

The atherosclerotic histopathology of IAs is associated with the aneurysm wall enhancement visible in magnetic resonance imaging (MRI), which is a marker of ongoing inflammation in the aneurysm wall. MMPs 2 and 9 are present in atherosclerotic IA histopathology (Zhong et al. 2021; Caird et al. 2006).

VSMCs switch phenotypes in IAs and atherosclerosis and promote pathological matrix remodelling. Macrophages are major players in innate immunity, and in both IAs and atherosclerosis, the pro inflammatory M1 phenotype is prominently present (Z. Wang et al. 2023; Shao et al. 2017; Gisterå and Hansson 2017; Bennett, Sinha, and Owens 2016).

MCs are present in both IAs and atherosclerosis. In IAs, they seem to associate with loss of endothelium and neovascularization, which is associated with IA rupture. In atherosclerosis MCs associate with plaque destabilization and increased inflammation as they promote inflammatory milieu, and they also affect ECs. MCs functions are mediated via multiple cascades, but ECs seem to be one important target of MCs. Abundance of MCs associates with clinical end points of atherosclerotic cardiovascular diseases (Ollikainen et al. 2014; Elieh-Ali-Komi et al. 2024; Mäyränpää et al. 2006).

Lipids are established, causative agents of atherosclerosis and they are also present in all IAs. Especially RIAs are often found with lipids and atherosclerotic lesions (Ollikainen et al. 2016; Zhong et al. 2021; Libby et al. 2019).

Overall, IAs and atherosclerosis' shared features are listed in Table 4.

Table 4. Common traits of atherosclerosis and Ias.

	INTRACRANIAL ANEURYSMS	ATHEROSCLEROSIS	SHARED
CYTOKINES AND MEDIATORS	Multiple	Multiple	Some inflammatory mediators and MMPs are shared
INFLAMMATORY CELLS	macrophages neutrophils T cells B cells mast cells VSMCs	macrophages neutrophils T cells B cells mast cells VSMCs	Mostly Yes
ENDOTHELIAL DYSFUNCTION	Yes	Yes	Yes
VSMC	Phenotype Switch, acquire pro-inflammatory phenotype, artery remodellation	Phenotype Switch, acquire pro-inflammatory phenotype, artery remodellation	Yes
LIPIDS	Present, role still unclear	Causative	No
RISK FACTORS	Hypertension, smoking, alcohol, sex, genetic background	Hypertension, smoking, alcohol, sex, cholesterol, genetic background, diabetes	Mostly yes
PREVALENCE	UIA 3%, RIA 10/100,000 person-years	CVD morbidity incidence 1000-1300/100,000 in Europe	Some co-existence
HEMODYNAMICS	Presents in bifurcations and sites with interrupted non-laminar blood flow	Non-laminar blood flow associates with stenoses	Yes

3 Aims

The primary hypothesis of this dissertation is that intracranial aneurysms are related to atherosclerosis and aortic calcification.

The first study aims to show that intracranial aneurysms are associated with greater aortic calcification.

The second study aims to show that aortic calcification is associated with the burden of atherosclerotic cardiovascular diseases and to illustrate aortic calcification's potential to be a practical tool for predicting risk among atherosclerotic disease patients.

The third study shows that low ankle-brachial index values are also associated with intracranial aneurysms.

The fourth study aims to illustrate inflammatory profile that presents with aortic calcification.

4 Materials and Methods

4.1 Study populations, data and patient categorization

The study populations consisted of consecutive patients who had been treated for an IA or PAD at the Turku University Hospital (TUH) or were examined in hospital's vascular laboratory as a part of their diagnostic workup. The data consists of patient information on clinical characteristics, medications, essential medical history (diagnosed diseases and essential biomarker values) and the required imaging.

The first study population consisted of patients with RIA or UIA. They were matched by age and sex with controls who had no history of IAs and whose cranial imaging showed no IA. The control patients were consecutive patients treated in emergency department for any reason unrelated to IA and they had no evidence of IA.

Of the IA patients, those with computed tomography imaging showing abdominal aorta were selected to study population for aortic calcification measurements. Control patients were included if they had undergone abdominal computed tomography during their visit to the emergency department.

The third study's population consisted of patients who had undergone ABI measurements and subsequent intracranial imaging at TUH. The intracranial imaging results were examined for IA and the patients were categorized according to ABI and IA prevalence.

The participants of the second and fourth studies were a cohort of patients with PAD. Their records were also reviewed for computed tomography studies showing abdominal aorta, and calcification indices were measured.

4.1.1 Intracranial aneurysms patients and matched controls (Study I)

Study I was a retrospective study based on register that contains data TUH's IA patients. Figure 15 presents the selection of aneurysm- and control patients. TUH is tertiary center and is responsible for the treatment of IA patients in its 870,000-inhabitant geographical catchment area.

The register data consisted of consecutive patients who were examined or treated in of TUH's department of neurosurgery between January 2003 and May 2018. IA

patients were categorized as patients with either UIA or as patients with RIA. Diagnostic imaging for IA was computed tomography (CT), computed tomography angiography (CTA), magnetic resonance angiography (MRA) or DSA (digital subtraction angiography).

The control patients were selected from the TUH emergency department's patient records. These patients' cranial imaging results showed no evidence of IA, but their abdominal computed tomography showed abdominal aorta. RIA and UIA patients were matched at a ratio of 1:3 to control patients on the basis of age and gender.

Both the IA and control patients' electronic patient records and PACS (Picture archiving and communication system) data were reviewed manually so that their radiological studies, comorbidities and cardiovascular risk factors could be studied. Patients with missing records or no adequate abdominal aorta imaging data were excluded. Patients with diagnosed connective tissue disorders (Marfan syndrome, Ehlers-Danlos syndrome type IV and Loeys-Dietz syndrome) were also excluded. The ACI of the IA patients and the controls was measured using the available computed tomography studies that showed abdominal aorta.

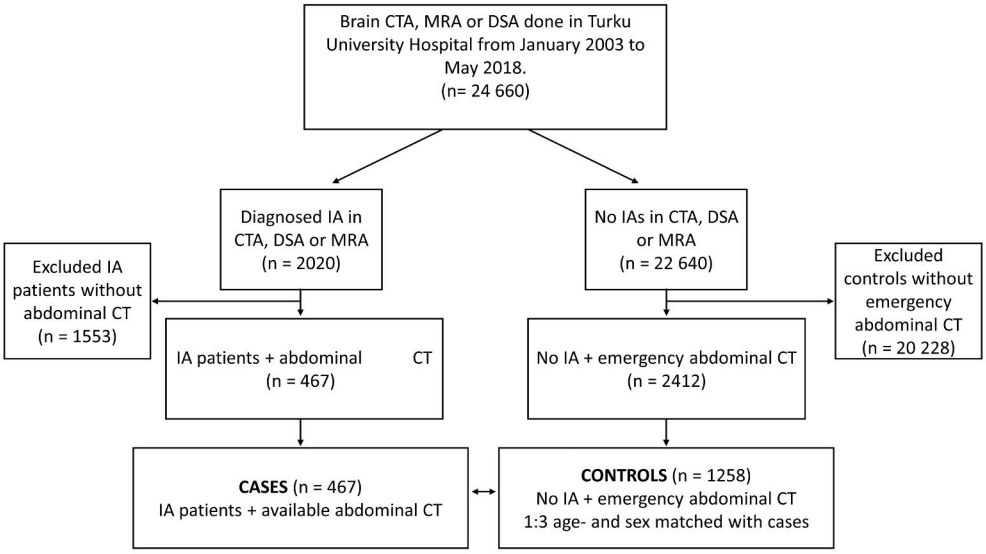

Figure 15. Flow-chart presenting aneurysm- and control patients' selection and exclusion. Reprinted with permission from (Rantasalo et al. 2021).

4.1.2 PURE ASO Cohort (Studies II and IV)

The PURE ASO (The Role of Purinergic Signaling in Atherosclerosis) cohort is a follow-up cohort based at the Department of Vascular Surgery at the TUH (Jalkanen et al. 2015). All 227 patients admitted to TUH's Department of Vascular Surgery for

elective invasive treatment of PAD during the enrollment period (February 2012 – March 2013) were screened (227 patients) and 226 were included. Initially conservatively treated patients were not included, and thus the cohort consists of patients with symptomatic and invasively treated PAD. These patients were diagnosed with PAD by a vascular surgeon at the department´s outpatient clinic. They gave their informed consent at the time of enrollment.

Baseline data: The cohort data consists of baseline demographic factors, relevant medical history, prescribed medication and previous invasive procedures for CVD. The baseline data was collected upon inclusion in the study. The cohort patients were categorized according to the clinical presentation of the PAD using the Rutherford classification. Rutherford classes I-III were considered intermittent claudication and classes IV-VI were considered critical limb-threatening ischemia. The lowest ABI and TBI measurements were noted (Jalkanen et al. 2015).

Cytokines: The cohort data includes the results of blood test for circulating cytokine levels. The multiplex assay kit (Bio-Plex Pro Human Cytokine 21- and -27-plex panels, Bio-Rad Laboratories) was used to assess cytokine levels from the serum samples collected from the enrolled participants at the time of enrollment. The blood tests were performed at the time of inclusion in the study. In short, the cytokines in these panels represent a cytokine profile associated with atherosclerosis (Jalkanen et al. 2016; Jalkanen et al. 2015). Table 5 presents the cytokines.

Follow-up data for Study II: The data on the overall survival and occurrence of cardiovascular (MACE) or leg-related (MALE) adverse events after initial treatment were collected by reviewing patient records. The data in the electronic patient records was complete for the purposes of this study, as this TUH is responsible for the invasive treatment of its catchment area populations' PAD patients. Therefore, patients were not contacted for follow-up data collection. Information on mortality was available fromthe national conjoined patient records. Mortality, MACEs and MALEs were recorded until December 2020, but the follow-up period was limited to five years from individual inclusion date. MACEs were categorized as myocardial infarct (MACE MI), heart failure (MACE HF), ischemic stroke (MACE IS) and all combined (MACE). MALEs were categorized as major amputation (MALE amputation, above ankle level) or revascularization (MALE revascularization) or MALE (amputation and revascularization, either or both). MALE revascularization includes both surgical and endovascular revascularizations. Study II analyzed the main categories (any MACE, any MALE and mortality).

ACI value measurements: Patient records were reviewed for computed tomography showing abdominal aorta. ACI was measured manually from those who had sufficient imaging studies available.

Patient categorization for Study IV: For Study IV, a sub-group of patients (n=156) was selected for analysis. Patients with no missing values (cytokines, ACI,

significant clinical variables) were included. The patients were reviewed and categorized according to ACI and their cytokine levels were correlated with ACI.

Table 5. Cytokines involved in Multiplex assay. MCP-3, IFN-α2, LIF, IL-1α, IL-3, IL-15, and TNFb were mostly below a detectable limit and therefore excluded from the analyses. Cytokine RANTES was constantly higher than the measurable range and therefore excluded from the analyses.

IL-1B	Interleukin-1 beta
IL-1RA	Interleukin-1 receptor antagonist
IL-2	Interleukin-2
IL-2RA	Interleukin-2 receptor alpha
IL-4	Interleukin-4
IL-5	Interleukin-5
IL-6	Interleukin-6
IL-7	Interleukin-7
IL-8	Interleukin-8
IL-9	Interleukin-9
IL-10	Interleukin-10
IL-12	Interleukin-12
IL-13	Interleukin-13
IL-16	Interleukin-16
IL-17	Interleukin-17
IL-18	Interleukin-18
EOTAXIN	Eotaxin
TNFa	Tumor necrosis factor alpha
IFN-G	Interferon gamma
IP-10	Interferon gamma induced protein-10
CTACK	Cutaneous T cell-attracting chemokine
MCP-1	Monocyte chemotactic protein-1
MIP-1A	Macrophage inflammatory protein-1 alpha
MIP-1B	Macrophage inflammatory protein-1 beta
MIF	Macrophage migration inhibitory factor
MIG	Monokine induced by interferon gamma
M-CSF	Macrophage colony stimulating factor
GM-CSF	Granulocyte-macrophage colony stimulating factor
G-CSF	Granulocyte colony stimulating factor
FGF	Basic Fibroblast Growth Factor
PDGF	Platelet derived growth factor
HGF	Hepatocyte growth factor
VEGF	Vascular endothelial growth factor
SCF	Stem cell factor
SCGF-B	Stem cell growth factor beta
SDF-1A	Stromal cell derived factor-1 alpha
B-NGF	Beta nerve growth factor
GROA	Growth regulated oncogene alpha
TRAIL	Tumor necrosis factor related apoptosis inducing ligand

4.1.3 Patients with measured ABI values (Study III)

The study population consisted of patients whose ABI value had been measured at the vascular laboratory at TUH's Department of Clinical Physiology between January 1st 2011 - December 31st 2013. These patients (n=2757) electronic records and radiological examinations up to January 1st 2023 were reviewed. Of the 2757 patients, 776 patients had available cranial imaging results (MRA, CTA or DSA) or a history of RIA were identified and were included to the analyses. Figure 16 shows the patient selection process.

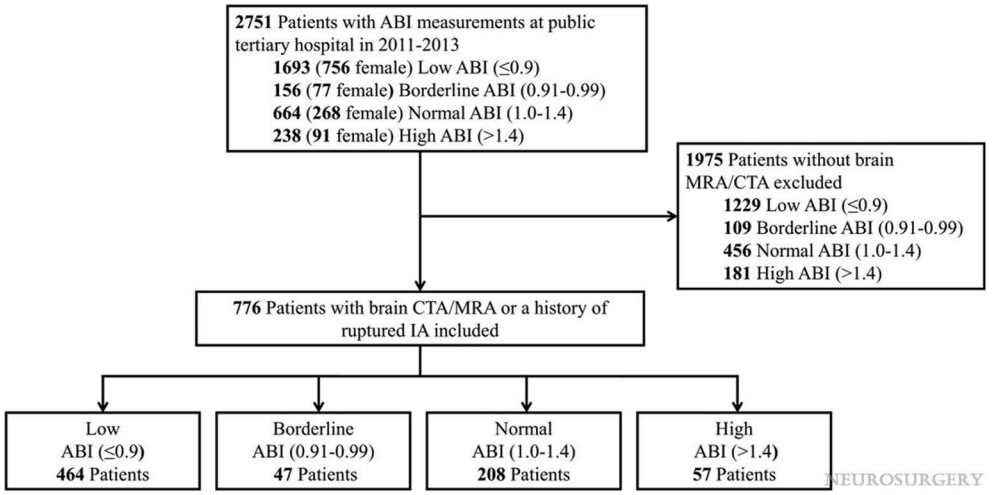

Figure 16. Flow diagram of study population. ABI = ankle brachial index, CTA = computed tomography angiography; IA = intracranial aneurysm; MRA = magnetic resonance angiography.

The baseline variables collected from the electronic patient records were smoking history (current/former/never smokers), hypertension (diagnosed hypertension and/or anti-hypertensive medication), hypercholesterolemia (diagnosed hypercholesterolemia and/or medication for hypercholesterolemia), type 1 diabetes, type 2 diabetes (diagnosed type 2 diabetes and/or medication for type 2 diabetes), coronary artery disease (diagnosed coronary artery disease, prior CABG or PCI, or prior myocardial infarction), malignancy (prior diagnosis of any malignancy), chronic obstructive pulmonary disease, rheumatoid arthritis or varicose ulcus.

4.2 ACI measurements

ACI-index: ACI were measured manually from the computed tomography studies with or without contrast enhancement and. The measurements were conducted on imaging studies already available in the TUH's Picture archiving and communication system (PACS). Thus, no additional imaging was performed for this study. The study populations of study I (IA and control patients) and PURE ASO cohort patients were reviewed for these imaging studies and the ACI values was measured from available studies.

Method: The measurement of the ACI included the whole length of the abdominal aorta from the level of the renal arteries to aortic bifurcation. ACI was calculated from axial CT-slices viewed in the multiplanar reconstruction mode (MPR). The slices were 5 mm apart. Each slice was given a value from 0 to 12 according to the degree of calcification visible in the slice. The degree of calcification, in turn, was estimated using the visual template shown in Figure 17. For example, 6 would mean that half of the circumference of axially viewed slice of aorta is covered in calcified plaque. Thus, every patient's ACI value consist of the number of slices 5 mm apart (n in the formula) and the sum of the calcification (the given values representing each slices degree of calcification were summed together). ACI was calculated using the following formula:

$$ACI = \frac{total\ sum\ of\ calcification\ in\ all\ slices}{12 * n} * 100$$

Aortic calcification (= calcified atherosclerotic plaque) was defined as a white, dense plaque greater than 1 mm^2 and over 300 Hounsfield units (HU) in the CT scan. The plaques were visually distinguished from contrast and adjacent structures.

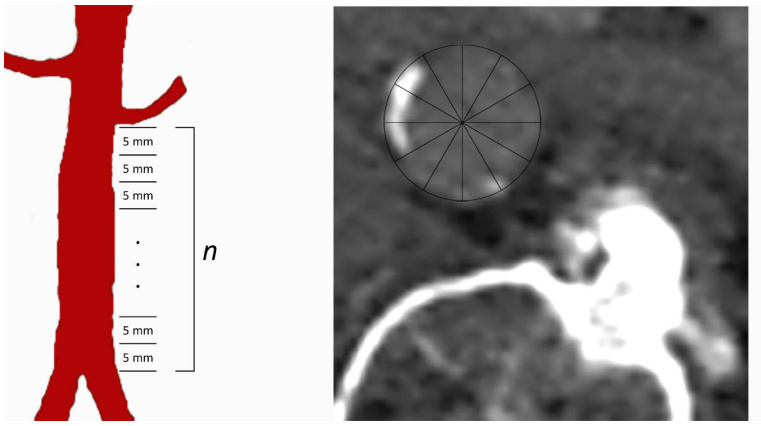

Figure 17. Aortic calcification index value measurement method. Left panel = number of slices 5 mm apart (=n), right panel = individual CT slice, 12-part pie-chart represents template which is used to estimate degree of calcification.

Inter-rater reliability: The manually measured ACI values were controlled between observers, using inter-rater reliability calculation. The measurements were conducted unblinded. Also, no significant difference was found when two random subsets of ACI value measurements by different observers were compared to each other.

4.3 ABI measurements

ABI values were measured in Study II and IV (PURE ASO Cohort) and in Study III. In studies II and IV, the ABI values were recorded as part of the diagnostic workup of the PURE ASO cohort patients during the cohort inclusion period from February 2012 to March 2013. All the cohort patients ABI values were measured. Study III patients' ABI values had been measured for any reason in TUH between January 1st 2011 and December 31st 2013.

All the ABI value measurements were conducted in TUH's Department of Clinical Physiology´s vascular laboratory, which provides non-invasive blood pressure measurements for the hospital's catchment areas population of 480,000 inhabitants. All the measurements were done by qualified sonographers. Systolic blood pressure was measured from the posterior tibial artery and the dorsalis pedis artery in both legs, as was systolic brachial pressure in both arms. The higher ankle pressure value and the higher arm pressure value were used to calculate the ABI value. The lower of the bilateral ABI values was used for the analyses. Toe pressure was also recorded when applicable, and these recordings were used to calculate TBI (Toe-Brachial index).

4.4 Intracranial aneurysm diagnosis and measurements

The presence of an IA was evaluated by the researchers of the study III and the presence of IA was confirmed by a neuroradiologist. Any disagreement regarding the IA diagnosis was resolved through consensus. The presence of an IA was determined using the CTA or MRA studies of the patients whose ABI value had been measured in the institution during the inclusion period.

IAs were considered true IAs when they had saccular morphology, when their largest diameter was greater than or equal to 2 mm and when they were located in the intracranial arteries. IA size was determined as the largest UIA or for the ruptured IA.

The location of the largest UIA or the RIA was recorded. The locations of the IAs were categorized in accordance with the Bouthillier classification (Bouthillier, van Loveren, and Keller 1996). IAs located distally to the clinoid segment (C5) were

defined as intradural and aneurysms located in the intracavernous segment (C4) were considered extradural. Aneurysms in segment C4 or proximal to it were not analyzed. UIAs were categorized by their location into anterior cerebral artery (ACA), internal carotid artery (ICA), middle cerebral artery (MCA) and posterior circulation UIAs.

4.5 Statistical methods

Common statistical methods: Statistical analyses were run using IBM SPSS statistics 27 software for Windows (IMB, Armonk, NY) and JMP® Version 16 and JMP® Version Pro 17 for windows (SAS Institute Inc., Cary, NC). GraphPad Prism version 9.5.1 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com. was used for data visualization.

P-values less than 0.05 were considered statistically significant. Categorical variable differences between groups were evaluated using the chi-square test for proportions. The selection of statistical methods used for analysing differences in the continuous variables between the groups were selected was on the basis of on each study's data distribution and variance.

Categorical variables were reported as n (%). Continuous variables were reported as mean (SD) or mean (SE) or median (IQR). Odds ratios (OR) were reported as OR (95 % confidence interval) and hazard ratios (HR) were reported as HR (95 % confidence interval). Regression coefficients were reported as and β (standard error, SE).

Study I, statistical analysis: Differences between the variances of the groups were evaluated using one-way ANOVA and the independent samples t-test. Levene's test was used to test variances equality. Binary logistic regression analysis was performed with all variables, applying backward selection (Wald). Classification and regression tree (CART) analysis was performed for the total population to identify the cutoff values for variables independently associated with IAs. Validation was assessed using cross-validation through 10 folds. The minimum number of patients for a parent node was set at 100 and at 50 for child nodes and maximum tree depth was set at 5. Gini's method was used to measure impurity and minimum change in improvement was set at 0.0001. Multiple imputation was used for missing data. The inter-rater reliability of the ACI value measurements was assessed using interclass correlation (ICC). A randomly selected subset of ACI value measurements were repeated by the neuroradiologist. ICC was interpreted according to following scale: poor (<0.5), moderate (0.5-0.75), good (0.75-0.9) and excellent (≥0.9).

The main variable of interest in this study was the abdominal ACI and the difference in its distribution between rIA and UIA patients. ACI values present a

continuous variable, but the patients were also categorized into two groups according to their ACI values: patients with ACI=0 meaning completely calcification-free aorta and ACI>0 meaning at least one calcification plaque identified in abdominal aorta. Another cutoff for binary categorization was an ACI value over 3, meaning that patients were categorized as patients with ACI>3 and patients with ACI<3.

Study II, statistical analysis: The primary outcomes of this study were ACI values among patients with MACE or MALE or among patients who died during follow-up, and the risk of these events according to the ACI values. The Kolmogorov-Smirnov test, the Shapiro-wilk test and visual observation were used to test the continuous variables' normal distribution assumption. The differences between the groups were analyzed using independent samples t-test assuming equal variances. Levene's test was used to test the equality of variances.

The survival analysis for MACEs, MALEs and mortality were conducted using Cox regression analysis. The variables representing clinically relevant risk factors and statistically significantly differing univariates for each event were included in the multivariate analyses. The log-rank test and respective Kaplan-Meier analysis were conducted for survival free of mortality, MACEs and MALEs.

To better categorize patients in relation to mortality according to ACI values, Classification and regression tree (CART) analysis was conducted. ACI was forced as the first variable in the tree to find the threshold value. This value was further used to categorize patients. Differences in survival among patients categorized by this threshold were also examined. Validation was assessed by cross-validation through 10 folds. The minimum number of patients for a parent node was set at 100 and for child nodes at 50 and maximum tree depth was set at 5. Gini's method was used to measure impurity and minimum change in improvement was set at 0.0001. CART-analysis uses surrogates as substitutes for missing values. Harrel's C, - "C-index" or "Harrel's C" - statistical analysis was used to investigate the ACIs relevance in the survival model, compared to ABI or TBI. The identic Cox proportional hazard model for total survival, including ACI or ABI or TBI, one at the time, were run separately. For each model, Harrel´s C index value was calculated and compared to the ACI, ABI and TBI values for their respective relevance in the prognostic model. The C-index was interpreted as follows: less than 0.5=very poor, 0.5-0.7=reasonable, 0.7-0.8=is good and above 0.8=excellent (Harrell, Lee, and Mark 1996; Therneau and Atkinson 2021).

Study III, statistical analyses: The prevalence of RIA and UIA were the primary outcomes of this study. The patients were classified into four groups on the basis of their ABI values: low ABI (≤0.9), borderline ABI (0.91-0.99), normal ABI (1.0-1.4), and high ABI (>1.4). They were further categorized into three groups according to IA presence: those with RIAs, those with UIAs, and those with no IAs.

If both RIA and UIA presented in same patient, they were categorised into the RIA group. History of smoking was categorized as a binary variable. Current and former smokers were categorized as smokers, and the other class was non-smokers. Those with no data on smoking were not included in the multinomial regression analysis.

The differences between the groups in continuous variables were analyzed using the Kruskal-Wallis test (nonparametric data) and one way ANOVA (normally distributed data).

The association between ABI groups and IAs was investigated using multinomial regression analyses. First, adjustments were made for clinically relevant variables. In the second phase, only variables that showed statistically significant differences ($p<0.05$) in the univariate analysis were included in the multivariate analysis. The associations between ABI groups and covariates were calculated. Both models were adjusted for sex and age.

Missing data were excluded from the analysis.

Study IV, statistical analyses: The continuous variables' normal distribution assumption was tested both visually and using the Shapiro-Wilks test. Logarithmic transformations of cytokine levels were used in univariate regressions because none of the cytokine levels were normally distributed. Simple linear regression was used to analyse the associations between the continuous variables. The clinically relevant risk factors were selected as variables for multivariate linear regression analysis. Univariate binary regression was used to determine the association between each cytokine and higher ACI values. Patients were also categorized in tertiles according to ACI, and in relation to ACI over or under 50. The key cytokines were analysed in these groups and binary regressions were run with the ACI over 50 and with the ACI higher than the lowest tertile as response variables. An ACI value over 50 was selected as the threshold level because the mean and median of ACI are both close to 50 (mean ACI = 51.27, median ACI is 52.26). The AUC_value of each binary regressions ROC-curve with their 95% confidence intervals were analyzed and plotted.

4.6 Ethical considerations

Studies I and III were based on register data. The study protocols were reviewed and accepted by the institutional review board and/or ethics committee, and neither study required informed patient consent due to their design.

Studies II and IV were based on an earlier created prospective cohort. Each patient gave their informed consent, and all the researchers were included to the study protocol and related permissions.

All the studies adhere to the local, institutional, and international guidelines and regulations concerning medical research's ethical and legal regulations on human subjects. All the study protocols adhered to the ethical guidelines of the 1975 Declaration of Helsinki.

The data were managed meticulously and only qualified staff with appropriate permission were allowed to access and process them. The data of the studies I, II, III or IV were not made publicly available in a repository due to confidentiality issues.

5 Results

5.1 Study I: Intracranial aneurysm is predicted by abdominal aortic calcification index

A total of 462 IA patients who also had available abdominal CT studies were included in the analyses. 216 (46.8 %) of them were categorized as rIA patients and 246 (53.2 %) as UIA patients. Control group consists of 1258 patients with abdominal imaging and patient records data. Control patients did not have a IAs. Baseline characteristics according to this categorization are shown in Tables 6, 7, 8 and 9.

Table 6. Study population demographics. Modified from Rantasalo et al., 2021.

	ALL CONTROLS MEAN (SD) OR N (%)	RIA MEAN (SD) OR N (%)	UIA MEAN (SD) OR N (%)	P-VALUE
PATIENTS	1258	216	246	
AGE, YEARS	63.0 (12.0)	62.9 (± 12.3)	62.5 (± 11.0)	0.683
ABDOMINAL CALCIFICATION INDEX	17.9 (22.7)	25.9 (22.7)	23.4 (24.2)	<0.001*
SMOKING	746 (67.0)	120 (67.0)	161 (70.3)	0.626
PRIOR PERCUTANEOUS CORONARY INTERVENTION	125 (9.9)	8 (3.8)	22 (9.0)	0.019
FEMALE	722 (57.4)	128 (60.7)	145 (59.7)	0.578
CORONARY ARTERY DISEASE	279 (22.2)	28 (13.4)	53 (21.7)	0.015*
PRIOR MYOCARDIAL INFARCTION	139 (11.0)	14 (6.7)	38 (15.6)	0.011*
TREATMENT FOR HYPERTENSION	791 (62.9)	153 (73.2)	185 (75.8)	<0.001*
TREATMENT FOR DYSLIPIDEMIA	460 (36.6)	58 (27.8)	102 (41.8)	0.007*
TYPE 2 DIABETES	317 (25.2)	45 (10.9)	49 (11.9)	0.158
TYPE 1 DIABETES	60 (4.8)	2 (3.1)	3 (1.2)	0.003*
DIALYSIS	34 (2.7)	5 (2.3)	17 (6.9	0.003*
CHRONIC-OBSTRUCTIVE PULMONARY DISEASE	170 (13.5)	30 (17.6)	43 (20.3)	0.02*
CALCIFICATION FREE AORTA	302 (24.0)	19 (8.8)	33 (13.6)	<0.001*
PRIOR CORONARY BYPASS	60 (4.8)	4 (1.9)	12 (4.9)	0.169
ASTHMA	317 (25.2)	33 (19.5)	37 (17.5)	0.02*
PERIPHERAL ARTERY DISEASE	92 (7.3)	15 (7.2)	16 (6.6)	0.918
ALCOHOL ABUSE	399 (31.7)	32 (15.5)	55 (22.5)	<0.001*

Age was noted as the patients' age at the time of abdominal aortic imaging.

Table 7. rIA patients and matched controls. Modified from Rantasalo et al., 2021.

	MATCHED CONTROLS MEAN (SD) OR N (%)	RIA MEAN (SD) OR N (%)	P-VALUE
ALCOHOL ABUSE	179 (32.0)	32 (15.5)	0.001*
CALCIFICATION FREE AORTA	144 (25.7)	19 (8.8)	0.001*
PRIOR PERCUTANEOUS CORONARY INTERVENTION	68 (12.1)	8 (3.8)	0.001*
TYPE 1 DIABETES	30 (5.4)	2 (1.0)	0.007*
CORONARY ARTERY DISEASE	122 (21.8)	28 (13.4)	0.01*
DYSLIPIDEMIA	211 (37.7)	58 (27.8)	0.011*
PRIOR CORONARY BYPASS	30 (5.4)	4 (1.9)	0.04*
PRIOR MYOCARDIAL INFARCTION	68 (12.1)	14 (6.7)	0.035*
ABDOMINAL CALCIFICATION INDEX	18.0 (22.7)	25.9 (22.7)	0.001*
CALCIFIED AORTA	416 (74.3)	196 (91.2)	0.001*
HYPERTENSION	351 (62.7)	153 (73.2)	0.006*
AGE, YEARS	63.6 (11.7)	63.9 (12.3)	0.448
ASTHMA	139 (24.8)	33 (19.5)	0.155
TYPE 2 DIABETES	150 (26.8)	45 (21.6)	0.158
MALE	236 (42.1)	83 (39.3)	0.578
FEMALE	324 (57.9)	128 (60.7)	0.578
DILAYSIS	18 (3.2)	5 (2.3)	0.64
PERIPHERAL ARTERY DISEASE	47 (8.4)	15 (7.2)	0.657
CHRONIC OBSTRUCTIVE PULMONARY DISEASE	80 (14.3)	30 (17.6)	0.283
SMOKING	321 (66.0)	120 (67.0)	0.853

Table 8. UIA patients and matched controls. Modified from Rantasalo et al., 2021.

	MATCHED CONTROLS MEAN (SD) OR N (%)	UIA MEAN (SD) OR N (%)	P-VALUE
HYPERTENSION	435 (63.1)	185 (75.8)	<0.001*
ABDOMINAL CALCIFICATION INDEX	17.8 (22.7)	23.4 (24.2)	<0.001*
DIALYSIS	16 (2.3)	17 (6.9)	<0.001*
CALCIFIED AORTA	533 (77.4)	210 (86.4)	0.003*
CALCIFICATION FREE AORTA	156 (22.6)	33 (13.6)	0.003*
ALCOHOL ABUSE	218 (31.6)	55 (22.5)	0.009*
CHRONIC OBSTRUCTIVE PULMONARY DISEASE	89 (12.9)	43 (20.3)	0.01*
TYPE 1 DIABETES	30 (4.4)	3 (1.2)	0.013*
ASTHMA	176 (25.5)	37 (17.5)	0.016*
PRIOR MYOCARDIAL INFARCTION	71 (10.3)	38 (15.6)	0.036*
DYSLIPIDEMIA	248 (36.0)	102 (41.8)	0.124
TYPE 2 DIABETES	166 (24.1)	49 (20.1)	0.216
PRIOR CORONARY BYPASS	30 (4.4)	12 (4.9)	0.412
FEMALE	392 (56.9)	145 (59.7)	0.497
MALE	297 (43.1)	98 (24.8)	0.497
SMOKING	421 (67.9)	161 (70.3)	0.507
PRIOR PERCUTANEUS CORONARY INTERVENTION	57 (8.3)	22 (9.0)	0.789
CORONARY ARTERY DISEASE	157 (22.8)	53 (21.7)	0.789
AGE, YEARS	62.6 (12.1)	62.5 (11.0)	0.841
PERIPHERAL ARTERY DISEASE	43 (6.2)	16 (6.6)	0.879

Table 9. UIA and RIA patients. Modified from Rantasalo et al. 2021.

	RIA MEAN (SD) OR N (%)	IUA MEAN (SD) OR N (%)	P-VALUE
DYSLIPIDEMIA	58 (27.8)	102 (41.8)	0.002*
PRIOR MYOCARDIAL INFARCT	14 (6.7)	38 (15.6)	0.003*
DIALYSIS	5 (2.3)	17 (6.9)	0.021*
CORONARY ARTERY DISEASE	28 (13.4)	53 (21.7)	0.021*
PRIOR PERCUTANEOUS CORONARY INTERVENTION	8 (3.8)	22 (9.0)	0.028*
ALCOHOL ABUSE	32 (15.5)	55 (22.5)	0.058
PRIOR CORONARY BYPASS	4 (1.9)	12 (4.9)	0.084
CALCIFICATION FREE AORTA	19 (8.8)	33 (13.6)	0.11
CALCIFIED AORTA	196 (91.2)	210 (86.4)	0.11
ABDOMINAL CALCIFICATION INDEX	25.9 (22.7)	23.4 (24.2)	0.242
SMOKING	120 (67.0)	161 (70.3)	0.479
CHRONIC OBSTRUCTIVE PULMONARY DISEASE	30 (17.6)	43 (20.3)	0.515
HYPERTENSION	153 (73.2)	185 (75.8)	0.524
ASTHMA	33 (19.5)	37 (17.5)	0.604
AGE, YEARS	62.9 (12.3)	62.5 (11.0)	0.658
TYPE 2 DIABETES	45 (21.6)	49 (20.1)	0.685
PERIPHERAL ARTERY DISEASE	15 (7.2)	16 (6.6)	0.784
TYPE 1 DIABETES	2 (1.0)	3 (1.2)	0.786
MALE	85 (39.4)	99 (40.2)	0.845
FEMALE	131 (60.6)	147 (59.8)	0.845

RIA patients vs matched controls: RIA Patients had higher mean ACI, 25.93 (SD 22.7 95% CI 18.6–21.8) than matched controls, 18.0 (SD 22.7, 95% CI 16.2–19.9, p <0.001). Fewer RIA patients had totally calcification free aorta (8.8% vs. 25.7%, p <0.001) than matched controls. RIA patients had hypertension more often (73.2% vs. 62.7%). RIA patients had less hypercholesterolemia (27.8% vs. 37.7%) and alcohol abuse (15.5% vs 32.0%) than matched controls. History of coronary artery disease, including previous percutaneous coronary interventions (PCI) and coronary artery bypass grafting surgery (CABG), was also less common among RIA patients than in matched controls.

Multivariate logistic regression analyses results are shown in Figure 18. Comparison of rIA patients and matched controls showed that hypercholesterolemia (OR 0.42, 95% CI 0.22–0.787), older age (OR 0.96 per year, 95% CI 0.93–0.99), prior PCI (OR 0.30 95% CI 0.10–0.86) and alcohol abuse (OR 0.41, 95% CI 0.19–0.86) reduced odds for RIA. Hypertension (OR 2.65, 95% CI 1.35–5.23), calcification in the aorta (OR 3.35, 95% CI 1.42–7.87) and ACI (OR 1.02, 95% CI 1.00–1.03 per increment) increased odds for RIA. ACI > 3 was associated with RIA with OR 5.77, 95% CI 3.29–10.11.

UIAs vs matched controls: Mean ACI for unruptured intracranial aneurysm (UIA) patients was significantly higher, 23.4 (SD 24.2, 95% CI 20.3–26.4) compared

to matched controls (mean ACI 17.8, 95% CI 16.1–19.5). UIA patients had more prior myocardial infarctions (15.6% vs. 10.3%), chronic-obstructive pulmonary disease (COPD) (20.3% vs. 12.9%) and hypertension (75.8% vs. 63.1%) compared to matched controls. Matched controls had significantly more often type I diabetes, history of alcohol abuse, asthma and dialysis treatment than UIA patients.

Alcohol abuse was associated with reduced risk of UIA compared to matched controls (OR 0.56 95% CI 0.31–0.99). Total aortic calcification (OR 2.10 95% CI 1.11–4.01), dialysis treatment (3.29 95% CI 1.46-7.45) and previous myocardial infarction (OR 1.87 95% CI 1.01–3.48) were associated with increased odds for UIA. ACI was not associated with odds for UIA when compared to matched controls. ACI>3 increased odds for UIA with OR 2.10, 95% CI 1.34–3.30. Odds ratios are plotted in Figure 18.

RIA vs UIA: Mean ACI was numerically higher in RIA patients than in UIA patients, although this difference was not statistically significant (25.9 95% CI 22.9–29.0 vs 23.4 95% CI 20.3–26.4), but when comparing RIA patients and UIA patients in regression model, ACI (OR 1.02 95% CI 1.00-1.03 per increment) was associated with increased odds for RIA. Dialysis treatment (OR 0.26 95% CI 0.08–0.86), hypercholesterolemia (OR 0.47, 95% CI 0.28–0.78) and previous CABG (OR 0.12, 95% CI 0.05–0.77) reduced odds for RIA compared to UIA. As a binary variable, ACI>3 was associated with increased risk for RIA with OR 2.73 95% CI 1.51–4.91. Patients with UIA and RIA were not matched with each other's. Odds ratios are plotted Figure 18.

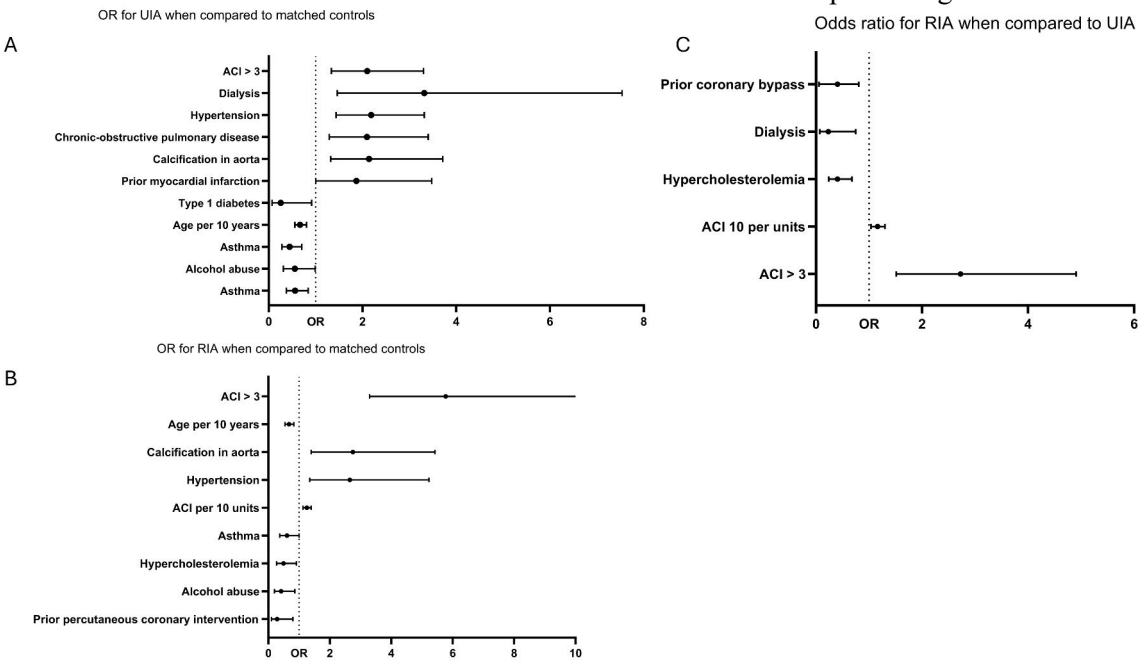

Figure 18. Diseases, risk factors and ACI: odds ratios for rIA and UIA. Panel A, patients with UIA vs. matched controls. Panel B, patients with RIA compared to matched controls. Panel C, RIA patients vs UIA patients.

Decision-tree and ROC: CART-analysis run for entire study population (including RIA and UIA patients and matched controls) showed that ACI over 3.1 (range from 0 to 134.0, mean 19.8) was associated with a two-fold risk of IA. RIA were more prevalent in patients who had ACI over 3.1, diagnosed hypertension and age above 63. Only on RIA and UIA patients, CART-analysis showed that ACI over 3.3 was associated with RIAs. Of 462 IA patients, 355 had ACI over 3.3, and in this group of patients, 179 of 355 (50.4%) were RIA patients, whereas in patients with ACI under 3.3 only 37 out of 107 (34.6%) had RIA. Thes two decision trees is shown in Figure 19.

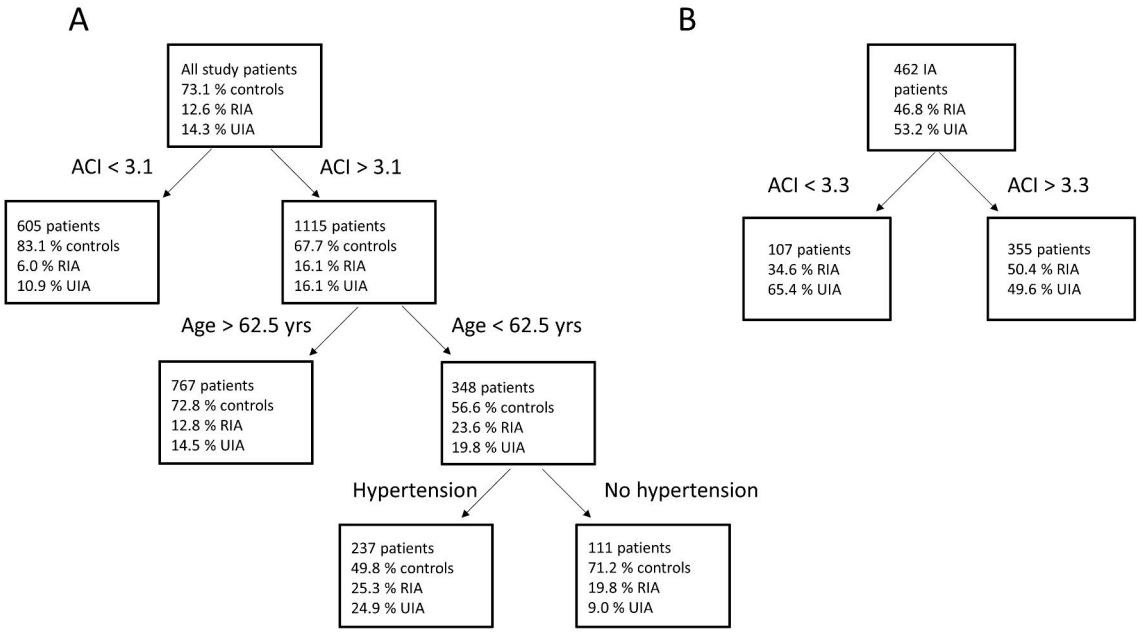

Figure 19. Panel A, CART-analysis including RIA, UIA and control patients. Panel B, CART-analysis including only RIA and UIA patients.

ROC analysis of ACI in rIA, UIA and matched control patients as well as between RIA and UIA patients showed biggest AUC (0.63 95% CI 0.59–0.67 $p \leq 0.0001$) in RIA patients compared to matched controls. Cut-off value of 3 in ACI showed sensitivity of 0.83 and 1-specificity was 0.61. Comparison of RIA and UIA patients AUC was 0.62 (95% CI 0.58-0.67, $p<0.0001$) and cut-off value of 3 yielded sensitivity of 0.83 and 1-specificity was 0.62. UIA patients vs their matched controls AUC was 0.58 (95% CI 0.54–0.62, $p=0.0002$). At the cut-off of 3, sensitivity was 0.73 and 1-specificity was 0.60. Figure 20.

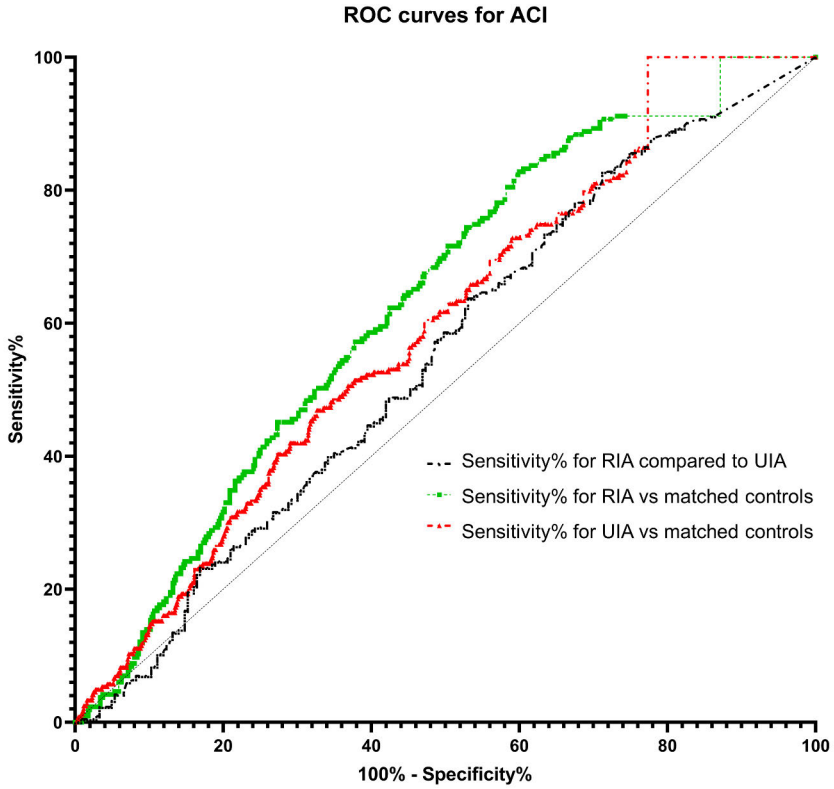

Figure 20. ROC analysis for RIA vs UIA, rIA vs matched controls and UIA vs matched controls. Authors´ own figure.

Inter-rater reliability: Inter-rater reliability of ACI measurements was excellent when comparing ratings against a board-certified neuroradiologist ratings (ICC value of 0.99, 95% CI 0.96–1.00).

5.2 Study II: Aortic calcification index predicts mortality and cardiovascular events in operatively treated patients with peripheral artery disease

Demographics and ACI: 226 patients were included in the study. 128 were male and mean age was 71.3 years. 126 patients (55.8%) died during follow-up. Mean survival time for non-survivors was 2.91 years (8 days to 5 years) and median survival was 3.27 years. Mean follow-up time was 3.82 years (all patients). 104 (46.2%) presented with intermittent claudication (IC) and 122 (54.0%) presented with critical limb threatening ischemia (CLTI) at the time of enrollment. Mean ACI was not significantly different between IC and CLTI patients (48.7 vs 44.4, p=0.251).

164 of 226 patients had available imaging studies for ACI measurements. Baseline characteristics at the time of enrollment in relation to survival and MACEs and MALEs are presented in Tables 10, 11 and 12.

Table 10. Baseline demographics. Modified from Rantasalo et al., 2022.

	ALIVE N (%) OR MEAN (SD)	DEAD N (%) OR MEAN (SD)	P-VALUE
PATIENTS	100 (44.2)	126 (55.8)	
OVERALL SURVIVAL MEAN, YEARS	5	2.91 (1.9)	
MEAN AGE AT THE IMAGING, YEARS	67.30 (9.31)	75.06 (8.6)	<0.001*
MEAN ACI	46.05 (25.7)	57.43 (23.4)	0.003*
MEAN SERUM CREATININE, µMOL/L	80.36 (25.5)	106.61 (83.3)	0.003*
LOW-DENSITY LIPOPROTEIN, MMOL/L	2.29 (1.0)	2.19 (0.9)	0.4589
TOE-BRACHIAL INDEX	0.34 (0.2)	0.25 (0.2)	<0.001*
MEAN ANKLE-BRACHIAL INDEX	0.60 (0.3)	0.71 (0.7)	0.106
ABI <0.5	41 (43.2)	59 (48.8)	0.022*
ABI 0.5-0.9	44 (46.3)	42 (34.7)	0.022*
ABI 0.9-1.4	9 (9.5)	8 (6.6)	0.022*
ABI >1.4	1 (1.1)	12 (9.9)	0.022*
MEN	57 (57.0)	71 (56.3)	0.922
INTERMITTENT CLAUDICATION	66 (66.0)	28 (30.2)	<0.001*
CRITICAL LIMB ISCHEMIA	34 (34.0)	88 (69.8)	<0.001*
HYPERTENSION	64 (64.0)	103 (81.7)	0.003*
ASTHMA	1 (1.0)	13 (10.3)	0.004*
CORONARY ARTERY DISEASE	22 (22.0)	45 (35.7)	0.025*
CONGESTIVE HEART FAILURE	0	37 (29.4)	<0.001*
ATRIAL FIBRILLATION	8 (8.0)	34 (27.0)	<0.001*
PREVIOUS ISCHEMIC STROKE	9 9.0)	19 (15.1)	0.168
HYPERCHOLSTEROLEMIA	32 (32.0)	40 (31.7)	0.968
TYPE 1 DIABETES	7 (7.0)	7 (5.6)	0.655
TYPE 2 DIABETES	24 (24.0)	41 (32.5)	0.159
NO DIABETES	69 (69.0)	78 (61.9)	0.267
RENAL INSUFFISIENCY	9 (9.0)	45 (35.7)	<0.001*
UREMIA	0	12 (9.5)	0.002*
RHEUMATOID ARTHRITIS	4 (4.0)	14 (11.1)	0.050*
STATIN PRESCIBED	66 (66.0)	77 (61.1)	0.449
ASPIRIN USE	64 (64.0)	76 (53.2)	0.102
CLOPIDOGREL USE	11 (11.0)	9 (7.1)	0.311
ANY ANTIPLATELET THERAPY	71 (71.0)	72 (57.1)	0.032*
WARFARIN	12 (12.0)	37 (29.4)	0.002*
BETA-BLOCKER	43 (43.0)	85 (67.5)	<0.001*
ANY RENIN-ANGIOTENSIN SYSTEM INHIBITOR	58 (58.0)	82 (65.1)	0.276
CALCIUM CHANNEL BLOCKERS	29 (29.0)	41 (32.5)	0.568
DIURETICS	11 (11.0)	53 (42.1)	<0.001*
NITROGLYCERIN	12 (12.0)	33 (26.2)	0.008*
GLUCOCORTICOIDS	12 (12.0)	30 (23.8)	0.023*
METFORMIN	21 (21.0)	20 (15.9)	0.321
BISPHOSPHONATES	3 (3.0)	12 (9.5)	0.050*

Total survival: All-cause mortality was 13.7% (31) at 1 year, 26.1% (59) at 3 years and 46.9% (106) at 5 years (SE 0.003, overall Kaplan-Meier analysis). Non-survivors had higher ACI than survivors (57.71 vs 45.59, p=0.002). TBI was also significantly lower in non-survivors (0.343 vs 0.249, p<0.001). Non-survivors also had more often coronary artery disease (CAD), renal insufficiency, congestive heart failure, atrial fibrillation, critical limb ischemia and hypertension at the baseline. Table 10 and Figure 21.

ACI>43 was associated with greater mortality in Log-rank test (p=0.005), Figure 21. Value of 43 is derived from CART-analysis, which is presented in Figure 22. Patients with ACI>43 had total mortality of 65.0% compared to 34.8% in patients with ACI≤43. Statistically significant risk factors for mortality in multivariate Cox regression model were ACI (HR 1.13, 95% CI 1.01-1.26 for 10 units), age (HR 1.05 per year, 95% CI 1.02-1.08), previous heart failure (HR 4.53, 95%CI 2.59-7.91), renal insufficiency (HR 2.19, 95% CI 1.27-3.79) and asthma (HR 2.86, 95% CI 1.23-6.61). ACI>43 had a HR for mortality was 1.83 (95% CI 1.01-3.32), Figure 23.

Mortality was highest among patients with high ABI (>1.4), 59.0%, and low ABI (<0.5), 92.3%, p=0.028) when compared to other ABI categories (normal = 0.9-1.4 and moderately low 0.5-0.9). Non-survivors had significantly lower TBI (0.249 vs 0.343, p<0.001) than survivors. Harrel´s C for Cox model including ABI as variable instead of ACI was 0.75. Harrel´s C for same model with TBI instead of ACI was 0.74. Harrel´s C for same model with ACI was 0.72. C-index for ACI as only variable in the Cox model was 0.58. For TBI, C-index was 0.61 and for ABI 0.52.

Table 11. Demographics and significant univariates in proportion for MACEs. Modified from Rantasalo et al., 2022.

	NO MACE N (%) OR MEAN (SD)	MACE N (%) OR MEAN (SD)	P-VALUE
PATIENTS	129 (57.1)	95 (42.0)	
OVERALL SURVIVAL MEAN, YEARS	3.72 (1.83)	3.97 (1.57)	0.287
MEAN AGE, YEARS	70.06 (9.6)	73.20 (9.73)	0.032*
MEAN ACI	46.31 (27.1)	59.70 (20.4)	0.001*
TBI	0.30 (0.2)	0.27 (0.2)	0.154
LOW-DENSITY LIPOPROTEIN, MMOL/L	2.29 (0.9)	2.16 (1.0)	0.331
CREATININE, µMOL/L	90.17 (40.2)	101.36 (90.0)	0.212
MEAN ABI	0.70 (0.6)	0.62 (0.5)	0.252
ABI <0.5	51 (41.5)	49 (53.8)	0.322
ABI 0.5-0.9	52 (42.3)	32 (35.2)	0.322
ABI 0.9-1.4	11 (8.9)	6 (6.6)	0.322
ABI >1.4	9 (7.3)	4 (4.4)	0.322
SURVIVED	67 (51.9)	31 (32.6)	0.004*
DECEASED	62 (48.1)	64 (67.4)	0.004*
MALE	66 (51.2)	60 (63.2)	0.074
FEMALE	63 (48.8)	35 (36.8)	0.074
CAROTID STENOSIS	3 (2.3)	9 (9.5)	0.019*
PREVIOUS ISCHEMIC STROKE	11 (8.5)	17 (17.9)	0.036*
CORONARY ARTERY DISEASE	29 (22.5)	36 (37.9)	0.012*
INTERMITTENT CLAUDICATION	68 (52.7)	35 (36.8)	0.018*
CRITICAL LIMB ISCHEMIA	61 (47.3)	60 (63.2)	0.018*
BETA BLOCKER	65 (50.4)	62 (65.3)	0.026*
NITROGLYCERIDE	14 (10.9)	31 (32.6)	<0.001*
MALE REVASCULARIZATION	43 (33.3)	47 (49.5)	0.015*

MACE-free survival: 95 (42.0%) patients suffered MACE during follow-up. Mean ACI was higher with patients who suffered MACEs (59.69 vs 46.31, p=0.001). Non-survivors suffered MACE more often (48.8% vs 34.4%, p=0.029) than survivors. Patients who suffered MACE had more often carotid stenosis, previous ischemic stroke, coronary artery disease or critical limb ischemia than those who survived without MACE. Demographic according to the MACEs are presented Table 11.

In multivariable Cox regression analysis, risk for MACE was associated with ACI (HR 1.10 per 10 units, 95% CI 1.00–1.22). Also, ACI>43 had HR 3.14 (95% CI 1.67–5.91 for MACE. Occurrence of MALE revascularization (HR 5.033, 95% CI 3.016–8.401) increased risk for MACE. Figure 23. In Log-rank test (Kaplan-Meier) ACI>43 was associated with MACEs compared to MACE-free survival, p=0.0012, Figure 21 and 23.

MALE-free survival: 103 patients suffered MALE (92 revascularizations and 27 major amputations, some in same subjects). ACI was not statistically significantly

different in patients who suffered MALE (54.65 vs 49.32, p=0.176) compared to those who did not. Type 2 diabetes (19.5% vs 39.8%, p=0.001), MACE MI (17.1% vs 32.0%, p=0.009) and critical limb threatening ischemia (46.3% vs 63.1%, p=0.012) were more common in patients who suffered MALE than those who did not. Demographic according to the MACEs are presented Table 12.

Risk for MALEs was not associated with ACI in multivariable Cox regression model. Figure 23. In Log-rank test and related Kaplan-Meier curve, ACI>43 was not significantly associated with MALE-free survival, p=0.117, Figure 21.

Patients who suffered MALE had lower mean TBI (0.26 vs 0.31, p=0.032) than those who did not suffer MALEs. Risk for MALE was associated with use of clopidogrel (HR 2.17, 95% CI 1.19-3.93) and type two diabetes (HR 1.66, 95% CI 1.05–2.63) in multivariate Cox regression model.

Table 12. Demographics and significant univariates in proportion to major adverse leg events (MALEs). Modified from Rantasalo et al., 2022.

	NO MALE N (%) OR MEAN (SD)	MALE N (%) OR MEAN (SD)	P-VALUE
PATIENTS	123 (54.4)	103 (45.6)	
AORTIC CALCIFICATION INDEX	49.45 (27.3)	54.78 (22.4)	0.175
SERUM CREATININE, µMOL/L	89.89 (35.1)	101.18 (89.3)	0.200
AGE AT THE IMAGING, YEARS	70.76 (10.2)	71.87 (9.16)	0.443
LOW-DENSITY LIPOPROTEIN LEVEL, MMOL/L	2.36 (0.87)	2.09 (0.99)	0.046*
TBI	0.31 (0.18)	0.26 (0.16)	0.032*
ABI	0.68 (0.56)	0.64 (0.5)	0.521
ABI <0.5	50 (43.1)	50 (48.5)	0.742
ABI 0.5–0.9	49 (42.2)	37 (36.0)	0.742
ABI 0.9–1.4	9 (7.8)	8 (7.8)	0.742
ABI >1.4	8 (6.9)	5 (4.9)	0.742
SURVIVED	55 (44.7)	44 (42.7)	0.763
DECEASED	68 (55.3)	59 (75.3)	0.763
MEN	62 (50.4)	66 (64.1)	0.039*
WOMEN	61 (49.6)	37 (36.0)	0.039*
TYPE 2 DIABETES MELLITUS	24 (19.5)	41 (39.8)	0.001*
UREMIA	3 (2.4)	9 (8.7)	0.035*
MACE MI	21 (17.1)	33 (32.0)	0.009*
INTERMITTENT CLAUDICATION	66 53.7)	38 (36.9)	0.012*
CRITICAL LIMB ISCHEMIA	57 (46.3)	65 (63.1)	0.012*
STATIN	70 (56.9)	73 (70.9)	0.030*
CLOPIDOGREL	4 (3.3)	16 (15.5)	0.001*
ANTI-PLATELET THERAPY	68 (55.3)	75 (72.8)	0.006*
GLIPTIN	9 (7.3.)	17 (16.5)	0.031*

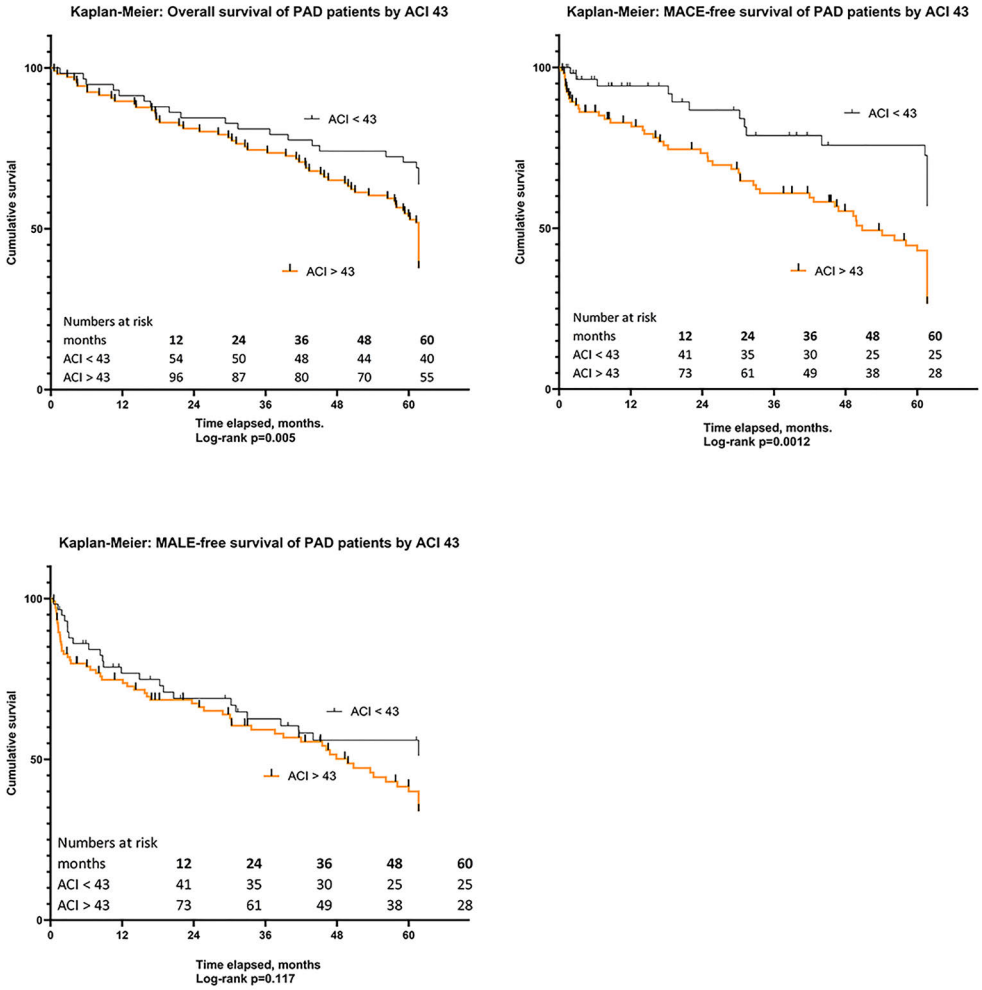

Figure 21. Kaplan-Meier curves showing MACE-free survival, MALE-free survival and overall survival of PAD patients categorized by ACI under or over 43. MACE-free survival and survival overall were statistically significantly better with those who had ACI under 43. Reproduced with permission of Elsevier ltd. from Rantasalo et al., 2022.

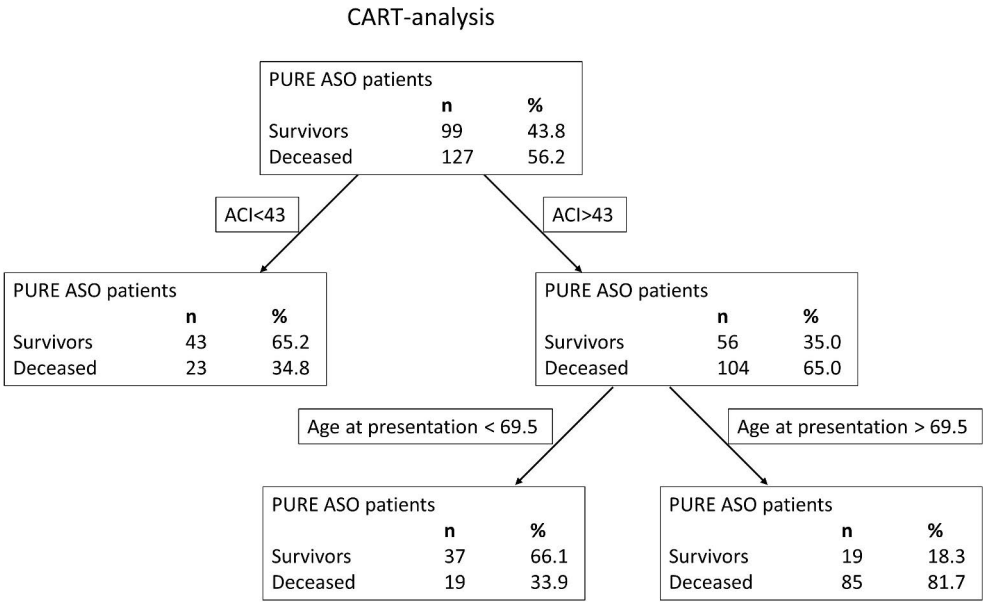

Figure 22. Classification and regression tree (CART) analysis of peripheral artery disease patients´ survival according to ACI threshold (over or under) 43. Reproduced with the permission of Elsevier ltd. from Rantasalo et al., 2022.

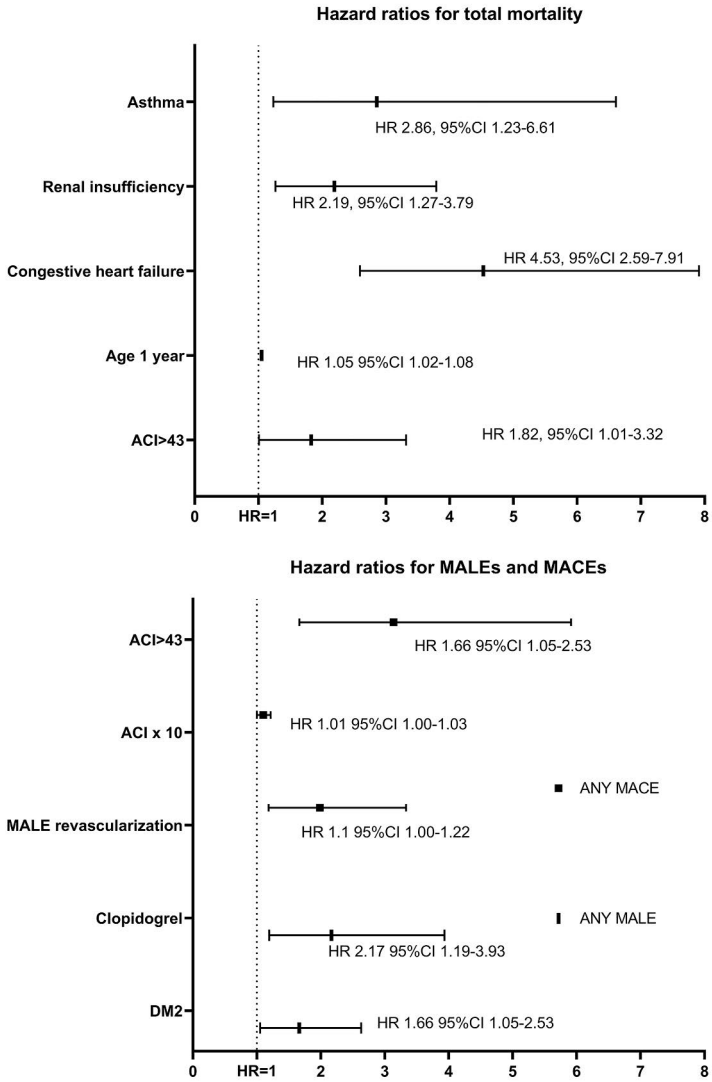

Figure 23. Hazard ratios (HRs) for total survival, major adverse cardiovascular evets (MACEs), and major adverse leg events (MALEs). Reproduced with the permission from Elsevier ltd. from Rantasalo et al., 2022. The model for mortality is adjusted for sex, serum creatinine, ankle-brachial index (ABI), hypertension, asthma, coronary artery disease atrial fibrillation, use of bisphosphonates, and rheumatoid arthritis. The model for MACE was adjusted for age, sex, and coronary artery disease. The model for MALE was adjusted for age, sex, toe-brachial index (TBI), aortic calcification index, stage of peripheral artery disease (intermittent claudication/critical limb ischemia), uremia, use of statins, type 2 diabetes, and the use of clopidogrel.

5.3 Study III: Low and borderline ankle-brachial index is associated with intracranial aneurysms

In total, 2751 patents underwent ABI measurements. Of them, 1,693 (61.5%) presented with a low ABI (≤0.9), 156 (5.7%) had a borderline ABI (0.91-0.99), 238 (8.7%) presented with a high ABI (>1.4), and 664 (24.1%) had a normal ABI (1.0-1.4). 776 had undergone brain MRA and/or CTA or had a confirmed diagnosis of RIA, and these patients were included in the study. Baseline characteristics are presented in Table 13.

Similar proportions of patients had undergone cranial imaging across ABI categories: 27.4% (464 out of 1693) in the low ABI group, 30.1% (47 out of 156) in the borderline ABI group, 23.9% (57 out of 238) in the high ABI group, and 31.3% (208 out of 664) in the normal ABI group.

Imaging study was performed due screening for IA in five patients. One of them was in the normal ABI (1.0-1.4) group, three were in the low ABI (≤0.9) group, and one was in the borderline ABI (0.91-0.99) group.

Overall, 464/776 (59.8%) had low ABI (≤0.9), 47/776 (6.1%) had a borderline ABI (0.91-0.99), 57/776 (7.3%) had a high ABI (>1.4), and 208/776 (26.8%) had a normal ABI (1.0-1.4).

There was no disagreement in the diagnosis of IA in cerebrovascular imaging studies between the interpreters.

Table 13. Baseline characteristics. Modified from Laukka et al. 2024.

BASELINE CHARACTERISTICS	NORMAL ABI (1.0-1.4) N (%) OR MEAN (SD) OR MEDIAN (IQR)	LOW ABI (≤0.9) N (%) OR MEAN (SD)	BORDERLINE ABI (0.91-0.99) N (%) OR MEAN (SD)	HIGH ABI (>1.4) N (%) OR MEAN (SD)	P VALUE
PATIENTS	208 (26.8)	464 (59.8)	47 (6.06)	57 (7.3)	
MEAN AGE AT ABI MEASUREMENT, YEARS	67.0 (10.8)	69.8 (9.6)	66.1 (13.1)	68.3 (11.5)	0.003
MEAN AGE AT CEREBROVASCULAR IMAGING, YEARS	69.4 (11.9)	72.2 (10.0)	68.6 (14.2)	68.0 (12.6)	0.010
IA SCREENING)	3 (1.4)	1 (0.2)	1 (2.1)	0 (0)	0.146
SEX, FEMALE	39.4	38.4	53.2	22.8	0.016
SMOKING HISTORY, NO. OF PATIENTS, TOTAL					<0.001
YES	115 (55.1)	365 (78.6)	32 (68.9)	34 (58.9)	
NO	93 (44.9)	99 (21.4)	15 (31.1)	26 (46.1)	
SMOKING HISTORY, MISSING DATA	14 (6.7)	29 (6.3)	2 (4.3)	5 (8.8)	0.815
HYPERTENSION	125 (60.1)	306 (66.0)	28 (59.6)	43 (75.4)	0.129

DIABETES TYPE 1 OR 2	73 (35.1)	171 (36.9)	22 (46.8)	36 (63.2)	<0.001
DIABETES TYPE 1	15 (7.2)	18 (3.9)	9 (19.2)	9 (15.8)	<0.001
DIABETES TYPE 2	58 (27.9)	155 (33.3)	12 (25.5)	29 (50.9)	0.008
HYPERCHOLESTEROLEMIA	59 (24.0)	142 (30.5)	13 (27.7)	21 (36.8)	0.19
CORONARY ARTERY DISEASE	62 (29.8)	167 (36.0)	16 (34.0)	24 (42.1)	0.27
CHRONIC HEART FAILURE	29 (13.9	76 (16.4)	10 (21.3)	16 (28.1)	0.069
ATRIAL FIBRILLATION	54 (26.0)	90 (19.4)	14 (29.8)	21 (36.8)	0.008
CHRONIC KIDNEY FAILURE	22 (10.6)	55 (11.9)	9 (19.2)	19 (33.3)	<0.001
CHRONIC OBSTRUCTIVE PULMONARY DISEASE	13 (6.3)	68 (14.7)	6 (12.8)	8 (14.0)	0.022
RHEUMATOID DISEASE	23 (11.1)	34 (7.3)	3 (6.4)	6 (10.5)	0.371
VARICOSE ULCUS	15 (7.2)	26 (5.6)	4 (8.5)	8 (14.0)	0.112
MALIGNANCY	37 (17.8)	90 (19.4)	6 (12.8)	18 (31.6)	0.070
INTRACRANIAL ANEURYSM CHARACTERISTICS					
PREVALENCE OF IAS, NO. OF PATIENTS	5 (2.4)	94 (20.3)	7 (14.9)	4 (7.0)	<0.001
UNRUPTURED	4 (1.9)	84 (18.1)	6 (12.8)	3 (5.3)	<0.001
RUPTURED	1 (0.5)	10 (2.2)	1 (2.1)	1 (1.7)	0.277
PREVALENCE OF UIA BY SEX, NO. OF PATIENTS					0.504
FEMALE	2 (2.4)	34 (19.1)	4 (16.0)	2 (15.4)	
MEN	2 (1.6)	50 (17.5)	2 (9.1)	1 (2.3)	
PROPORTION OF MULTIPLE IAS, NO. OF PATIENTS					0.213
AMONG UIA	2 (50)	9 (10.7)	0	0	
AMONG RIA	0	5 (50)	0	0	
MEDIAN SIZE OF THE LARGEST IUA/RIA	4.0 (3.0-5.0)	4.0 (3.0-5.0)	3.0 (3.0-8.0)	4.0 (3.0-7.0)	0.929
DISTRIBUTION OF LARGEST UIA OR RIA					0.817
ANTERIOR CEREBRAL ARTERY	1 (20.0)	24 (25.8)	2 (28.6)	2 (50.0)	
INTERNAL CAROTID ARTERY	3 (60.0)	29 (31.2)	1 (14.3)	1 (25.0)	
MIDDLE CEREBRAL ARTERY	1 (20.0)	20 (21.5)	2 (28.6)	0	
POSTERIOR CIRCULATION	0	8 (8.6)	2 (28.6)	0	
INTRACAVERNOUS INTERNAL CAROTID ARTERY	0	12 (12.9)	0	1 (25.0)	

ABI and intracranial aneurysms: Baseline demographics across ABI groups are present in Table 13. IA prevalence in the study population is presented in Figure 24, where RIA and UIA prevalence is categorized by ABI groups.

The prevalence of unruptured IAs was 18.1% in the low ABI (≤0.9) group, 12.8% in the borderline ABI (0.91-0.99) group, 5.3% in the high ABI (>1.4) group,

and 1.9% in the normal ABI (1.0-1.4) group (p<0.001). One patient in the low ABI (≤0.9) group was identified with an unruptured IA through screening and managed conservatively.

Within the low ABI (≤0.9) group, 2.2% had ruptured IAs, whereas in the borderline ABI (0.91-0.99) group, 2.1% had ruptured IAs. In the high ABI (>1.4) group, 1.8% had ruptured IAs, and in the normal ABI (1.0-1.4) group, 0.5% had ruptured IAs (p=0.277).

Demographics are presented according to IA presentation in Table 14. The median ABI was 0.59 (IQR 0.45-0.75) in patients with unruptured IAs, 0.57 (IQR 0.51-0.79) in patients with ruptured IAs, and 0.80 (IQR 0.53-1.13) in patients without unruptured IAs (p<0.001).

Low ABI (≤0.9) (OR 13.02; 95% CI 4.01-42.24; p<0.001), borderline ABI (0.91-0.99) (OR, 8.68; 95% CI 2.05-36.69; p=0.003), and smoking history (OR 2.01; 95% CI 1.07-3.77, p=0.030) were associated with unruptured IAs in a multinomial regression analysis adjusted for age, sex, and clinically significant variables. Second model adjusted for age, sex and statistically significant variables showed similar results. Regression models are presented in Table 15.

Associations with ABI Groups: Age (OR 1.06; 95% CI, 1.04-1.08; p<0.001), smoking history (OR 4.19; 95% CI, 2.77-6.34; p<0.001) and atrial fibrillation (OR 0.59; 95% CI, 0.38-0.91; p=0.018) were associated with low ABI (≤0.9) in the multinominal regression analysis.

Borderline ABI (0.91-0.99) was associated with female sex (OR, 2.18; 95% CI, 1.10-4.31; p=0.026) and smoking history (OR, 2.35; 95% CI, 1.11-4.96; p=0.025)

High ABI (>1.4) was associated with diabetes type 1 or 2 (OR, 2.73; 95% CI, 1.36-5.49; p=0.005) and chronic kidney failure (OR, 3.96; 95% CI, 1.79-8.76; p<0.001). Females had a reduced risk (OR 0.39; 95% CI, 0.17-0.88; p=0.024) of high ABI (>1.4).

Table 14. Baseline characteristics of patients with ruptured IAs, unruptured IAs and without IAs.

VARIABLE	UNRUPTURED IA MEAN (SD) OR N (%) OR MEDIAN (IQR)	RUPTURED IA MEAN (SD) OR N (%) OR MEDIAN (IQR)	WITHOUT IA MEAN (SD) OR N (%) OR MEDIAN (IQR)	P VALUE
PATIENTS	97	13	666	
AGE, YEARS	69.5 (8.7)	68.9 (8.9)	68.6 (10.6)	0.753
FEMALE	42 (43.3)	6 (46.1)	250 (37.5)	0.467
MULTIPLE IAS	11 (11.3)	5 (38.5)	—	0.022
SMOKING HISTORY				0.013
YES	80 (82.8)	8 (61.5)	454 (68.2)	
NO	17 (17.2)	5 (38.5)	212 (31.8)	
SMOKING HISTORY, MISSING DATA	4 (4.1)	0	46 (6.9)	0.229
HYPERTENSION	66 (68.0)	10 (76.9)	43 (64.0)	0.477
DIABETES TYPE 1 OR 2	38 (39.2)	2 (15.4)	262 (39.4)	0.213
DIABETES TYPE 1	5 (5.2)	0	46 (6.9)	0.508
DIABETES TYPE 2	33 (34.0)	2 (15.4)	218 (32.8)	0.396
HYPERCHOLESTEROLEMIA	33 (34.0)	4 (30.8)	188 (28.3)	0.508
CORONARY ARTERY DISEASE	29 (29.9)	1 (7.7)	239 (35.9)	0.061
CHRONIC HEART FAILURE	19 (19.6)	0	112 (16.8)	0.207
ATRIAL FIBRILLATION	16 (16.5)	1 (7.7)	162 (24.3)	0.096
CHRONIC KIDNEY FAILURE	15 (15.5)	2 (15.4)	88 (13.2)	0.820
CHRONIC OBSTRUCTIVE PULMONARY DISEASE	18 (18.6)	1 (7.7)	76 (11.4)	0.118
RHEUMATOID DISEASE	6 (6.2)	3 (23.1)	57 (8.6)	0.121
VARICOSE ULCUS	6 (6.2)	3 (23.1)	44 (6.6)	0.064
MALIGNANCY	17 (17.5)	1 (7.7)	13 (20.0)	0.475
ABI	0.59 (0.45-0.75)	0.57 (0.51-0.79)	0.80 (0.53-1.13)	<0.001

Table 15. Multinominal regression models for unruptured IAs adjusted with age and sex.

VARIABLE	OR	95%CI	P VALUE
MODEL 1			
AGE	1.02	0.99–1.04	0.209
SEX (FEMALE VS MALE)	1.53	0.95–2.46	0.083
HYPERTENSION	1.23	0.75–2.02	0.417
SMOKING HISTORY (YES VS NO)	2.01	1.07–3.77	0.030
CORONARY ARTERY DISEASE	0.61	0.37–1.02	0.058
CHRONIC KIDNEY FAILURE	1.46	0.74–2.84	0.273
NORMAL ABI 0.9–1.4	ref.		
LOW ABI ≤0.9	13.02	4.01–42.24	<0.001
BORDERLINE ABI	8.68	2.05–36.69	0.003
HIGH ABI >1.4	3.97	0.76–20.76	0.103
MODEL 2			
AGE	1.01	0.99–1.04	0.295
SEX (FEMALE VS MALE)	1.58	0.99–2.54	0.056
SMOKING HISTORY (YES VS NO)	1.97	1.05–3.68	0.035
NORMAL ABI 0.9–1.4	ref.		
LOW ABI ≤0.9	12.69	3.91–41.12	<0.001
BORDERLINE ABI	8.80	2.09–36.98	0.003
HIGH ABI >1.4	4.47	0.83–22.00	0.083

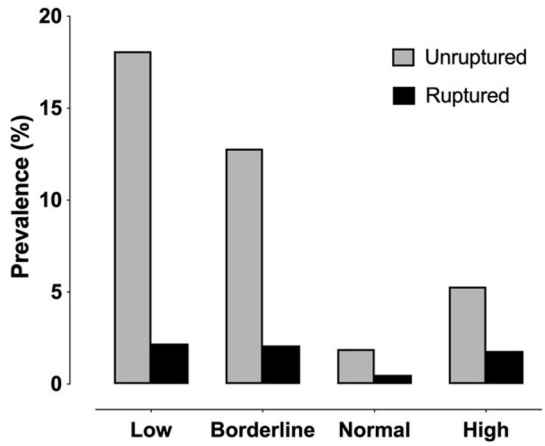

Figure 24. Reproduced with permission from Wolters Kulwer Health inc. from Laukka et al., 2024. Prevalence of unruptured and ruptured intracranial aneurysms by ABI group. ABI, ankle-brachial index.

5.4 Study IV: Association between aortic calcification and cytokine levels in patients with peripheral artery disease

Clinical characteristics: Subgroup of this cohort included 156 patients. Mean age was 70.7 years, and 64 (41.0%) were women. 100 (64.1%) of patients were ex-smokers or current smokers. Mean ABI was 0.64. Of 156 patients, 69 (46.3%) patients had ABI 0.5-0.9 and 64 (42.2%) had ABI <0.5. Mean ACI was 52.3. Baseline characteristics are presented in Table 16. From baseline characteristics, age was associated with ACI (β 0.88 per year, SE 0.20, p<0.001) in univariate linear regression.

Cytokines and ACI: Logarithmic transformations of cytokine levels were used in univariate regressions because none of the cytokine levels were normally distributed. Logarithmic transformation values of cytokines MIP 1a (β 12.54, SE 5.81, p=0.033), CTACK (β 23.08, SE 5.22, p<0.001) and MIG (β 9.40, SE 2.82, p=0.001) were associated with increased ACI. Cytokines are presented in Table 5 and 17. Univariate regression analyses are presented in Table 18.

Logarithmic transformation value of CTACK was significantly higher in the highest ACI tertile, compared to the two lower tertiles (logCTACK 7.29 (SE 0.05) in first tertile, 7.25 (SE 0.05) in second tertile and 7.53 (SE 0.05) in third tertile, ANOVA p-value=0.002, Tukey´s HSD p-value=0.002 for third vs first tertile and 0.023 for third tertile vs. second tertile. Also, Logarithmic transformation of cytokine MIG value was significantly higher in the highest tertile (7.65) compared to the lowest tertile (7.3), Tukey´s HSD p-value=0.028.

Logarithmic transformation of CTACK was associated with increased ACI in multivariate regression analysis adjusted for hypertension, smoking, sex, diabetes type 1 or type 2, age, dyslipidemia, and chronic kidney disease. Regression estimate β for un-transformed value of CTACK was 0.011 (SE 0.003, p=0.001) per one unit increase in ACI, and the regression estimate β for logarithmic transformation of CTACK was 17.90 (SE 5.55, p=0.002).

Regression estimate β was 1.08 (SE 0.32, p=0.001) for 100 units in untransformed CTACK value. Range for cohort subgroup CTACK values was 664.9 – 3663.4.

In multivariate analysis, age was also associated with ACI with regression estimate β 0.91 (SE 0.22, p<0.001), per one year.

Multivariate analysis with same adjustments with MIP-1a instead of CTACK showed that MIP-1a was not statistically significantly associated with ACI. When same model was run with MIG instead of CTACK, MIG showed significant association to ACI with regression estimate β 0.003 (SE 0.001, p=0.030) per one unit of untransformed MIG value. For logarithmic transformation of MIG, regression

estimate β was 6.80 (SE 3.33, p=0.043). Multivariate regression analyses are presented in Table 18.

Cytokines associations with ACI was also investigated by analyzing AUC/ROC values derived from binary regressions. These regressions were run with binary variable "ACI over 50", coded as 1 = ACI is over 50 and 0 = ACI is less than 50. Each cytokine was individually analyzed in this model and each cytokines binary regressions respective AUC-value with respective 95% confidence intervals were determined and plotted together in Figure 25, which shows how cytokines CTACK, MIG, IFNg, IL-5 and MIP1a were associated statistically significantly with ACI over 50, as these cytokines binary regressions' respective AUC-values did not cross 0.5.

Table 16. Baseline demographics.

	N (%) OR MEAN (SD)
AGE	70.7 (9.5)
WOMEN	64 (41.0)
HYPERTENSION	110 (70.5)
DYSLIPIDEMIA	50 (32.0)
TYPE 2 DIABETES	43 (27.6)
TYPE 1 DIABETES	10 (0.06)
NO DIABETES	103 (66.0%)
CORONARY ARTERY DISEASE	45 (28.8)
CONGESTIVE HEART FAILURE	26 (16.7)
CHRONIC KIDNEY FAILURE	33 (21.1)
PREVIOUS STROKE	19 (12.2)
ATRIAL FIBRILLATION	26 (16.7)
COPD	38 (24.4)
SMOKING (CURRENT AND POST-CESSATION)	100 (64.1)
SERUM	
LDL	2.24 (0.97)
HDL	1.44 (0.54)
CREATININE	93.8 (72.7)
ACI	51.3 (25.3)
ABI (LOWER)	0.64 (0.48)
TBI	0.31 (0.17)

Table 17. Key Cytokines (pg/ml).

	MEAN	MEDIAN	MIN	MAX	SE	SD	25% QUARTILE	75% QUARTILE
CTACK	1730.1	1634.0	664.98	3663.4	49.87	622.9	1219.3	2143.4
MIG	2222.2	1739.8	249.02	14697.6	143.42	1791.3	1161.0	2732.5
MIP1A	45027	45566	32203	35.67	0.35	11414	8.18	12.56

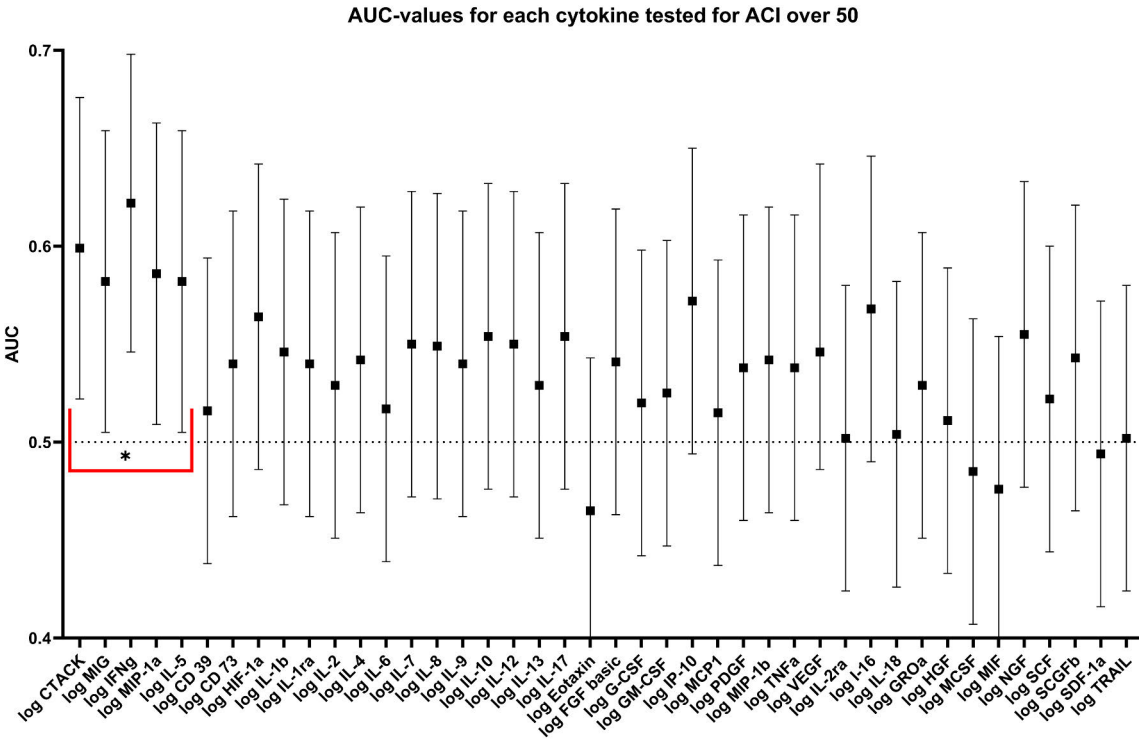

Figure 25. AUC-values with 95% confidence intervals for each ROC curve derived from binary regression run for binary variable "ACI over 50" with each cytokine. AUC-value is derived from binary univariate regression where response variable was binary variable of ACI over 50 (code as 1, positive level). Each cytokine was tested separately against this binary variable and ROC-curve was analyzed for each test and AUC-value for each ROC-curve was plotted here. * AUC-values 95% confidence intervals do not cross 0.5.

Table 18. Univariate and multivariate and binary regressions for ACI.

UNVARIATE LINEAR	UNADJUSTED β (SE)	P-VALUE
LOG MIP-1A	12.54 (5.81)	0.033
LOG CTACK	23.08 (5.22)	<0.001
LOG MIG	9.40 (2.82)	0.001
AGE	0.88 (0.20)	<0.001
MULTIVARIATE LINEAR	**ADJUSTED β (SE) ***	
LOG MIP-1A	5.22 (5.90)	0.377
LOG CTACK	17.90 (5.55)	0.002
CTACK **	0.11 (0.003)	0.001
100 UNITS CTACK **	1.08 (0.32)	0.001
LOG MIG	6.80 (3.33)	0.043
AGE	0.91 (0.22)	<0.001
MULTIVARIATE BINARY FOR ACI OVER 50		
LOG MIP-1A	0.21 (0.54)	0.693
LOG CTACK	0.97 (0.53)	0.068
LOG MIG	0.38 (0.31)	0.221
MULTIVARIATE BINARY FOR ACI OVER LOWEST TERTILE		
LOG MIP-1A	1.28 (0.62)	0.038
LOG CTACK	1.01 (0.55)	0.068
LOG MIG	0.63 (0.34)	0.063

* multivariate linear regression adjusted for age, sex, smoking, hypertension, diabetes type 1 or type 2 and chronic kidney disease.
** no logarithmic transformation
B, β (beta) = Regression coefficient
SE = Standard Error

6 Discussion

6.1 Study I: Intracranial aneurysm is predicted by abdominal aortic calcification index

The result of this study is that increased aortic calcification is associated with an increased overall risk of IAs. Mean ACI was significantly higher among patients with RIA than their respective matched controls. The risk of RIA was also associated with greater ACI when compared to matched controls. Mean ACI was higher among patients with UIA than matched controls.

The independent association between ACI values and IAs was further confirmed by CART analysis, in which RIAs in particular were associated with ACI. CART analysis showed that the highest overall risk of IA was among patients with ACI values greater than 3.1, who had hypertension and were older than 63. Further, a threshold ACI value of over 3, based on CART analysis was, tested in the regression models and was found to be associated with a higher risk for RIA compared to the matched controls. The same threshold value was also associated with a higher risk of RIA than the UIA.

While the higher ACI values were not associated with increased risk of UIA compared to their matched controls when ACI was used as a continuous variable, the ACI threshold value over 3 was associated with increased odds for UIA compared to matched controls.

Aortic calcification can be used as a marker of systemic atherosclerosis. It is reproducible, simple, and relatively unbiased index, that directly describes the extent of arterial wall calcification which represent atherosclerosis (New and Aikawa 2011). The index is able to show atherosclerosis regardless of recorded risk factors and previous studies have found ACI values to be associated with atherosclerotic cardiovascular and cerebrovascular diseases. For example, greater carotid IMT, incidence and severity of CAD and cardiovascular events in patients with CAD are associated with higher ACI values (Tsushima et al. 2008; Takayama et al. 2016; An et al. 2014; Tatami et al. 2015). The results of this study suggest that high ACI values might also indicate an increased risk for RIAs and UIAs.

The pathophysiology of atherosclerosis is a multifactorial cascade which is based on inflammation, genetic factors and environmental risk factors. Some of these

factors might also be involved in IA pathophysiology. (Rutsch et al. 2011; Tulamo et al. 2018; Libby et al. 2019). Inflammation plays a crucial role in the pathophysiology of IA formation and rupture (Chalouhi et al. 2012). The role of inflammation is also well established in atherosclerosis (Raggi et al. 2018). Therefore, there is a variety of possible explanations for the association between ACI values and IAs. Even though the morphological changes in IA and atherosclerosis are different, there are similarities in their inflammatory profiles. Inflammatory triggers might be different – currently it is thought that oxidized low-density lipoprotein can trigger atherosclerotic inflammation and mechanical shear stress and hemodynamic disturbance can trigger inflammation leading to IA developement (Raggi et al. 2018; Frösen et al. 2019). In IAs and atherosclerosis, the initial phases of pathophysiology take place in the endothelium. Dysfunctions in the ECs and the signalling related to them is a feature present in both IAs and atherosclerosis, and the severity of endothelial dysfunction correlates with arterial calcifications (Torngren et al. 2020; Tulamo et al. 2018).

The results of some studies already support the idea of common features in pathophysiology if IA and atherosclerosis. Zhong et al. found that atherosclerotic lesions and immunohistochemical signs of inflammation in IA were both associated with aneurysm wall enhancement in imaging (Zhong et al. 2021). IA and atherosclerotic plaques both harbour T helper lymphocytes and macrophages, and the same types of cytokines (IL-1b, TNFa) are found in both (Kataoka et al. 1999; Hasan et al. 2012; Chalouhi et al. 2012; Moriya 2019; Raggi et al. 2018; Caird et al. 2006). Also, lipids are found in IAs, and dysregulatory mast cells and endothelial dysfunction are present in IAs and atherosclerosis. Common features of both are presented in Table 4 in review of literature (Elieh-Ali-Komi et al. 2024; Ollikainen et al. 2014; 2016; Sheinberg et al. 2019; Gimbrone and García-Cardeña 2016).

VSMCs phenotype switches in IAs from the normal contractile type to the synthetising and pro-inflammatory type. They lose their contractile abilities and begin to produce ECM. A similar phenomenon takes place in atherosclerosis (Nakajima et al. 2000; Starke et al. 2014; Bennett, Sinha, and Owens 2016). Inflammatory markers are present in UIAs and RIAs, but more prominent in RIAs than in UIAs. RIAs have more pro-inflammatory cells than UIAs and macrophages polarization to M1 type pro-inflammatory cells or M2 type anti-inflammatory cells might be altered in IAs. (Frösen et al. 2004; Cebral et al. 2017; Rodemerk et al. 2020).

As in this study, Kang et al. reported lower prevalence of CAD among RIA patients (Kang et al. 2015). However, Huhtakangas et al. have reported that mortality was higher at younger age among their IA patients than their matched control patients due to cardiovascular and cerebrovascular diseases (Huhtakangas et al. 2015). They also found multiple IAs to be related to IA patients long-term mortality

which could mean more widespread inflammation of the cerebral arteries. Similarly to Kang et al. 2015, the RIA patients in our data also had the fewest markers of CAD but within each IA patient group (RIA, UIA) and the control patients, the mean ACI values were actually higher among patients with CAD or history of coronary interventions than among those without.

Therefore, these results support the hypothesis that IAs are related to atherosclerotic diseases. Method of this study utilises ACI to expose the atherosclerotic burden of the subjects, and an association between IAs and atherosclerotic burden was found, even though no other specific, heart-related atherosclerotic endpoints were present.

These results did not show increased risk for IAs with hypercholesterolemia (OR 0.42, 95 % CI 0.27 – 0.91 for RIA) or alcohol abuse (OR 0.41, 95 % CI 0.20 – 0.86 for RIA) which are known to have an association with RIA (Feigin et al. 2005; Can et al. 2018). Even though smoking is well established risk factor for IAs, in this data smoking did not exhibit an increased risk for IAs (Can et al. 2017). Several factors might explain these findings. This study was based on register data, which is susceptible to bias generated by insufficient reporting. The IA patients' risk profiles might have improved (reduced risk behaviour, lipid profile improvement) after diagnosis, but the method of this study was not able to show this kind of change. Also due to the study design, current and ex-smokers were categorised as smokers and reported together. It can be hypothesized that established hypercholesterolemia might be associated with contemporary statin treatment and possibly with other preventive methods, and it is possible that hypercholesterolaemia acts as a surrogate marker of preventive methods applied for CVD.

Finally, alcohol abuse may not emerge as a statistically significant risk factor, because it might be associated with other risk factors for IA (Szőllősi et al. 2023). Hence, the risk of IA might be a resultant of multiple risk factors that are increased in concordance with the alcohol abuse, and this phenomenon might render alcohol abuse as non-significant variable while other risk factors act as surrogate markers for it.

Older age also showed inverse association to the risk of IAs. A large study by Kaneko et al. 2023 found that the cardiovascular risk factors were more robustly associated with cardiovascular risk in younger people than older. While some of these cardiovascular risk factors are shared with IAs, it is possible that these risk factors pose a higher risk for IA in younger participants. However, this study I does not investigate age as a independent risk factor, but rather aims to use ACI as a surrogate marker for total risk for IA (Vlak et al. 2013a; 2013b; Kang et al. 2015; Rasmussen, Chong, and Alter 2007; Szőllősi et al. 2023; Kaneko et al. 2023).

It is noteworthy that smoking was quite common in this study population, as 67.5% of all the participants were categorised together as ex-smokers or current

smokers. Present data on smoking relies on patient records and it carries difficulties in interpreting the findings with it. Used methods did not allow classification of smokers according to the smoking intensity or determine the duration since cessation. Reporting heavy smokers together with those who barely are identifiable as smokers might have falsely increased the number of patients categorised as smokers. Separating smokers from ex-smokers could produce in the same way biased information on smoking. Although the risk of RIA is increased by the duration and intensity of smoking, a recent study has found that smoking cessation or duration since cessation do not revert the risk to baseline level (Can et al. 2017). Therefore it was seen most fit to report ex- and current smokers together. Most importantly, these inverse findings with recorded risk factors underline the relevance of the ACI as a surrogate marker of atherosclerotic burden. The ACI is not dependent on reported risk factors but rather it summarises individual's atherosclerotic vascular disease burden.

The inversed statistical finding concerning RIA and atherosclerotic CVD may also be explained by the RIA patients having been asymptomatic until their IA rupture and not having been previously diagnosed with CVD. Patients with incidentally found UIAs have been evaluated for IA in part due to known risk factors and CVD which might have been the reason for cranial imaging or suspected cerebrovascular diseases, and hence UIA patients might have presented with a more complete risk profile. Based on these data, IA rupture and the resulting aSAH could be seen as the first presentation of vascular disease. Finally, potential survivor bias might also have occurred as the RIA patients might have had asymptomatic but unrecorded CVD, and also because some RIA patients are likely to develop atherosclerotic burden after IA rupture.

One potential factor is that patients with cardio- or cerebrovascular diseases usually receive optimal medical therapy (statins, antithrombotic medication, antihypertensive medication) and counselling in preventive lifestyle modifications as a part of secondary prevention, and they might also have undergone invasive treatment of affected vascular beds. These methods might affect the emergence of cardiovascular events, which may consequently reduce the prevalence of CVD in RIA patients. Therefore, RIA might be seen as a surrogate marker of lacking optimal preventive pharmacotherapy.

Further, the categorisation of IA patients forces bias into the study design, as some UIA patients may be RIA patients in the future, but others' aneurysms may be treated electively, preventing rupture. Thus, some patients with atherosclerotic burden and inflammation possibly leading to aneurysm rupture are categorised as UIA patients due to an early diagnosis rather than a diagnosis of a different disease entity. This is plausible, as it has already been established that inflammation-

extinguishing medication such as aspirin and statins might reduce the risk of IA rupture and growth (Hasan et al. 2011; Cheng et al. 2019; Can et al. 2018).

In conclusion, abdominal aortic calcification was more common among patients with IAs than their matched controls. In addition, higher abdominal aortic calcification may be associated with RIAs. These results suggests that IAs and especially RIA could be both a marker and a result of increased atherosclerotic burden, and careful consideration of primary prevention could be reasonable in the case of IA patients.

6.2 Study II: Aortic calcification index predicts mortality and cardiovascular events in operatively treated patients with peripheral artery disease

The mortality and cardiovascular morbidity of operatively treated PAD patients was associated with ACI values, but ACI values were not associated with MALEs. Patients with ACI > 43 exhibited a greater risk for mortality according to the CART analysis. ACI over this threshold value was also related to greater risk for MACEs during follow-up. The ACI showed similar ability in all-cause mortality risk analysis as the ABI and TBI, according to Harrell's C-statistics. In this study design, Harrel's C results are interpreted as none of these were greatly superior or inferior (Longato, Vettoretti, and Di Camillo 2020).

PAD patients outcome is assessed by mortality, limb patency (after initial treatment) and survival free cardio- or cerebrovascular events. The current classifications of patient outcomes have certain deficits. Disease severity is estimated by the distribution of atherosclerotic lesions causing stenoses in the iliac and lower limb arteries and distal aorta. The latest classification system Global Anatomic Staging System (GLASS) offers guidance for choosing revascularisation method but does not perform patient risk stratification very well (El Khoury et al. 2021; P. Liang et al. 2021; Kodama et al. 2020; Hicks and Zhang, et al. 2021; Conte et al. 2019). PLAN (Patient risk estimation, Limb staging and ANatomic distribution) is a risk assessment tool offered by the same guidelines. It is based on factors associated with reduced survival, but it does not include any patient- or disease specific measurements (Conte et al. 2019).

The Transatlantic Inter-society Consensus for the management of PAD (TASC II staging) is an older but still more widely used classification system for PAD. It is also based on anatomic disease distribution, but lacks information on infra-popliteal vasculature and describes survival narrowly. Studies have found that TASC II classes seem to have no association with PAD patients' overall survival or limb patency (Norgren et al. 2007; Kumakura et al. 2015).

PAD patients' risk of amputation and benefit from revascularisation can be assessed on more detail using the Wound, Ischemia and Foot Infection (WIfI) classification, but this also lacks tools for overall survival estimation. The survival of PAD patients in relation to MALEs, MACEs and limb patency seems to depend on antithrombotic pharmacotherapy strategies. This might render bias in event-free survival analyses (Bauersachs et al. 2021).

Aortic calcification's association with CVD-related morbidity and mortality is well established and the results of this study resemble those of studies investigating the ability of ABI to be a prognostic marker. It has been found that aortic calcification and low ABI are associated with higher total healthcare costs (Criqui et al. 2014; Lewis et al. 2018; Levitzky et al. 2008; Oishi et al. 2020; Aboyans et al. 2005; Escofet Peris et al. 2020; Bonaca et al. 2020; Tullos et al. 2013; Criqui et al. 2010; Schousboe et al. 2020). Greater ACI values have also been found among patients with renal insufficiency, a disease that accelerates vascular calcification (Hanada et al. 2010). However, in this study, serum creatinine was not statistically significantly different in patients with MALE or MACE compared to those who had an event. Serum creatinine was not correlated to ACI, and serum creatinine was not statistically significantly higher with patients who had diagnosed renal insufficiency compared to patients with normal renal function. Furthermore, Study I shows the association between ACI values and IAs. ACI is also associated with PAD incidence (Levitzky et al. 2008). Aortic calcification is utilised as a marker of atherosclerotic disease, which is caused by systemic low-grade inflammation that eventually builds calcification in artery walls (Raggi et al. 2018).

Based on these results, the ACI can be a disease-specific biomarker for visualising patients' atherosclerotic disease burden and a risk factor for cardiovascular events and mortality. Subsequent studies are warranted to establish further reference intervals for the ACI in order for it to be used alongside other metrics and risk factors in patients' risk evaluation. ACI measurements are easily reproducible, non-invasive, relatively free of bias and available with minimal resources. The imaging studies needed for ACI measurements is often readily available without additional visits, because diagnosis and classification of patients with CVD is often based on vascular imaging, which sometimes includes abdominal aorta. Abdominal CT is also quite common on the population level (Pola et al. 2018). In clinical practice, the ACI can be used to obtain additional information when assessing patients' risk after imaging has been performed. The power of ACI in relation to other metrics used in risk assessment is yet to be established.

In conclusion, this study found that ACI values were associated with PAD patients' overall survival and MACE-free survival. Based on present results, the ACI can improve the risk assessment of PAD patients.

6.3 Study III: Low and borderline ankle-brachial index is associated with intracranial aneurysms

This study showed that low ABI (≤0.9) and borderline ABI values (0.91-0.99) were associated with an increased risk of an IA than normal ABI (1.0-1.4). The prevalence of UIAs in the low ABI group (≤0.9) was 18.1%, whereas the prevalence of RIAs was 2.2%. The borderline ABI group presented the prevalence of 12.8% of UIAs, whereas the prevalence of RIAs was 2.1% in this group. The subjects in normal the ABI group had a much lower prevalence of UIAs (1.9%) and RIAs (0.5%). These data suggest that patients with lower ABI values are at substantially increased risk of an IA

This study finds a nine-fold prevalence of UIAs in the low ABI group and an approximately seven-fold prevalence in the borderline ABI group compared to those in the normal ABI group (ABI 1.0-1.4). It should be noted that the prevalence of UIAs in the normal ABI group was similar to that reported for the general population (Vlak et al. 2011).

The UIA prevalence in the low and borderline ABI groups was similar to that observed in specific populations known to be at a high risk of an IA, such as patients with polycystic kidney disease or with at least two first-degree relatives with IAs. These are populations for which IA screening is recommended because they are at a high risk (Thompson et al. 2015). High IA prevalence has also been reported among females who smoke, ranging from 12% to 19% (Huhtakangas et al. 2021; Ogilvy et al. 2020).

Low ABI values (≤0.9) and high ABI values (>1.4) are both markers of vascular disease and both predict cardiovascular mortality beyond known risk factors (Criqui et al. 2010; Resnick et al. 2004; Fowkes et al. 2008). Indicators of systemic atherosclerosis (such as coronary artery calcification and abdominal aortic calcification) correlate highly with low ABI values. The ABI is an especially advantageous marker in this context because it does not require imaging or invasive investigation/exploration, and is thus readily available indicator. IAs may also be associated with an increased burden of atherosclerosis. However, the association between IAs and CVD has hitherto received relatively little attention (Allison et al. 2006; Tullos et al. 2013; Cho et al. 2019; Huhtakangas et al. 2015; Uehara, Tabuchi, and Mori 1998; Rantasalo et al. 2021).

Hypertension and smoking are both risk factors for IA and low/borderline ABI values, which may partly explain these findings (Karhunen et al. 2021; Vlak et al. 2013a; Song et al. 2019). Smoking history, low ABI values, and borderline ABI values each emerged as independent risk factors for IAs in this study. However, in the multinomial regression, the relationship between smoking and IAs appeared to be comparatively weaker than that between smoking and low/borderline ABI values, suggesting that the association with IA might be explained by different underlying

mechanisms. Low ABI values indicate a combination of several different risk factors, including genetic factors, and serves as an objective marker of vascular disease - regardless of the recorded risk factors (Aboyans et al. 2012; Resnick et al. 2004; Fowkes et al. 2008). Low ABI values may be associated with systemic inflammation and endothelial dysfunction which could increase the risk of IAs (Frösen et al. 2012; Brevetti et al. 2010).

This study found no statistically significant difference between the prevalence of UIAs among females and males in the different ABI groups. However, trend towards a heightened risk of UIAs was observed among females. A larger sample might have potential to reveal a difference. Consistent with earlier research, it has been reported that in the general population, females exhibit a 1.5-2 times higher prevalence of UIAs when compared to males, suggesting possible distinct risk factors for IAs in males and females. These kind of factors may not be reflected in ABI (Cras et al. 2020; Laukka et al. 2024; Fuentes, McGuire, and Amin-Hanjani 2022; Vlak et al. 2011).

Recommendations for IA screening based on ABI values will require a prospective study to confirming these results. Nonetheless, the results of this study show that the prevalence of IAs in the low ABI and borderline ABI groups was exceptionally high among patients who had undergone brain CTA or MRA. Beyond the established criteria for IA screening, the ABI emerges as a potential, straightforward screening tool for identifying patients at high risk of IAs. In this study, only five patients underwent imaging due to IA screening indications, and thus this study does not conclusively show that using the ABI for individuals with recognised indication for IA screening is plausible. Individuals who present with indications for IA screening should undergo screening in accordance with prevailing standards, even if their ABI values are normal.

This study population was too small to draw conclusions about any differences between the ABI groups in terms of RIAs in the ABI groups. The difference between the number of RIAs in the ABI groups was not statistically significant, only 0.5% of patients had an RIA in the normal ABI group, whereas in the low and borderline ABI groups, 2% had an RIAs. Therefore, it is intuitive to think that patients with increased atherosclerotic burden would also carry increased risk factors for RIA and therefore these kind of patients would exhibit greater RIA risk compared to patients with no significant atherosclerotic disease burden.

In conclusion, the prevalence of unruptured IAs was nearly 9-fold higher in the low ABI group and nearly 7-fold higher in the borderline ABI group compared to the normal ABI group. Notably, the prevalence of unruptured IAs in the normal ABI group was similar to that reported in the general population. ABI measurements could be clinically relevant for identifying individuals at higher risk of IAs and may help guide screening and preventive strategies in addition to established criteria for IA screening.

6.4 Study IV: Association between aortic calcification and cytokine levels in patients with peripheral artery disease

The cytokines CTACK, MIG and MIP-1a were associated with higher ACI values in the unadjusted analysis. MIG and CTACK remained associated with higher ACI values in the adjusted multivariate model. These three cytokines were distinguished from 39 cytokines in the AUC/ROC analyses derived from regression models, in which CTACK, MIG and MIP-1a showed association with higher ACI values.

CTACK also known as C-C motif chemokine ligand 27 or CCL27, is a chemotactic cytokine that is predominantly expressed in skin keratinocytes. It is induced by TNFa and it is associated with skin and other barrier tissues (Vestergaard et al. 2005). Its primary function is to recruit resident T cells and participate in T cell mediated immune homeostasis of the skin. CTACK has not been directly connected to atherosclerotic CVD, but an association between them has been found. For example, it has been used as a biomarker variable in the CHDRA model which was created for predicting the risk of CVD, which in turn can be compared to the Framingham risk score. In the CHDRA model, CTACK demonstrated a significant association with the risk of CVD (Cross et al. 2012). Previous results of the same PURE ASO cohort studies have shown CTACK to be associated with age and with Th1-IFN-γ induced cytokines. It is also associated with PAD present in the proximal lower extremity arteries (Jalkanen et al. 2019).

Dysregulation in CTACK-related signalling have been linked with inflammatory skin diseases such as atopic dermatitis and psoriasis (Vestergaard et al. 2005; Davila et al. 2022; Garzorz-Stark et al. 2016). Psoriasis, in turn, is associated with systemic inflammation, and a higher risk of CVD has been reported among psoriasis patients. The inflammatory mechanisms of psoriasis and CVD also share some details (Teague et al. 2023; Terui and Asano 2023). In atherosclerosis and psoriasis, T helper cells types 1 and 17 (Th1, Th17) as well as interleukins 1 and 17 play crucial roles. This is supported by results showing that novel pharmacotherapy targeted at these inflammatory mechanisms can reduce atherosclerotic signs among psoriasis patients (Terui and Asano 2023; Tsiogka et al. 2023). Monokine induced by Gamma Interferon (MIG; CXCL9) has been studied even less. MIG is a chemoattractant cytokine for Th1 cells and is induced by IFNg. MIG is associated with atherosclerosis and CVD, and MIG expression is excessive in inflammatory skin conditions, primarily in others than psoriasis, although MIG is associated with psoriasis to some degree (Yu et al. 2015; Y. Liang et al. 2017; Goebeler et al. 1998; Flier et al. 2001).

The pathophysiology of atherosclerosis varies according to the affected vascular beds (lower extremities, coronary and carotid arteries). Clinically, PAD manifestations mainly occur due to thrombosis without plaque rupture, whereas

CAD events occur mainly due to plaque rupture. The influence of the risk factors vary between the vascular beds, and atherosclerotic inflammation is more pronounced in the coronary and carotid arteries than in the peripheral arteries. Peripheral artery atherosclerosis presents with fibroproliferative pathology instead of lipid-rich cores and thin fibrous caps, which in turn are seen in clinically unstable CAD. The coronary, carotid and peripheral arteries face distinct blood flow conditions and luminal shear stress, which may also affect pathological alterations. The miRNA profiles in the different arterial beds of atherosclerosis also vary. Aortic atherosclerosis biomarkers have been sought in relation to atherosclerosis-related aneurysms, and proteomics have revealed differences in aortic and coronary atherosclerosis, but no detailed soluble inflammatory profile has yet been established for aortic atherosclerosis even though aortic calcification is connected to CVD burden (Narula, Olin, and Narula 2020; Poredoš, Cevc, and Blinc 2021; Teixeira et al. 2022; Jalkanen et al. 2019; Schousboe et al. 2020)

In conclusion, these findings provide a valuable insight into the potential link between the dysregulation of inflammation and aortic atherosclerosis. Based on what is already known about these cytokines as discussed above, it can be hypothesised that the skin, as a barrier tissue, might act as one of the initial sites of inflammation dysregulation that eventually contributes to the development of atherosclerosis and CVD. The significant associations between CTACK and MIG and ACI values support this. These results warrant further systematic investigation of skin-related inflammatory changes in relation to the pathogenesis and endpoint events of CVD and aortic atherosclerosis. Understanding these associations could potentially revea the underlying mechanisms of CVD and open avenues for targeted therapeutic interventions in the future.

6.5 Limitations

Retrospective studies are prone to selection bias. Bias caused by insufficient reporting is possible, but it would have minor effect on results, because such errors in the data would probably be evenly distributed and patient categorisation was not dependent on missing data or data quality.

The geographical catchment area of TUH is mostly populous, and these study populations described the typical demographics of the area's patients risk profile. These populations were not heavily exposed to geographic bias.

The data on smoking was categorised as smokers, ex-smokers and non-smokers because the study design would make more detailed data (smoking intensity, smoking duration on duration since cessation) unreliable, as self-reported data on smoking is known to differ from electronic health record data (Patel et al. 2020). In Studies I and III, only those who had never smoked were categorized as non-

smokers. In Studies II and IV (PURE ASO Cohort) the ACI value was not associated with smoking even though it is an established risk factor for atherosclerosis. Therefore, the vascular disease burden attributable to smoking alone could not be established. Such a phenomenon should have a minor influence on the results of these studies, because the ACI summarizes individual atherosclerotic burden regardless of the recorded risk factors. In Study III, smoking history data were collected in 94% of the patients and missing data on smoking history had a similar distribution in the different ABI groups.

The participants abdominal imaging was not performed to determine their atherosclerotic burden, and therefore the ACI value does not carry a significant risk of selection bias related to it.

The inflammatory mechanism of atherosclerosis has been found in earlier research, and therefore, the samples that would be examined for inflammatory markers were not evaluated in studies I, II and III. This examination was not deemed necessary for these studies, because the ACI is cumulative in nature, i.e. it summarizes the results of individual atherosclerotic process regardless of other kind of measurements.

The mean ACI value in PURE ASO cohort was higher than it was among the participants of Study I showing that ACI values were associated IAs. As the PURE ASO cohort patients had symptomatic, advanced atherosclerotic disease and were older, it is credible that their ACI values were higher.

Interobserver reproducibility was only measured in Study I, in which inter-rater reliability using interclass correlation found good agreement. For the PURE ASO cohort, a random subset of measurements was compared to other observer and no statistically significant difference was found. All the ACI value measurements were conducted using identical methods.

In addition, in Study I, some demographic variables (smoking, alcohol abuse, CVD) revealed controversial findings, as discussed above. The IA and control patients were hospitalized patients, admittedly in part due their risk profile. However, the IA patients did not undergo abdominal imaging for a diagnosis or suspicion of IAs, and the control patients were selected from among emergency departments patients who had undergone abdominal imaging when there. Therefore, selection bias presumably occurred, but it was estimated to have a minor effect on the results, as abdominal imaging is rarely connected to atherosclerotic cardiovascular events or IA events.

In study II, the ACI values of 62 cohort members were missing. However, the cohort demographics did not alter the availability of imaging studies. The patients are not categorized by the invasive treatment (open bypass surgery or endovascular treatment). It was estimated that categorization by invasive treatment methods would interfere with the study design, as categorization by revascularization strategy would

have emphasized the effect of the current strategies on the possible outcome during follow-up and could simultaneously have undermined the relevance of the ACI. Similarly, the chosen treatment strategy was not affected by the ACI.

The patients of the Study II were not categorized according to the clinical presentation of PAD (IC or CLTI) because CLTI describes a very broad spectrum of patients from those with ischemic rest pain to major tissue loss. The PAD of the cohort's patients was severe enough to be symptomatic and indicated invasive treatments in the initial setting. The survival of IC and CLTI patients relative to the ACI cutoff value of 43 is presented in the supplementary material. Total survival in relation to ACI cut-off value was not significantly different among patients presented who had IC but according to the abovementioned bias, this statistical finding was not considered clinically significant in this setting. Furthermore, the cutoff of ACI in the CART analysis was determined by analyses that use substitute values. This has no major effect on the use of these results as in this study, the CART-analysis was used to show that it is possible to find an ACI cutoff value that best divides populations in relation to end-points. More data are needed to establish diagnostic or prognostic reference values for ACI.

Study II was unable to assess patients in relation to two other major peripheral artery disease classifications – the WIfI (wound, ischemia and foot infection) and the GLASS (global limb anatomic staging system), because these classifications were only introduced after this study cohort was found. The approach of this study did not allow us to evaluate these patients efficiently or reliably afterwards in relation to these classifications.

In study III, of the 2757 patients, only 32% underwent cerebrovascular imaging or had RIAs, and they were included in the analyses. Imaging can have various indications, which could also lead to selection bias. There is a risk that a part of the population who did not undergo cerebrovascular imaging might have IA and therefore they might present as a false negative for IA. This might have some influence on the differences between groups, but as this data represents otherwise comprehensive population in the normal ABI group and the low ABI group and the normal ABI group presents with similar IA prevalence as general population, occurrence of false negatives in patients without cranial imaging would not be sufficient to reverse these findings. Therefore, the conclusion of this study, exceptionally high prevalence of IA in low and borderline ABI groups, remains credible.

In study IV the used method was merely explorative, and findings should be validated in a controlled or comparative setting. Moreover, due to the explorative approach, the results should be interpreted with caution regarding causality and correlation, as the latter would require a comparative setting and a different categorization of subjects. No control group or validation cohort was available for

the purpose of this study, and as the aim of the study was to explore and generate a hypothesis, none were needed. This study found no significant results in all of the classical interleukins and cytokines associated with atherosclerosis. This may be due to the lack of a comparison group or due to the fact that all the participants suffered from symptomatic atherosclerotic disease and had heavy atherosclerotic burden, and thus all the compared groups already had high values so differences were not significantly noticeable. Several comorbidities can elevate cytokine levels, and the approach used did not allow all of them to be controlled. The soluble cytokine profile of aortic atherosclerosis can be examined in various settings, also with patients with different degrees of clinical and subclinical atherosclerosis, but as this study cohort did not reach such categorization, these results may not be generalizable to different patient categories.

7 Conclusions

7.1 Conclusions

Study I – Aortic calcification is greater among patients with ruptured or unruptured intracranial aneurysms. Greater aortic calcification increases the risk of IA rupture.

Study II – Aortic calcification index is a risk factor for mortality and cardiovascular morbidity among patients with peripheral artery disease. Aortic calcification index can be utilised to predict the risk of cardiovascular diseases in a cohort of patients with atherosclerosis.

Study III – Low and borderline ankle-brachial index values are associated with a significantly increased intracranial aneurysms prevalence.

Study IV – Aortic calcification is associated with the CTACK and MIG cytokines.

The primary hypothesis is supported by the results of Studies I and III. The ACI and ABI value data in these studies showed an association between IA and atherosclerosis.

In Study I, aortic calcification increased the risk of an RIA and was higher among IA patients than among the control patients. In Study III, IA prevalence was substantially higher among patients with low or borderline ABI values than among those with normal ABI values, and higher than IA prevalence in general.

Study II showed that the ACI is a plausible tool for risk evaluation even in a cohort of patients ridden with advanced atherosclerosis, as ACI was associated with reduced overall- and MACE-free survival of PAD patients. Aortic calcification increased the risk of mortality and cardiovascular events in patients with PAD. This evidence was further supported by the results of Study IV, which presented the distinctive features of a soluble inflammatory profile of atherosclerosis severity relative to aortic calcification. The CTACK and MIG cytokines were associated with increased aortic calcification.

The related risk of CVD among patients with IA is considerable and non-invasive ABI and ACI value measurements can be used to visualize this risk beyond demographic modifiable and non-modifiable risk factors, as both methods present

cumulative risks, despite previously recorded risk factors or estimates of exposure to them.

The clinical perspective of this dissertation is risk stratification. Currently, the risk of IA is not evaluated among patients with atherosclerotic diseases, and vice versa. The results presented in this dissertation suggest that there may be a subgroup of populations who suffer from atherosclerosis and might benefit from IA screening, and that IA patients might benefit from more robust treatment or assesment of the risk factors of atherosclerosis. The shared risk factors, co-prevalence, and concomitant disease progression measured by ACI and ABI, together with the similarities in pathophysiology, highlight the need for studies of preventive methods and screening for IAs and CVDs among these patients.

The ACI and ABI provide a clinically detectable, non-invasive, and easily available method for finding a link between IAs and atherosclerosis. However, the ACI is measured by CT which uses radiation, and hence mere screening for the presence of atherosclerosis without clinical symptoms would most likely be deemed inappropriate. When CT is performed due other reasons, ACI data could be collected and used as a surrogate marker for atherosclerosis instead of or in addition to ABI or TBI if appropriate reference values were established. The ACI provides a linear measure, with increasing values representing increased disease burden and, according to the results of this dissertation, an increased risk of events. The ACI shows the cumulative impact of risk factors, regardless of which of them are known. The ABI in turn is more difficult to interpret, as abnormally high values are also indicative of an increased risk, and ABI values can be deceivingly normal or borderline abnormal in the presence of advanced atherosclerosis in very distal peripheral arteries (Wickström et al. 2017).

Considering screening leads to considering potential harm of preventive methods. Methods to prevent atherosclerotic CVDs include non-pharmacological strategies which are not likely to cause adverse effects. Risk-reducing pharmacotherapies might cause adverse effects, and hence, screening options require studies of the probability of the harm and benefits in populations screened for CVD using the ACI or ABI. Moreover, screening should be cost effective, and while some results have claimed that aortic calcification is associated with health care costs, the cost-effectiveness of CVD screening using ACI or ABI should be investigated (Schousboe et al. 2020).

Risk-reducing therapies have beneficial effect on atherosclerotic CVDs. However, it is not known how these therapies would affect ACI in follow-up. Coronary artery calcifications might increase and the risk for events decrease with the use of statins, which cause plaque stabilization and simultaneous increase in calcification. ABI value measurements during follow-up may not be reliable, as ABI value can increase when PAD is treated with revascularization, but the ACI could be

an incorruptible marker for disease progression in clinical research However, there are no studies clearly stating the progression of the ACI according to atherosclerosis, and hence studies regarding ACI values progression are warranted. Using the ACI as a research tool in this way could eventually lead to finding threshold values for ACI. For example, in Study I, CART analysis revealed that an ACI value of 3 or greater was indicative of an IA, whereas in the PURE ASO cohort, CART analysis showed that an ACI value 43 could distinguish patients with high likelihood of mortality. This indicates high variability in the ACI in different study settings, and subsequent research is needed to establish more accurate threshold values for the ACI (Amarenco et al. 2004; Harris, Roos, and Landry 2016; Xian et al. 2021; Shin et al. 2013; Raitakari, Pahkala, and Magnussen 2022).

ACI and ABI value measurements had interesting results in the context of atherosclerotic diseases progression in relation to the risk of IA. In Study I, only little calcification in the aorta increased the risk of IAs. However, in Study II, a much higher ACI of 43 was needed to categorize patients by survival. In Study III, even moderately reduced ABI values were associated with a significant risk of IA. This might mean that the different stages of atherosclerotic disease pose a different kind of risk for patients: Based on these data, the risk of IA is higher even in the early stages of atherosclerosis, but advanced atherosclerosis has an impact on survival and mortality. Low ABI values represent advanced atherosclerosis, which was associated with a high risk of IA. Low ABI values, atherosclerosis and IA share risk factors and are all caused by inflammation. These results support the idea of concomitant disease progression. In the context of the ACI and ABI, it can be hypothesized that more advanced vascular inflammation is associated with worsening indices, which might in turn be associated with IAs and CVD events. Hence, future research might investigate whether ABI or ACI values could be used as surrogate markers for the vascular inflammation and disease progression that eventually results in IAs or classical CVD events. Currently, disease progression is only being noted when new end points occur or IA is found (Kennedy et al. 2005; Vlak et al. 2013a).

Using ACI and ABI values in cases of IA could improve the categorization of vascular disease phenotypes. The ABI and ACI have been used to categorize patients according to cardiovascular risk, but these results show that IA presence could be an additional categorization for patients with CVDs. The ABI and ACI are known to be associated with CAD, PAD and strokes. Present data suggest that IA presence could be added to the list, and that IA presence can predict IA-related clinical outcomes that should be prevented or treated. The results of Studies I and III suggest that the coexistence of IAs and atherosclerotic CVDs may show a common vascular disease underlying the degenerative, acquired arterial lesions.

It is not known why certain individuals with the same risk factors present with IA while others present with atherosclerotic plaques. There might be common

genetic reasons and different proportions of the non-genetic risk factors can lead toward either or both of these clinical manifestations. Inversely, the effects of the non-genetic risk factors may be similar, but certain, yet unknown genetic factors guide the process towards aneurysm and/or plaque formation. However, it must be acknowledged that the concept of systemic atherosclerosis-related disease deserves more research. The genetic background of both diseases has not yet been established comprehensively and the proportion and the duration of exposure to risk factors and the possible synergy of the risk factors and genetic predispositions should be investigated thoroughly (Bakker and Ruigrok 2021; Björkegren and Lusis 2022).

Study IV investigated the inflammatory markers in peripheral blood and correlated them with ACI. Study IV distinguished cytokines CTACK and MIG from the panel of multiple cytokines in a cohort of atherosclerotic patients. Soluble inflammatory profile or aortic atherosclerosis has not yet been defined, as studies of the subject are lacking. It is known that aneurysms and atherosclerosis in different vascular beds present with slightly different risk factor profiles and comorbidities. Aneurysms in different arterial beds also have differences in pathophysiologies, although their cellular-level histopathological findings and faulty signaling of inflammatory mediators are shared to some extent (Kuzmik et al. 2010; Ruigrok et al. 2008; Ito et al. 2008). Atherosclerotic lesions are more like each other, whereas few distinctive features have been found in in lesions different arterial beds (Narula, Olin, and Narula 2020). Therefore, the results of Study IV concerning the features of soluble inflammatory profiles in relation to aortic calcification may assist in phenotyping CVDs. More research is still warranted to determine whether the aortic calcification-related soluble inflammatory profile is distinguishable from other atherosclerotic phenotypes.

Prevention is the cornerstone of CVD treatment. Primary prevention measures are carried out when patients are at an increased risk of CVDs, and secondary prevention is warranted when patients have already suffered an end-point of atherosclerosis. While discussing IA and atherosclerosis and their common traits, one should concentrate on the matter of primary and secondary prevention. IA patients´ cardiovascular risk factors should be addressed accordingly via the means of primary prevention as per current society guidelines. On the other hand, these data show that IA patients could be categorized as already having a complicated cardiovascular or cerebrovascular disease, and therefore they could be seen as a target for secondary prevention. This would mean more robust medical therapy measures and closer surveillance of their effectiveness, and would require additional resources. If proven effective, increased resources could be a productive investment if they reduce IA patients' risk of vascular complications. These kind of measures are already being taken, as a randomized controlled trial PROTECT-U is currently

investigating acetylsalicylic acid and intensive blood pressure treatment as secondary prevention for UIA (Vergouwen et al. 2018).

The risk of end points is a subtle concept. Individual experience the risk of 0% or a risk of 100%, but a physician or center should see the statistics change over time in relation to applied therapies in a treated population, and, moreover, they should experience the increasing survival of patients with a positive prognosis due to more accurate patient selection and improving treatment options. At the same time, patients who have no realistic chances of benefiting from aggressive treatment should be increasingly spared from these treatments. Similarly, the risk of adverse effects should be weighed against the risk of the disease, but also against the patients' overall risk of other comorbidities and their end points. In the context of this dissertation, this means that the ACI and ABI might prove valuable in IA patients overall risk estimation, because it seems that both can visualize the risk beyond known clinical diseases. Both are associated with IAs, which cause an additional risk of morbidity and mortality in patients with atherosclerotic CVD (Korja, Lehto, and Juvela 2014).

CVDs pose a challenge in interpreting risk. For example, the survival percentages for most cancers are often more easily comprehendible than the risk of CVDs manifesting later in a patient with atherosclerosis or risk factors. Many patients with diagnosed atherosclerotic disease suffer multiple end-points each with a risk of increased costs, morbidity and mortality (Steen et al. 2022). Hence, predicting the whole burden of CVDs is paramount. The most novel approach in this endeavor is to apply AI in risk prediction. The VASCUL-AID initiative is a European, multinational consortium that is investigating cardiovascular risk in patients with CVD, applying AI, close monitoring of patients, automated imaging analyses and personalized preventive strategies (VASCUL-AID 2024). Predicting the course and outcome of concomitant CVDs could help in planning treatment. Invasive treatment in particular should be considered carefully if there is a substantial risk of death or disability despite the planned treatment for other vascular diseases.

7.2 Future perspectives

Inflammation is established as a driver for IA formation and atherosclerosis, but the trigger for the inflammation is yet to be discovered. Ox-LDL has shown potential in this research, but there are also novel ideas regarding the trigger of inflammation. Most recent advances concern the microbiological environment of the human. Gut microbiota and oral bacteria have been associated with IAs and atherosclerosis, and these might offer productive research fields for the future (Chhibber-Goel et al. 2016;

M. J. Pyysalo et al. 2013; Witkowski, Weeks, and Hazen 2020; Lindskog Jonsson and Bäckhed 2017; Shikata et al. 2019).

This dissertation establishes aortic calcification as a prognostic biomarker for cardiovascular risk. ABIs association with the risk of CVDs has been known for long. This dissertation examined these two separately, but in the future, the cumulative data on the atherosclerotic burden of both these biomarkers should be investigated together with other known or suspected risk factors to build more accurate tools for risk estimation of IA patients and CVD patients.

Aortic calcification is still greatly overlooked as a biomarker. When imaging indicates calcification in the aorta is, this is not reported in any standardized manner if at all, and the information certainly does not reach clinical decision-making or risk estimation, even though the data is available to radiologists and clinicians. For example, Pola et al (2018) found that the number of abdominal CT scans had increased during time period from 2004 to 2014. Undeniably, all these studies show abdominal aortic calcification. AI and machine learning could assist in creating treatment algorithms and risk-assessment tools based on large amounts of clinical and imaging data in the future, although challenges in applying AI in medical sciences have been recognized (Pola et al. 2018; Varoquaux and Cheplygina 2022; Mohsen et al. 2022; VASCUL-AID 2024). ACI, ABI, blood pressure and demographic factors or other measurements could be added to these kinds of risk assessment tools. It must be stated that acquirement of this data as a mere indication for computed tomography imaging would not be acceptable. Only data that is acquierd regardless of the suspected risk would be considered here (Alkadhi and Euler 2020).

Finally, this dissertation calls for future study designs that investigate screening. Screening for IA in patients with low or borderline ABI values is one obvious study setting for the future. Similarly, IA patients with no CVD diagnoses could be examined for concomitant atherosclerotic vascular diseases and risk factors.

Furthermore, no comprehensive studies or guidelines on screening for atherosclerotic calcification and development of atherosclerosis in general currently exist. There are guidelines stating the relevance of screening for asymptomatic clinical diseases such as PAD, but the atherosclerotic diseases process as a systemic inflammatory disease is not currently screened (Nordanstig et al. 2024). The ACI, ABI and deeper knowledge on vascular disease phenotypes, including IA presence, could help future studies identify populations who might benefit from screening for atherosclerosis.

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