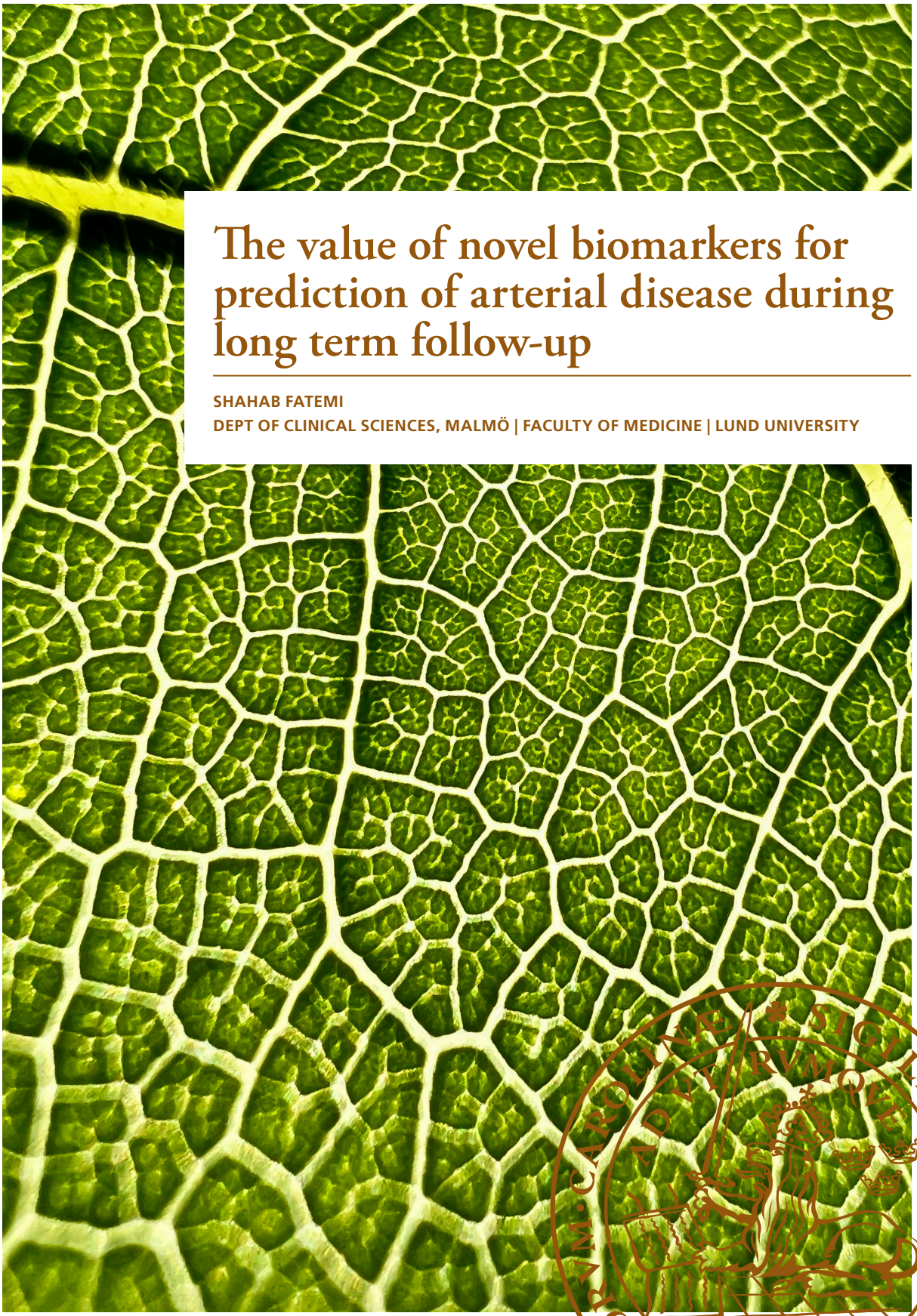
The value of novel biomarkers for prediction of arterial disease during long term follow-up

Fatemi, Shahab

2024

Document Version:
Förlagets slutgiltiga version



The value of novel biomarkers for prediction of arterial disease during long term follow-up

SHAHAB FATEMI
DEPT OF CLINICAL SCIENCES, MALMÖ | FACULTY OF MEDICINE | LUND UNIVERSITY

The value of novel biomarkers for prediction of arterial disease during long term follow-up

Shahab Fatemi

LUND
UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the faculty of Medicine at Lund University to be publicly defended on 24 May 2024 at 09.00 in Hoodsalen, Department of Medicine, Skåne University Hospital, Malmö

Faculty opponent
Elias Johansson, Göteborg University

Organization LUND UNIVERSITY	Document name Doctoral dissertation
Faculty of Medicine Department of Clinical Sciences, Malmö	Date of issue May 24th 2024
Author Shahab Fatemi	Sponsoring organization

The Value of novel biomarkers for prediction of arterial disease during long term follow-up

Introduction: Plasma biomarkers may be useful to detect healthy individuals at increased risk for development of peripheral arterial disease (PAD), carotid artery stenosis (CAS), or abdominal aortic aneurysm (AAA). The subset of plasma biomarkers studied regarding associations with incident cardiovascular disease during long-term follow-up in this thesis were the inflammatory markers lipoprotein - associated phospholipase A2 (Lp - PLA 2) activity and mass, proneurotensin, and C-reactive protein (CRP) and hemodynamic markers cystatin C, copeptin, N- terminal pro-B-type natriuretic peptide (N-BNP), midregional proatrial natriuretic peptide (MR-proANP) and midregion al proadrenomedullin (MR-proADM). As patients with type 2 diabetes run lower risk for abdominal aortic aneurysm (AAA), we also evaluated relationships between glucose metabolism and arterial stiffness in 65-year old men with and without screening detected AAA.

Methods: The prospective cardiovascular cohort of the Malmö Diet and Cancer study (MDCS; n=5550 middle- aged individuals examined for biomarkers in 1991 - 94) was used in papers I-V. The diagnoses of incident PAD, CAS, and AAA during median 23.4 years of follow-up were obtained as International Classification of Disease (ICD) codes from national registries, and validated by scrutinization of randomly selecting 100 patient records for each of the different diagnoses. PAD, CAS, and AAA were confirmed in 98%, 99%, and 95% of cases, respectively. In paper V, additional validation of acute myocardial infarction and ischemic stroke confirmed the diagnosis in 97% and 89% of cases, respectively. Cox regression analysis was used to calculate hazard ratios (HR) per 1 standard deviation increment of each respective log transformed plasma biomarker with 95% confidence intervals (CI). In paper VI, 48 men with screening detected AAA and 115 men with normal aortic diameter underwent examination of glucose metabolism and arterial stiffness.

Results: During the 23.4 years of follow-up the cumulative incidences of PAD, CAS, and AAA were 4.4% (men 5.9%, women 3.3%), 2.3% (men 3.4%, women 1.5%), and 1.6% (men 3.1%, women 0.6%), respectively. In paper V, the cumulative incidence for any atherosclerotic disease was 22.2% (men 28.6%, women 17.9%). Adjusting for several traditional risk factors, Lp-PLA 2 activity (HR 1.33; CI 1.17 - 1.52), Lp-PLA 2 mass (HR 1.20; CI 1.05 - 1.37), CRP (HR 1.55 ; CI 1.36 - 1,76), copeptin (HR 1.46; CI 1.19 - 1.80), N-BNP (HR 1.28; 1.11 - 1.48), and cystatin C (HR 1.19; 95% 1.10 - 1.29) were associated with incident PAD. N-BNP (adjusted HR 1.36; CI 1.12 - 1.65) and MR-pro ADM (adjusted HR 1.30; CI 1.03 - 1.65) were associated with incident CAS and incident surgery for CAS, respectively. N-BNP (adjusted HR 1.59; CI 1.20 – 2.11), MR-proADM (adjusted HR 1.40; CI 1.13 - 1.73), cystatin C (adjusted HR 1.21; CI 1.02 - 1.43), and CRP (adjusted HR 1.53; CI 1.13 - 1.73) were associated with incident symptomatic CAS, whereas no plasma biomarker was associated with incident asymptomatic CAS. In paper V, MR-proADM was independently associated with incident isolated atherosclerotic disease and incident isolated AAA, and adjusted HR were higher for Lp PLA 2 (mass) for incident isolated AAA compared to for isolated atherosclerotic disease. No individual with diabetes mellitus (DM) at baseline developed isolated AAA, whereas DM was associated with an increased risk of isolated atherosclerotic disease (adjusted HR 2.57; CI 2.08 - 3.18). Paper VI showed that hyperinsulinaemia as a marker of insulin resistance, but not hyperglycaemia or aortic stiffness, was associated with AAA and abdominal aortic diameter.

Conclusions: Plasma biomarkers are independent predictors of cardiovascular disease during long-term follow-up, supporting the view that components of subclinical vascular inflammation and cardiovascular stress are related to development of atherosclerotic disease and AAA. In contrast to atherosclerotic disease, DM appears to protect from the development of AAA, but we were not able to detect direct relationships between AAA and hyperglycaemia in 65-year old men.

Key words: peripheral arterial disease, carotid artery stenosis, atherosclerosis, abdominal aortic aneurysm, plasma biomarkers, lipoprotein-associated phospholipase, c-reactive protein, pro-adrenomedullin, B-type natriuretic peptide, proatrial natriuretic peptide, copeptin, cystatin C, proneurotensin, risk factors, diabetes mellitus, hyperinsulinaemia.

Classification system and/or index terms (if any)

Supplementary bibliographical information		Language English
ISSN and key title 1652-8220		ISBN 978-91-8021-570-1
Recipient's notes	Number of pages 89	Price
	Security classification	

Signature Date 2024-04-10

The value of novel biomarkers for prediction of arterial disease during long term follow-up

Shahab Fatemi

LUND
UNIVERSITY

Coverphoto photo: Picture of a leaf from a mulberry tree, representing the complex but yet elegant vascular system of nature. By Annelie Torstensson

Faculty of Medicine
Department of Clinical Sciences, Malmö

ISBN 978-91-8021-570-1
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2024

Dedicated to My beloved family,

Your love brings boundless joy to my heart. Grateful for each and every one of you.

Table of Contents

Abbreviations

AAA	Abdominal aortic aneurysm
ACAS	Asymptomatic carotid atherosclerosis study
ACST	Asymptomatic Carotid Surgery Trial
AD	Atherosclerotic disease
ADMA	Asymmetric dimethylarginine
AGE	Advanced glycation end products
AMI	Acute myocardial infarction
ANOVA	Analysis of variance
BMI	Body mass index
CAS	Carotid artery stenosis
CI	Confidence interval
CLTI	Chronic limb threatening ischaemia
CT	Computer tomography
CTA	Computed tomography angiogram
CPT	Copeptin
CRP	C-reactive protein
CV	Cardiovascular
Cyst C	Cystatin C
DBP	Diastolic blood pressure
DM	Diabetes mellitus
EVAR	Endovascular aneurysm repair
FPG	Fasting plasma glucose
GLP-1-RA	Glucagon-like peptide 1 Receptor Agonist
HbA1c	Glycated hemoglobin a1c
HOMA-IR	Homeostatic model assessment for insulin resistance
IC	Intermittent claudication
ICD	International Classification of Diseases
IFN	Interferon
LDL	Low density lipoprotein
Lp	Lipoprotein
Lp-PLA2	Lipoprotein phospholipase 2
MACE	Major adverse cardiovascular events
MDCS	Malmö diet cancer study
MDPI	Multidisciplinary Digital Publishing Institute
MMP	Matrix metalloproteinase
MR-proADM	Midregional proadrenomedullin
MR-proANP	Midregional proatrial natriuretic peptide
NASCET	North American Symptomatic Carotid Endarterectomy Trial
OGTT	Oral glucose tolerance test
OR	Odds ratio
PAD	Peripheral arterial disease
PAI-1	Plasminogen activator inhibitor 1
PNT	Proneurotensin
PWV	Pulse wave velocity

RR	Relative risk
SBP	Systolic blood pressure
SGLT2	Sodium-glucose co-transporter 2
TNF	Tumour necrosis factor
TIA	Transitory ischemic attack
tPA	Tissue plasminogen activator
US	Ultrasound
VEGF-A	Vascular endothelial growth factor A
WHR	Waist-hip ratio

Thesis at a glance

Paper	Aim	Method	Main results
I. Lp-PLA_2 activity and mass and CRP are associated with incident symptomatic peripheral arterial disease	Relationships between inflammatory biomarkers and incident symptomatic PAD	Prospective cohort study. MDCS	CRP, Lp-PLA_2 (activity and mass) were associated with incident symptomatic PAD
II. Copeptin, B-type natriuretic peptide and cystatin C are associated with incident symptomatic PAD	Relationships between hemodynamic biomarkers and incident symptomatic PAD	Prospective cohort study. MDCS	Biomarkers copeptin, BNP, and Cystatin C were associated with incident symptomatic PAD
III. Pro B-type Natriuretic Peptide and Midregional Proadrenomedullin are associated with incident carotid stenosis during long term follow-up	Relationships between biomarkers and incident CAS	Prospective cohort study. MDCS	BNP was associated with incident CAS and MR-proADM was associated with incident surgery for CAS
IV.Circulating biomarkers predi ct symptomatic but not asymptomatic carotid artery stenosis	Relationships between biomarkers to incident symptomatic and asymptomatic CAS	Prospective cohort study. MDCS	BNP, MR-proADM, cystatin C and CRP were associated with incident symptomatic CAS
V. Prospective comparison of plasma biomarker and traditional risk factor profiles for incident isolated atherosclerotic disease and incident isolated AAA	Comparison of biomarkers and conventional risk factors for incident isolated atherosclerotic disease and incident isolated AAA	Prospective cohort study. MDCS	MR-proADM were linked to AAA and AD. DM lower risk for AAA but increased for AD. Lp-PLA_2 (mass), male sex and current smoking had higher risk for AAA than AD
VI. Hyperglycemia and arterial stiffness in 65-year-old men with and without abdominal aortic aneurysm (AAA) – a cross sectional study	Evaluate relationships between disturbances in glucose metabolism and arterial stiffness in 65-year old men with and without screening detected AAA	Cross sectional study	No inverse associations could be confirmed between the presence of an AAA and hyperglycemia or aortic stiffness. Hyperinsulinemia was related to both AAA and abdominal aortic diameter

AD, Atherosclerotic disease; AAA, abdominal aortic aneurysm; PAD, peripheral arterial disease; CAS, Carotid artery stenosis; DM, Diabetes Mellitus; CRP, C-reactive protein; Lp-PLA_2, lipoprotein-associated phospholipase A_2; BNP, B-type natriuretic peptide; MR-proADM, midregional proadrenomedullin, MDCS, Malmö Diet and Cancer Study.

List of Publications

The following papers are included in this thesis as indicated by Roman numerals. Permission was granted from respective publishers for reprinting.

Paper I.

Fatemi S, Gottsäter A, Zarrouk M, Engström G, Melander O, Persson M, Acosta S. Lp-PLA_2 activity and mass and CRP are associated with incident symptomatic peripheral arterial disease. Sci Rep 2019;9:5609.

Paper II.

Fatemi S, Acosta S, Gottsäter A, Melander O, Engström G, Dakhel A, Zarrouk M. Copeptin, B-type natriuretic peptide and cystatin C are associated with incident symptomatic PAD. Biomarkers 2019;24:615-621.

Paper III.

Fatemi S, Acosta S, Zarrouk M, Engström G, Melander O, Gottsäter A. Pro B-type natriuretic peptide and midregional proadrenomedullin are associated with incident carotid stenosis during long term follow-up. J Stroke Cerebrovasc Dis 2021;30:105403.

Paper IV.

Fatemi S, Acosta S, Zarrouk M, Engström G, Melander O, Gottsäter A. Circulating biomarkers predict symptomatic but not asymptomatic carotid artery stenosis. Cerebrovasc Dis 2022;51:623-629.

Paper V.

Acosta S, Fatemi S, Melander O, Engström G, Gottsäter A. Prospective comparison of plasma biomarker and traditional risk factor profiles for incident isolated atherosclerotic disease and incident isolated abdominal aortic aneurysm. Front Cardiovasc Med 2022;8:818656.

Paper VI.

Fatemi S, Acosta S, Zarrouk M, Nilsson P, Gottsäter A. Hyperglycemia and arterial stiffness in 65-year-old men with and without abdominal aortic aneurysm (AAA) – a cross sectional study. Cardiovasc Endocrinol Metab 2023;12:e0290.

Introduction

Atherosclerosis

The word atherosclerosis is derived from the Greek words athero – meaning gruel or paste and sclerosis meaning hardness. The pathophysiology of atherosclerosis (*Pentikäinen et al, 2000; Libby et al, 2019; Öörni et al, 2021; Lorey et al, 2022)* is summarized below (Fig 1).

The arterial vessel is composed of different layers:

- Lumen: circulating blood containing red and white blood cells, proteins, and lipids.
- Endothelium and tunica intima: the innermost layer, composed of endothelial cells in contact with circulating blood allowing it to flow smoothly.
- Tunica media: composed mainly by smooth muscles enabling vascular contraction.
- Tunica externa: containing a connective tissue framework.

Plaque formation in atherosclerosis begins as endothelial dysfunction, mechanical damage due to hypertension, diabetes mellitus, or any factor causing rupture of the endothelial lining. Such injury enables permeability for lipids which normally cannot cross the endothelial membrane. This sparks a complex cascade of events leading to plaque formation, reduced circulation, plaque rupture, thrombosis, embolism, and potential organ damage and death.

1. Accumulation of low density lipoprotein (LDL)-cholesterol in the tunica intima *(Lorey et al, 2022)*. LDL molecules undergo oxidation and modifications causing activation of vascular endothelial cells, expressing adhesion molecules binding to and engaging circulating immune cells such as monocytes and T cells (*Pentikäinen et al, 2000)*. After monocytes have migrated to the tunica intima they differentiate to macrophages and engulf LDL molecules, growing into foam cells *(Öörni et al, 2021)*.
2. Scavenger receptors expressed by macrophages promote and identify oxidized LDL antigens, further increasing LDL uptake, foam cell formation, and inflammatory activity *(Lorey et al, 2022)*.

3. As LDL is not metabolized in high enough rate, the increasing concentration of cholesterol esters damages immune and endothelial cells and activates apoptotic pathways. This results in increased concentration of foam cell debris, apoptosis, and further release of inflammatory mediators in a vicious cycle within the arterial wall (*Pentikäinen et al, 2000)*.
4. This inflammatory cascade *(Bhattacharya et al, 2022)* together with increased concentration of lipids and apoptosis within the wall causes plaque formation.
5. In late stages of atherosclerosis plaque might destabilize and rupture *(Libby et al, 2019),* as a cause of enzyme release by foam cells degrading connective tissue in tunica externa. The plaque is further undermined by cytokines released by tumour necrosis factor (TNF)-α and interferon (IFN)-γ.

This slow multifaceted atherosclerotic process can occur anywhere within the arterial tree, but is more common where damage occurs frequently such as at arterial bifurcations (*Lorey et al, 2022)*. The process might depending on its extent and localization cause either no symptoms at all or tissue ischemia *(Camm et al, 2019; Libby et al, 2019)*. If circulation is not restored quickly enough to guarantee oxygen supply, the consequences might be irreversible and life-threatening. Atherosclerosis is the most common cause of diseases such as *(Roth et al, 2020)*:

- Peripheral arterial disease (PAD)
- Carotid artery stenosis (CAS) and ischemic stroke
- Coronary artery disease and acute myocardial infarction

The two first of these conditions will be further explored in this thesis, together with another important arterial disease, abdominal aortic aneurysm (AAA).

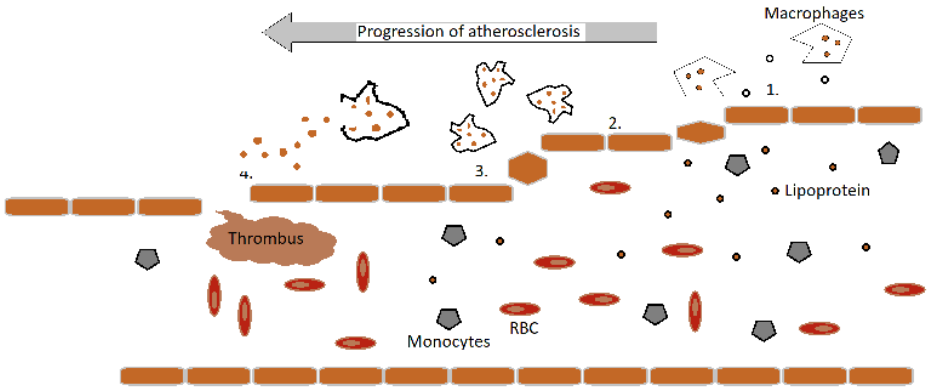


Figure 1. Process of atherosclerosis. © Shahab Fatemi

Peripheral arterial disease (PAD)

Background

PAD refers to atherosclerotic *(Libby et al, 2019)* narrowing or blockage of the vessels carrying blood from the aorta to different organs. PAD can occur in any blood vessel, but it is more common in for example arteries to the lower extremities than to the upper (Fig. 2). In this thesis, PAD is defined as occlusive atherosclerosis of the lower extremity arteries *(Hiatt et al, 2008)* or the arteries distal to the aortic bifurcation. Both men and women are affected by PAD; however, African Americans have an increased risk of PAD, whereas Hispanics may have similar to slightly higher rates of PAD compared with non-Hispanic Whites *(Fowkes et al, 2013)*. Approximately 6.5 million people age 40 and older in the United States have PAD *(Fowkes et al, 2013).* Other health conditions and disorders of arteries can mimic the symptoms of PAD, and not all PAD is due to atherosclerosis *(Aboyans et al, 2017)*.

The condition can be either asymptomatic, or cause symptoms such as pain inflicted by walking, intermittent claudication (IC) or critical limb threatening ischemia (CLTI), defined as either rest pain, ulceration, or both *(Aboyans et al, 2017)*. Furthermore, PAD is associated with an extensive atherosclerotic burden in coronary, carotid and cerebral arteries and increased cardiovascular and overall mortality *(Weir-McCall et al, 2016; Aboyans et al, 2017)*.

The diagnosis of PAD is presently based on case history, clinical evaluation, and measurement of ankle brachial index (ABI) *(Aboyans et al, 2017)*. These methods have certain limitations in elderly or diabetic patients and in those with renal

insufficiency, however. Other causes of lower extremity pain such as neuropathy and orthopaedic conditions might obscure the diagnosis, and heavily calcified stiff arteries might cause inaccurate ABI measurements *(Wilkes et al. 2015).*

PAD prevalence is increasing *(Fowkes et al, 2013)*, and plasma biomarkers measured in healthy individuals might as discussed later in this thesis be of relevance both to detect individuals at increased risk for developing PAD, and to identify factors influencing disease progression.

C-reactive protein (CRP) is a well established biomarker for atherosclerosis *(Li et al, 2017)*, which has been shown to predict worse outcome in PAD patients with PAD *(Singh et al, 2017)*. It would be relevant to investigate the utility of several other plasma biomarkers relevant for prediction of atherosclerosis at other localisations also in relation to long-term incidence and prognosis in PAD.

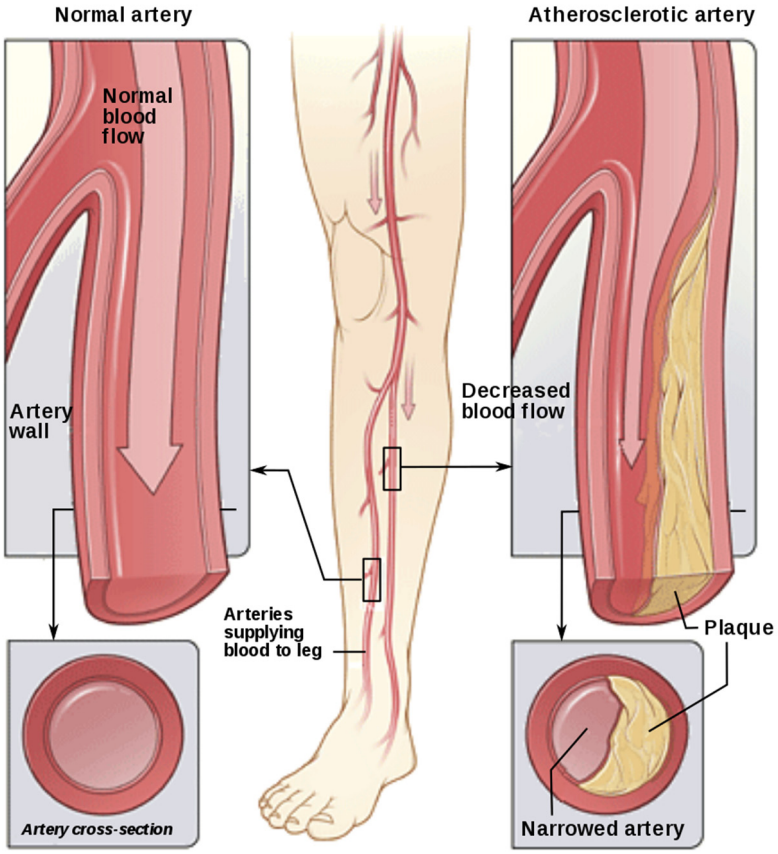


Figure 2. Normal blood flow (left) with and decresead blood flow in peripheral arterial disease (right). © Wikimedia Commons

Carotid artery stenosis (CAS)

Background

The carotid arteries are the main blood vessels carrying blood and oxygen to the brain. Narrowing of these arteries mainly caused by atherosclerosis *(Libby et al, 2019)* might result in CAS.

Carotid artery disease might affect the flow of oxygen to the brain (Fig. 3). Even a brief interruption in cerebral blood supply can cause cerebral ischaemia. Embolization from an ipsilateral CAS accounts for approximately 10-15% of ischaemic stroke cases *(Marulanda-Londoño et al, 2016).*

The stroke preventive benefits of carotid endartertomy are well established in patients with ≥70 % stenosis of the internal carotid artery graded according to the North American Symptomatic Carotid Endarterectomy (NASCET) trial - method *(North American Symptomatic Carotid Endarterectomy Trial Collaborators, 1991)* which has caused symptoms such as transitory ischemic attack (TIA), amaurosis fugax (transient monocular blindness), or stroke *(North American Symptomatic Carotid Endarterectomy Trial Collaborators, 1991; European Carotid Surgery Trialists Collaborative Group, 1991; European Carotid Surgery Trialists Collaborative Group, 1998; Barnett et al, 1998; Mayberg et al, 1991)*.

Most atherosclerotic lesions in the internal carotid artery are asymptomatic, however, and ≥50% CAS occurs in 2.5-4% of subjects aged 25–84 years *(Mathieson et al, 2001; Ghilardi et al 1994)*. This prevalence increases with age, total- and LDL-cholesterol, systolic blood pressure, and smoking *(Mathiesen et al, 2001a*). Among individuals between 60 and 79 years of age, CAS prevalences of 10.5 % in men and 5.5 in women have been reported *(Kiechl* et al, 1994), whereas the prevalence of CAS ≥30 % in PAD patients is 57% *(Alexandrova et al, 1996)*.

The risk for TIA or stroke in subjects with previously asymptomatic ≥50% CAS is related to degree of stenosis *(O'Holleran et al, 1987*) and plaque ecogenicity assesed by ultrasound or magnetic resonance tomography (MR) *(O'Holleran et al, 1987; El Barghouty et al, 1995; Mathiesen et al 2001b)*. Patients with high degree of stenosis and soft echolucent plaques run a higher risk of neurologic events *(O'Holleran et al, 1987; El Barghouty et al, 1995; Mathiesen et al 2001b)*.

Carotid endarterectomy has been evaluated also in patients with asymptomatic CAS. Two large randomised multinational studies, Asymptomatic carotid atherosclerosis study (ACAS) (*Executive committee for the Asymptomatic Carotid Atherosclerosis Study, 1995)* and Asymptomatic carotid surgery trial (ACST) *(MRC Asymptomatic Carotid Surgery Trial [ACST] Collaborative Group, 2004)*, confirmed that carotid

surgery combined with medication which was considered as ”best medical treatment” during the respective study periods almost halved the yearly risk for stroke from 2% to 1% in patients up to 75 years of age with 60-99% degree of stenosis compared to ”best medical treatment” alone. Recently, carotid artery stenting was proven to confer equal benefits and risks as endarterectomy in this situation. The long-term effects of these two carotid artery procedures on fatal or disabling stroke in the ACST-2 trial were comparable *(Halliday et al, 2021*).

After ACAS *(Executive committee for the Asymptomatic Carotid Atherosclerosis Study, 1995)* and ACST *(MRC Asymptomatic Carotid Surgery Trial [ACST] Collaborative Group, 2004)*, the concept of ”best medical treatment” of patients with asymptomatic carotid artery stenosis has been further developed in accordance with guidelines *(Naylor et al, 2018*) referred to below, however. Modern pharmacological treatment resulting in lower LDL-values is related to a decreased risk for microembolisation from an asymptomatic carotid artery stenosis *(Spence et al, 2010)* and stroke rates in asymptomatic patients have decreased *(Abbott, 2009; Naylor et al, 2009; Marquardt et al, 2010)* as a consequence of this.

It is therefore still highly relevant to look for methods to help select the right candidates for carotid endarterectomy or stenting among the group with asymptomatic CAS, i.e. those with the highest risk for neurological symptoms if treated only by non-invasive means.

Assessment of plaque composition by ultrasound *(Goncalves et al, 2003; Gronholdt et al, 2001; Kölbel et al, 2010; Stenudd et al, 2020)*, MR *(Kwee et al 2008; Kwee et al, 2009, Kwee et al, 2010)*, positron emission tomography (*Kwee et al, 2010)*, and transcranial doppler *(ACES Investigators, 2009; Wong et al, 2010)* have all proven useful in such risk prediction, but there is still need to evaluate assessment of biomarkers in this situation *(Carballo-Perich et al, 2022)* as discussed later in this thesis.

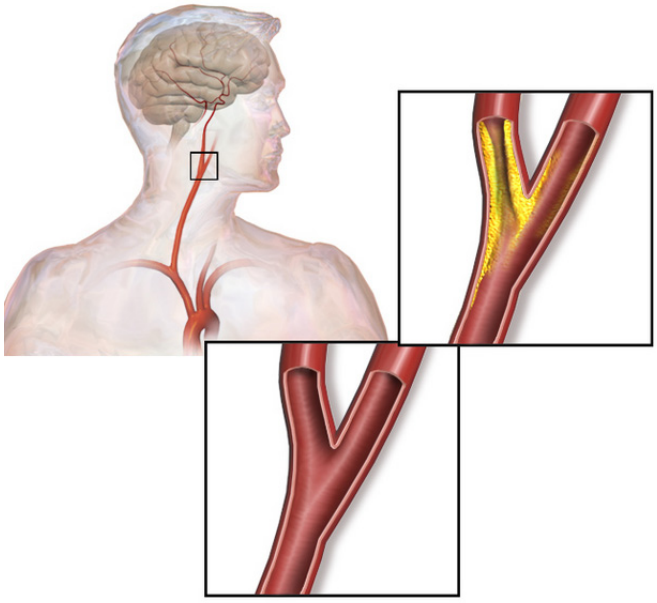


Figure 3. Carotis artery stenosis. © Wikimedia Commons

Abdominal aortic aneurysm (AAA)

Background

The aorta is the largest artery in the human body, transporting oxygenated blood from the heart. The mean diameter of the infrarenal aorta is 19-22 mm in men *(Norman et al, 2004)* and 10-15 mm in women *(Bux et al, 2009)*. Aortic diameters normally increase with age and are related to body size *(Starck et al, 2019)*. The aortic wall might expand further in the radial directions (dilate) at a localized point in the chest or abdomen (Fig. 4). Such enlargement can be an ongoing process for years and even decades. A dilatation of ≥ 30 mm of the abdominal aorta is classified as an abdominal aortic aneurysm (AAA) *(Wanhainen et al, 2019)*. A fusiform aneurysm comprises the circumferential vessel wall, whereas a saccular aneurysm is a localized diatation on one side of the vessel.

The dilatation might remain unnoticed, or might cause symptoms including pain in the abdomen, lower back, and loins, or abdominal pulsations. The most feared consequence of an AAA is rupture, however, a life-threatening condition requiring acute surgery *(Wanhainen et al, 2019)*. As rupture risk increases with aneurysm diameter, smaller aneurysms are kept under regular ultrasound surveillance

(Wanhainen et al, 2019). Abdominal aortic diameter ≥ 5 cm in women or ≥ 5.5 cm in men are the recommended thresholds for elective operation to prevent rupture *(Wanhainen et al, 2019).*

As discussed later in this thesis, many different biomarkers reflecting immune activation, coagulation, inflammation, and connective tissue degradation have been reported to correlate with aortic diameter and prescence of AAA *(Juvonen et al, 1997; Flondell-Sité et al 2009; Gottsäter et al, 2009; Villard et al, 2012; Lindberg et al, 2016; Yagi et al, 2020; Li et al; 2021),* whereas prospective biomarker data in relation to future AAA development are partly contradictory. We found no correlation between biomarkers and aortic diameter or AAA prevalence at ultrasound screening 14-19 years later *(Taimour et al, 2017),* whereas biomarkers such as lipoprotein phospholipase 2 (Lp-PLA2) activity and mass *(Acosta et al, 2017)* and mid-regional pro-adrenomedullin (MR-proADM) *(Acosta et al, 2018)* were markers of incident AAA hospitalization, mainly caused by the need for aneurysm surgery, during long term follow-up.

It is not known whether biomarkers are relevant only close to AAA diagnosis, or only for prediction of large AAA requiring hospitalization and surgery *(Acosta et al, 2017; Acosta et al, 2018)*. To enable use of biomarkers to help select proper candidates for active treatment and strict surveillance, it would also be of large clinical value to establish if and in which way biomarker patterns predicting AAA development *(Memon et al, 2020)*, growth (*Memon et al, 2020; Nana et al, 2021),* and rupture differ from the corresponding patterns predicting atherosclerotic disease.

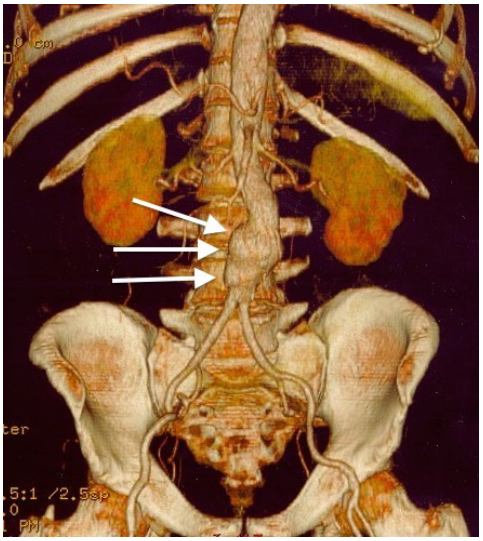


Figure 4. CT reconstruction image of an abdominal aortic aneurysm (white arrows) © Wikimedia Commons

Type 2 diabetes mellitus (DM)

The pancreatic beta cells produce insulin which is an amino acid hormone composed of two peptide chains joined together by a bond *(van Lie et al, 2017)*. This vital hormone is responsible for anabolic processes by regulating absorption of ingested glucose in various human cells. Pancreatic insufficiency, shortage of or total absence of insulin, leads to development of type 1 or 2 DM. Individuals which lack the ability to produce insulin have type 1 diabetes, whereas type 2 diabetes is disability to generate sufficient insulin via pancreatic beta cells or the disability to utilize glucose due to insulin resistance *(DeFronzo et al, 2015)* caused by reduced amount of insulin receptors on the cell surface. Impaired or lacking insulin receptors lead to increased glucose levels in circulating blood, hyperglycemia. Prevalences of both type 1 and 2 DM2 are increasing dramatically worldwide; it is estimated that 9.3 % of the worldwide population suffers from this disorder and that this number will exceed 10 % by 2030 *(Saeedi et al, 2019).* Type 2 DM is increasing at an alarming rate in younger individuals due to inadequate physical activity and poor lifestyle habits *(Classification and diagnosis of Diabetes: Standards of Medical Care in Diabetes 2020;43:S14-S31).* The main causes of DM2 in older individuals are genetics, inactive lifestyle, and obesity *(Schnurr et al, 2020).*

The inverse relationship between diabetes and AAA *(Avdic et al, 2018; Raffort et al, 2018; Xiao et al, 2021)* is discussed in the section on risk factors below.

Arterial stiffness

The stiffness of the arterial system can be indirectly assessed by measurement of the speed with which pressure pulses propagate through the arteries, the pulse wave velocity (PWV). PWV of a healthy vascular system is dependent on age, and increased in conditions such as hypertension, smoking, type 2 DM, and obesity *(Safar et al, 2006; Elewa et al, 2015).*

Diagnosis of PAD, CAS, AAA, diabetes mellitus, and arterial stiffness

The following diagnostic tests for of PAD, CAS, AAA, DM, and arterial stiffness can be used both in clinical routine and for research purposes:

- Ankle brachial index (ABI) is a noninvasive test measuring blood pressure in the ankles in relation to blood pressure in the arms at rest and after exercise *(Aboyans et al, 2017)*.
- Carotid artery duplex vascular ultrasound uses high-frequency sound waves to visualize carotid *(Naylor et al, 2018*) or peripheral *(Aboyans et al, 2017)* arteries, and is the most used test to evaluate the presence of CAS *(Naylor et al, 2018*) (Fig. 5). Duplex ultrasound is also an inexpensive and noninvasive initial imaging method for detection and surveillance of AAA in asymptomatic patients *(Lindholt et al, 1999)*.
- Angiography is an invasive imaging procedure during which a thin, flexible cathether is inserted into a blood vessel in the arm or leg, and guided to the carotid *(Naylor et al, 2018*) or peripheral *(Aboyans et al, 2017*) arteries. Contrast is injected through the catheter before imaging. The test may be performed to evaluate or confirm the presence of narrowing in the carotid *(Naylor et al, 2018*) or peripheral *(Aboyans et al, 2017*) arteries, and evaluate the need for future treatment such as stenting or surgery in carotid *(Naylor et al, 2018*) or peripheral *(Aboyans et al, 2017)* arteries.
- Magnetic resonance angiogram (MRA) uses a magnetic field and radio waves to provide pictures of the carotid *(Naylor et al, 2018*) or peripheral *(Aboyans et al, 2017*) arteries. MRA can provide information which cannot be obtained from an X-ray, ultrasound, or computed tomography (CT) scan about the degree of stenosis in carotid and vertebral *(Naylor et al, 2018*) and peripheral *(Aboyans et al, 2017*) arteries, and can also be used for visualisation of AAA *(Petersen et al, 1995).*
- Computer tomography (CT) of the brain may be performed if ischaemia is suspected. A CT scan produces three-dimensional images on a computer screen. Contrast might be injected intravenously to reveal areas of damage in the brain (Naylor et al, 2018),
- Computer tomography angiogram (CTA) uses advanced CT technology, along with intravenous contrast material to obtain high-resolution, three-dimensional pictures of the carotid *(Naylor et al, 2018*) or peripheral *(Aboyans et al, 2017*) arteries enabling physicians to determine the degree of stenosis (Fig. 5) and assess more distal arteries *(Naylor et al, 2018*). Additionaly, CTA has the ability to provide detailed information about an AAA and its surrounding anatomy *(Chien et al, 2010)*.

- Diagnostic criteria for DM are the following (*Classification and Diagnosis of Diabetes, 2020)*: Fasting plasma glucose >7 mmol/l (2-3 measurements separated in time), glycated haemoglobin >48 mmol/mol, non-fasting plasma glucose >11.1 mmol/l, or plasma glucose during oral glucose tolerance test >11.1 mmol/l.

PWV can be assessed with instruments measuring the time (*Δt)* elapsing between the passage of a pulse wave at the carotid and femoral artery or from the right carotid to the right femoral artery (cfPWV). The distance (*d*) between the measurement points is divided by the time difference Δt, whereafter PWV is defined as: *PWV = d / Δt (Laurent et al, 2006).* This equation has been slightly modified in the Sphygmocor® device used in paper VI in this thesis *(Laurent et al, 2006; Sugawara et al, 2008; Weber et al, 2009; Butlin et al, 2016).*

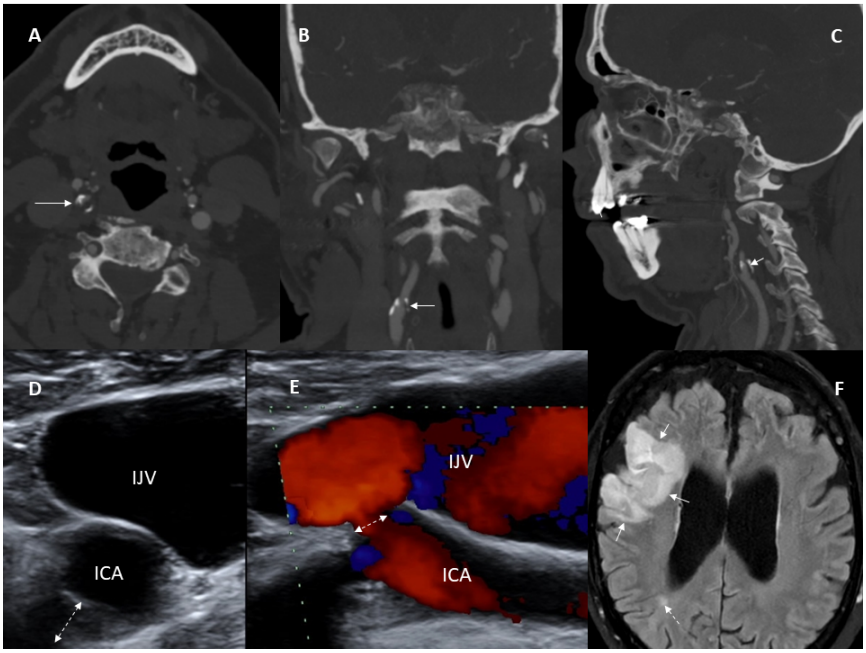


Figure 5. A 72-year-old man with hypertension and previous smoking was admitted with acute left-sided central facial palsy and palsy of the arm, and visual neglect to the left. CT of the brain showed an extensive established frontal infarction on the right side within the supply of the middle cerebral artery, and supplementary CT angiography visualized a carotid stenosis of 70% on the right side (arrows in Fig 5A-horizontal view, B- coronal view, C-sagittal view). The backwall plaque at the origin of ICA was seen on ultrasound (dashed arrow – Fig 5D) and the grade of stenosis (dashed arrow) was reproduced on color doppler ultrasound (Fig 5E). A concomitant acute myocardial infarction complicated management and prolonged time to thrombendarterectomy of the symptomatic carotid stenosis. A magnetic resonance tomography of the brain six days after the cerebral event showed a 6 x 5 cm large, current ischemic lesion in the right frontal lobe (multiple arrows – Fig 5F), and a 0.6 cm large, current cortical ischemic lesion in the right posterior central gyrus, posterior lobe (dashed arrow – Fig 5F). ICA, internal carotid artery; IJV, internal jugular vein. © Department of Radiology, Skane University Hospital.

Conventional risk factors

Risk factors for PAD and CAS

The strongest risk factors for PAD, CAS, and other manifestations of atherosclerotic disease are smoking, hypertension, dyslipidaemia, DM, adiposity and inflammatory states *(Aboyans et al, 2017; Visseren et al, 2021).*

Risk factors for AAA

The strongest risk factors for AAA are family history, smoking, age, atherosclerotic disease, hypertension, and ethnicity *(Wanhainen et al, 2019).* The genetic component is reflected by the fact that having a first-degree relative with AAA doubles the risk compared to in subjects without family history (Larsson et al, 2009). Paradoxically, type 2 DM seems to be associated with a decreased risk for aneurysm development, growth, and rupture *(Avdic et al, 2018; Raffort et al, 2018; Xiao et al, 2021).*

It has been suggested that effects of metformin, the most common drug for treatment of type 2 DM might partly explain these negative relationships between AAA and DM *(Raffort et al, 2020)*. Metformin affects pathophysiological mechanisms relevant for AAA growth *(Raffort et al, 2020)*, and randomized clinical trials are ongoing to evaluate whether metformin reduces growth of AAA in non-diabetic patients *(Wanhainen et al, 2021)*.

Biomarkers

Medical practice requires accurate diagnosis of diseases and conditions. Diagnostic biomarkers are formally defined by the U.S. National Institute of Health and the Food and Drug Administration as "A defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention" *(F-NBWGroup, 2016)*. Biomarkers can be used for determination of whether a patient has a particular medical condition for which treatment may be indicated, or whether an individual should be enrolled in a clinical trial studying a particular disease. Many diseases have subtypes with different prognoses or treatment responses, as indicated by various prognostic biomarkers.

The clinical performance of diagnostic biomarker tests must be ascertained. Typically, a test is evaluated against a reference diagnosis to calculate clinical sensitivity, i.e., the fraction of people with disease who test positive, and specificity, i.e., the fraction of people without the disease who test negative. For a perfect

diagnostic biomarker test, all patients with the disease or disease subset would be detected (100% sensitivity) and no patients without the disease would be diagnosed with the disease (100% specificity). In clinical practice, no biomarker test has such perfect clinical and analytical performance.

To be of clinical value in relation to PAD, CAS, and AAA, a biomarker must be stable, reliably measurable with reasonable cost and effort, and give additional relevant information on diagnosis or prognosis when combined with the diagnostic methods already used in clinical practice. It might be beneficial to combine assessment of several different biomarkers, due to large intra-individual variations *(Engelberger et al, 2015; Matsushita et al, 2018)*.

Biomarkers studied in this thesis

C-reactive protein (CRP)

C-reactive protein (CRP) is produced by the liver in response to a range of inflammatory cytokines occurring in the blood plasma and binds to dead or dying cells as a part of the immune system *(Sproston et al, 2018)*. Concentration of CRP increase rapidly in reaction to trauma, inflammation, and infection and decrease just as rapidly with the resolution of the condition. Measurement of CRP is commonly used to monitor various inflammatory states.

CRP is also a well-established plasma biomarker for atherosclerosis *(Li et al, 2017)* and a predictor of worse outcome in patients with PAD *(Singh et al, 2017)*. Previous studies have also reported a potential or significant link to AAA *(Badger et al, 2009)*.

Lipoprotein-Associated Phospholipase A_2 (mass and activity)

Lipoprotein-Associated Phospholipase A_2 (Lp-PLA_2) is an enzyme produced by the inflammatory cells in the blood vessels. It is released by monocytes, but can be synthesized by all hematopoetic and hepatic cells. High concentrations of the enzyme LP-PLA2 participates in progress of atherosclerosis by contributing to the modification of oxidated LDL in the atherosclerosis process, expressed by macrophages in atherosclerotic lesions. Lp-PLA_2 is considered a biomarker for atherogenesis *(Charniot et al, 2013)*, and levels have been cross-sectionally associated with PAD *(Li et al, 2013)* and carotid artery stenosis (*Liu et al, 2018*), whereas results are conflicting regarding its potential predictive role. Lp-PLA_2 activity and mass have been shown to predict both the risk for PAD in elderly individuals *(Garg et al, 2016)* and hospitalisation for PAD *(Garg et al, 2018)*. The

predictive value not been corroborated in all studies, however, Lp-PLA_2 activity and mass were not related to increased risk of incident PAD in an American study *(Garg et al, 2017)*.

Proneurotensin (PNT)

Proneurotensin produced by endocrine cells (N cells) scattered throughout the jejuno-ileal mucosa is a precursor to to the gut hormone neurotensin, a hormone in the central nervous system promoting fat absorption, diet-induced weight gain, and liver steatosis. Proneurotensin is linked to satiety, obesity regulation, and cardiometabolic disease *(Motiwala et al, 2014).* It is believed that this hormone promotes cardiocasvular disease in middle-aged populations.

Midregional proatrial natriuretic peptide (MR-proANP) and midregional proadrenomedullin (MR-proADM)

MR-proANP is a natriuretic peptide related to inflammation and hemodynamic stress (*Öner et al, 2019)* and MR-proADM is a peptide produced by vascular smooth muscle, vascular endothelial cells, cardiomyocytes, and many other cell types. MR-proADM is present in lungs, heart, and gastrointestinal regions *Öner et al, 2019),* and elevated in disease states in response to the increased leakage into the extracellular space and is a potent vasodilator. Both markers are potentially associated with microvascular endothelial dysfunction in relation to falls and fragility fractures *(Johansson et al, 2019)*, and elevated levels have been reported in PAD *(Kollerits et al, 2013)*.

N-terminal pro-B-type natriuretic peptide (NT-proBNP)

N-terminal pro-B-type natriuretic peptide (NT-proBNP) produced by cardiac atria is a marker of cardiac failure and myocardial ischemia. As heart failure progresses, there is an increase ventricular NT-proBNP synthesis causing diuresis, vasodilatation, and decreased renin and aldosterone secretion. Levels of the peptide have previously been reported to be elevated in PAD patients *(Falkensammer et al, 2015)* and cross-sectionally related to parameters reflecting common carotid artery lesions *(Sasaki et al, 2020)*. Additionaly, NT-proBNP has been shown to predictive both incident PAD *(Matsushita et al, 2018)* and higher all-cause mortality in PAD patients *(Kremers et al, 2020).*

Copeptin

Copeptin, the C-terminal fragment of proarginine vasopressin, is produced by the hypothalamus and released in response to several inflammatory stimuli *(Siong Chan et al, 2018)* is a marker of acute illness and disease and plasma concentrations are elevated in acute cardiac conditions *(Mueller et al. 2018).* Plasma levels are highly correlated to proarginine vasopressin and easier to measure *(Fenske et al, 2009).*

Cystatin C

Cystatin C is a protein expressed in all nucleated body fluid cells responsible for inhibiting proteinases *(Herget-Rosenthal, 2012).* As it is excreted and freely filtered by the glomerulus, high concentrations of Cystatin C are associated with kidney failure. Reduced kidney function reflected by cystatin C is associated with incident PAD *(Yang et al, 2017),* and PAD patients with elevated cystatin C have increased mortality *(Urbonaviciene et al, 2011).* Cystatin C levels have also been cross-sectionally associated with extracranial carotid artery stenosis in noncardioembolic stroke *(Umemura et al, 2016)* and negatively linked to AAA growth *(Lindholt et al, 2001)*.

Other biomarkers of potential relevance

Apart from the biomarkers studied in the present thesis, several other biomarkers have either been evaluated or are under current evaluation regarding their potential relevance in PAD *(Ziegler et al, 2022),* CAS *(Carballo-Perich et al, 2022), and* AAA *(Nana et al, 2021).*

No baseline samples from MDCS subjects were available for studies of inflammatory biomarkers such as interleukin 6 *(Engelberger et al, 2015; Pande et al, 2015; Signorelli et al, 2012; Signorelli et al, 2016)*, neopterin *(Signorelli et al, 2016; Caesovschih et al, 2020, Signorelli et al, 2013)*, and tumour necrosis factor-α *(Pande et al, 2015; Signorelli et al, 2016; Gardner et al, 2014)*, adhesion molecules such as selectins *(Engelberger et al, 2015; Signorelli et al, 2012; Signorelli et al, 2016)*, intracellular CAM (sICAM-1) and vascular CAM (sVAM-1) *(Engelberger et al, 2015; Signorelli et al, 2012; Signorelli et al, 2016; Signorelli et al, 2003; Edlinger et al, 2019)*, markers of oxidative stress such as asymmetrical dimethylarginine (ADMA) *(Dowsett et al, 2020)*, markers of platelet activation such as the tissue factor and von Willebrand factor *(Lee et al, 1999; Vieczor et al, 2020)*, or coagulation markers such as plasminogen activator inhibitor 1 (PAI-1) *(Engelberger et al, 2015; Mustonen et al, 1998; Lee et al, 1999; Vieczor et al, 2020)*, fibrinogen *(Unlu et al, 2006; Mustonen et al, 1998; Lee et al, 1999; Vieczor et al, 2020)*, D-dimer *(Engelberger et al, 2015; Mustonen et al, 1998; Lee et al, 1999;*

Vieczor et al, 2020), and tissue plasminogen activator (tPA) *(Mustonen et al, 1998; Lee et al, 1999; Vieczor et al, 2020)*. Neither were we able to study degrading enzymes such as matrix metalloproteinases *(Engelberger et al, 2015; Signorelli et al, 2012; Signorelli et al, 2016)* or markers of angiogenesis such as vascular endothelial growth factor A (VEGF-A) (*Vieczor et al, 2019; Findley et al, 2008; Stehr et al; 2010)*. No results regarding these biomarkers are therefore reported in this thesis, and no conclusions can be drawn regarding their potential relevance for prediction of PAD, CAS, or AAA in the studied cohort.

Table 1. Overview of plasma biomarkers studied in this thesis. For references, please see text on preceding pages 26-28.

Name	Cell origin	Type of marker	Disease marker
CRP	Liver	Inflammatory	Cardiovascular Obesity Diabetes
Lp-$PLAS_2$ (mass)	Monocytes	Inflammatory	Atherogenesis
Lp-$PLAS_2$ (activity)	Monocytes	Inflammatory	Atherogenesis
Proneurotensin	Gut	Fat metabolism	Satiety Obesity regulation
MR-proADM	Vascular smooth muscle and endothelial cells, cardiomyocytes	Vasodilatation	Endothelial dysfunction
MR-proANP	Atria	Cardiac	Heart failure, volume overload
NT pro-BNP	Atria	Cardiac	Heart failure, volume overload
Copeptin	Hypothalamus	Inflammatory	Heart failure, acute myocardial infarction, ischemic stroke
Cystatin C	Body fluids	Renal	Kidney failure

Treatment of arterial disease and diabetes

Non pharmacological and pharmacological medical treatment of PAD, CAS, AAA, and DM

Irrespectively of symptoms, patients with atherosclerotic PAD have widespread atherosclerosis and high rates of cardiovascular (CV) events *(Steg et al, 2007)*, and a low ankle-brachial index (ABI) is related to increased risk for CV events and mortality *(Heald et al, 2006)*. Atherosclerotic disease is also a common co-morbidity in subjects with AAA *(Wanhainen et al, 2019)*. Efficient treatment of

atherosclerotic risk factors should therefore be offered to patients with PAD *(Aboyans et al 2017; Frank et al, 2019),* carotid artery stenosis *(Naylor et al, 2018*), and AAA. *(Wanhainen et al, 2019).*

Diet and exercise

Healthy diet and physical exercise is recommended for all patients at risk for or with prevalent atherosclerotic vascular disease or DM *(Cosentino et al, 2020; Visseren et al, 2021)*, including those with PAD *(Aboyans et al 2017; Frank et al, 2019)* or carotid artery stenosis *(Naylor et al, 2018)*. A Mediterranean diet with low content of saturated fats, cholesterol, and sodium, but rich in fish, fibre, and nuts *(Visseren et al, 2021)* is recommended, and desirable weight level should be maintained. The amount of alcohol should be restricted to no more than 100 g of pure alcohol per week *(Visseren et al, 2021)*. These recommendations should also be considered in patients with AAA *(Wanhainen et al, 2019).* At least 150 - 300 min a week of moderate intensity or 75 - 150 min a week of vigorous intensity physical activity is recommended. If possible, exercise training in PAD should be supervised *(Fakhry et al, 2015; Mazari et al, 2017; Jansen et al 2019).*

Smoking cessation

Smoking cessation is recommended in all smokers with atherosclerotic vascular disease or DM, as well as in those at risk *(Aboyans et al 2017; Frank et al, 2019; Naylor et al, 2018; Cosentino et al, 2020)*, and lowers disease progression *(Willigendael et al, 2004; Aboyans et al, 2010).* As smoking is also associated with increased growth rate of AAA, this recommendation applies also to AAA patients *(Wanhainen et al, 2019)*. In this group, smoking cessation confers reduction in both growth rate and the risk of aneurysm rupture *(Sweeting et al, 2012)*. Nicotine replacement therapy, bupiron, and formal cessation support programs can be used *(Jorenby et al 1999; Steinberg et al, 2009; Hennrikus et al. 2010).*

Lipid lowering

Increased levels of total cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, and lipoprotein (a), and decreased levels of high-density lipoprotein *(Tunstall-Pedoe et al, 2017)* are risk factors for atherosclerotic vascular disease. Risk reductions in major adverse CV events (MACE) and all-cause mortality have been documented in PAD patients both with and without symptoms *(Ramos et al, 2016)*. Additionaly, statins improve walking distance in claudication *(Aronow et al, 2003; Mohler et al, 2003)*, reduce amputation rates *(Sohn et al, 2013; Hsu et al, 2017)* and improve patency of vascular grafts *(Abbruzzese et al, 2004)* in PAD, as well as reduces plaque lipid levels and inflammatory activity *(Crisby et al, 2001)*,

and the risk of TIA and stroke *(Collins et al, 2004; Crouse et al, 1997; Hegland et al 2010)* in patients with CAS. There is no definite proof of statin effects upon AAA growth, but statin prescription has been associated with survival benefits in AAA patients *(Bahia et al, 2016; Risum et al, 2021).*

Statin therapy is therefore warranted in all atherosclerotic patients with or without symptoms (*Mach et al, 2019*), and should be considered also in patients with AAA *(Wanhainen et al, 2019)*. An LDL goal of <1.4 mmol/L (55 mg/dL) and an LDL reduction of >50% from baseline is recommended for subjects with atherosclerotic disease (*Mach et al, 2019)*, and follow-up is important to ensure that these targets are reached. If targets are not reached with maximal tolerable statin doses, combination with ezetimibe *(Cannon et al, 2015)* or proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors *(Oyama et al, 2021; Jukema et al, 2019)* may be used. PCSK9 inhibitors decrease CV events in patients with PAD *(Oyama et al, 2021)* and polyvascular disease *(Jukema et al, 2019)*, but their use is hampered by cost issues in many countries.

Antihypertensive treatment

Antihypertensive therapy reduces CV events and mortality, and current guidelines on blood pressure (BP) lowering *(Mancia et al, 2023)* are applicable also for patients with atherosclerotic vascular disease and AAA *(Wanhainen et al, 2019)*. Medication is recommended at BP ≥140/90 mmHg, and target BP, if tolerated, is <130/80 mmHg in patients 18-64 years old and < 140/80 mmHg in ages 65-79 years *(Mancia et al, 2023)*. Most patients need two or more drug classes to achieve target BP, and angiotensin converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB), calcium channel blockers, and thiazide diuretics are recommended as first choices *(Mancia et al, 2023)*.

Antithrombotic treatment

Asymptomatic PAD

As antiplatelet treatment has no beneficial effects in asymptomatic PAD *(Belch et al, 2008; Fowkes et al, 2010)*, it is not recommended *(Aboyans et al 2017; Frank et al, 2019; Twine et al, 2023)* in PAD patients without leg symptoms or other symptomatic manifestations of atherosclerosis.

Symptomatic PAD

Antiplatelet therapy reduces vascular morbidity in PAD by 23 % *(Antithrombotic Trialists' Collaboration, 2002)*. In the PAD subgroup of the CAPRIE trial *(CAPRIE Steering Committee, 1996)* clopidogrel reduced both CV mortality and MACE

compared to low-dose aspirin. Meta-analysis showed that neither aspirin, cilostazol, picotamide, ticagrelor, ticlopidine, nor vorapaxar were superior to clopidogrel in PAD *(Katsanos et al, 2015)*. Single antiplatelet treatment with clopidogrel or aspirin or is therefore recommended in symptomatic stable PAD *(Aboyans et al 2017; Frank et al, 2019; Twine et al, 2023)*.

The combination of rivaroxaban 2.5 mg twice daily and aspirin 100 mg daily reduces both a composite of CV death, myocardial infarction, or stroke and rates of amputation and other major adverse limb events in patients with either stable PAD or CAS compared to aspirin alone *(Eikelboom et al 2017; Anand et al, 2018)*. Both European PAD guidelines *(Aboyans et al 2017; Twine et al, 2023)* and guidelines for CTLI *(Conte et al, 2019)* therefore advocate consideration of this combination in patients with stable PAD without high bleeding risk or other contraindications.

Evidence for double antiplatelet therapy (DAPT) after endovascular revascularization in lower limbs is lacking *(Peters Weem et al, 2016)*. The above mentioned combination of rivaroxaban 2.5 mg twice daily and aspirin 100 mg daily is beneficial compared to aspirin alone also after endovascular, hybrid, or open infrainguinal revascularization in PAD *(Bonaca et al, 2020; Hiatt et al, 2020)*, whereas no additive effects of combining aspirin with clopidogrel were detectable after open peripheral bypass surgery, except for in a subgroup receiving prosthethic grafts *(Belch et al, 2010)*.

CAS

Low dose aspirin is recommended as first line treatment in asymptomatic CAS, with clopidogrel as an alternative for patients with aspirin intolerance. In symptomatic cases not undergoing intervention, either clopidogrel 75 mg daily or aspirin 75 mg daily plus modified release dipyridamole 200 mg twice daily is considered as first choice stenosis *(Naylor et al, 2018).*

AAA

Direct evidence for beneficial effects of antithrombotic treatment in AAA is lacking, but antiplatelet therapy has been associated with better survival in this group *(Bahia et al, 2016)*, and should therefore be considered *(Wanhainen et al, 2019*), particularly in patients with concomitant atherosclerotic disease.

Treatment of type 2 diabetes

Tight glucose control has beneficial cardiovascular effects in diabetic patients *(Adler et al, 2002; Gaede et al, 2003)* and HbA_{1c} <7% or <53mmol/mol is recommended in diabetic patients with atherosclerotic disease *(Cosentino et al,*

2020). Treatment targets are individual, however, and less ambitious in elderly patients to reduce their risk for hypoglycemia *(Cosentino et al, 2020)*.

In addition to dietary changes and reduction of obesity, different glucose-lowering agents are available for treatment of type 2 diabetes: metformin, acarboses, glitazones, sulfonylurea, dipeptidyl peptidase-4 inhibitors, sodium-glucose linked transporter 2 (SGLT2) inhibitors, glucagon-like peptide-1 receptor agonists (GLP1-RA), and insulin. The GLP1-RA liraglutide, semaglutide, and dulaglutide *(Sattar et al, 2021)* as well as the SGLT2 inhibitors empaglifozin, canaglifozin, and dapaglifozin *(McGuire et al, 2021)* reduce the risk for CV events in type 2 diabetes. Increased risk for minor amputations was reported for canaglifozin *(Neal et al, 2017)*, however, but this finding could not be corroborated in metaanalysis *(McGuire et al, 2021)*. GLP1-RA or SGLT2 inhibitors are therefore recommended in type 2 diabetic patients with cardiovascular disease *(Cosentino et al, 2020)*, but canaglifozin should be used with caution in diabetic patients with CLTI *(Conte et al, 2019)*. Neither GLP1-RA nor SGLT2 inhibitors have been formally evaluated for treatment of type 2 DM in patients with PAD or AAA, however *(Liarakos et al, 2023)*.

Operations for PAD, CAS, and AAA

PAD

Most patients with PAD will be treated conservatively, especially those with asymptomatic disease and claudication. Development of CLTI requires more close attention, and the target population includes objectively documented PAD and ischemic rest pain, diabetic foot ulcer or any lower limb ulceration > 2 weeks of duration or gangrene involving any portion of the lower limb or foot *(Conte et al, 2019)*. Depending on patient comorbidity, patient preferences and vascular surgery competence at each centre, a variety of endovascular and open vascular surgical procedures can be offered.

CAS

Most patients with CAS will have asymptomatic disease, which if discovered should be medically treated. Asymptomatic CAS patients may be enrolled in randomized trials to evaluate if endovascular stenting or operation will be beneficial in preventing future cerebrovascular accidents. Patients with probable recent symptomatic CAS should be discussed in multidisciplinary conferences, and if considered symptomatic with 50-99% stenosis, offered carotid artery surgery (thrombendarterectomy with patch or eversion thrombendarterectomy) *(Naylor et al, 2018)*.

AAA

There is an indication for elective operative repair of AAA, either open or endovascular, when aneurysm diameter is ≥ 55 mm in men and ≥ 50 mm in women or emergency repair when there is a rupture of the AAA. In most patients in high income countries with suitable anatomy and reasonable life expectancy, endovascular aneurysm repair (EVAR) should be considered as the preferred treatment modality. In patients with long life expectancy, open AAA repair should be considered as the preferred treatment modality *(Wanhainen et al, 2019).*

Despite diagnostic and therapeutical advances in both atherosclerotic and aneurysmal disease, many patients with PAD, CAS, and AAA remain undetected until late disease stages. Prediction of these diseases therefore has to be improved. Additionaly, the paradoxal relationship between AAA and type 2 DM warrants further studies on relationships between AAA, glucose metabolism, and arterial stiffness.

Overall aim

To better clarify the utility of biomarkers in prediction of atherosclerotic and aneurysmal disease.

To evaluate disturbances in glucose metabolism and arterial stiffness in patients with abdominal aortic aneurysm.

Specific aims in papers I-VI

To evaluate inflammatory biomarkers in relation to established risk markers for incident PAD during long term follow-up (paper I).

To investigate the association between hemodynamic biomarkers and symptomatic PAD during long term follow-up (paper II).

To investigate novel biomarkers in relation to conventional risk factors and CRP for incident CAS during long term follow-up (paper III).

To clarify relationships between biomarkers and symptomatic and asymptomatic CAS during long term follow-up (paper IV).

To compare biomarkers and conventional risk factors for incident isolated atherosclerotic disease and incident isolated AAA during long term follow-up (paper V).

To evaluate disturbances in glucose metabolism and arterial stiffness in 65-year old men with and without screening detected AAA (paper VI).

Subjects and methods

Subjects (Paper I-V)

Malmö Diet and Cancer study (MDCS)

All subjects studied in this thesis were recruited from the cardiovascular arm of the population-based Malmö Diet and Cancer Study (MDCS) *(Hedblad et al, 2000)*. For the original MDCS study, a total of 71,104 middle-aged men and women from Malmö born between 1923 and 1950 (age range 45 to 73 years) were invited, but only 30,000 underwent all examination procedures *(Wirfält et al, 2002)*. This was caused by the following reasons:

- Death occurring between invitation and examination
- Relocation or unknown address of subject
- Refusal to participate
- Lack of literacy in the Swedish language
- Return of incomplete questionnaire
- Lack of answer to questionnaire

Among the MDCS participants, a random sample of 50 % was invited to undergo an additional more extensive evaluation; the MDCS cardiovascular arm (MDCS-CV). Of the 14,000 invited individuals, the 6,103 participating also in this extended investigation 1991-1994 *(Hedblad et al, 2000)* were included in the analyses presented in this thesis.

Subjects (Paper VI)

Sixty-five year old men examined within the national ultrasound screening program for AAA *(Zarrouk et al, 2013)*. Out of 193 men with screening detected AAA undergoing extended examination and blood sampling *(Lindberg et al, 2016; Memon et al, 2020)*, a subgroup of 48 men consented to a further extended examination of glucose homeostasis and aortic stiffness in this study. As a control group, we examined 115 screened 65-year-old men with aortic diameter <30 mm at the screening examination, matched for dates of examination and blood sampling.

Methods (Paper I-V)

The following definitions were used in the baseline examination in MDCS-CV.

Current smoking was defined as self-reported regular smoking or smoking cessation within the last year.

Diabetes mellitus was defined as self-reported physician´s diagnosis, use of anti-diabetic medication, or fasting whole blood glucose >6.0 mmol/l.

Hypertension was defined as use of antihypertensive medication or blood pressure ≥140/90 mmHg.

Weight (kg) and height (m) were measured in light indoor clothing and BMI

calculated as kg/m^2.

Ultrasound examination of the right carotid artery was performed at baseline.

Laboratory measurements

Glycosylated hemoglobin (HbA1c) was determined by ion exchange chromatography, using the Swedish Mono-S standardization system; reference values were 3.9–5.3% in non-diabetic individuals.

Plasma (p-) triglycerides, low-density lipoprotein (LDL-C) and high-density lipoprotein (HDL-C) cholesterol levels were obtained utilizing a Cobas device (Roche Diagnostics).

Methods (Paper VI)

Aortic ultrasound

Aortic ultrasound examinations were performed by biomedical scientists and registered nurses using the LOGICe (General Electric Healthcare Inc, Chalfont S. Giles, UK). The maximal infrarenal anteroposterior diameter of the aorta was evaluated, and an AAA was defined as an aortic diameter of ≥30 mm, from the leading edge of the adventitia in the anterior wall to the leading edge of the intima of the posterior wall.

Oral glucose tolerance test (OGTT)

After an oral 75 g glucose load, p-glucose, serum (s-) insulin, and p-glucagon were analysed from blood samples at 0, 60, and 120 min. The Hemocue Glucose System (HemoCue AB, Ängelholm, Sweden) was used to analyse p-glucose and the DakoELISA kit (Glostrup, Denmark) to analyse s-insulin (minimum detection level 3 pmol/l, intra- and interassay coefficients of variation 5.1–7.5% and 4.2–9.3%,

respectively). P-glucagon was assayed with radioimmunoassay GL-32K (Merck Millipore, Dermstadt, Germany, minimum detection level 18.5 pg/ml, intra- and interassay coefficients of variation 3.6–6.2 % and 8.7–14.7%. respectively). Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was defined as (fasting p-glucose in mmol/L x fasting p-insulin in mU/L) /22.5.

Skin autofluorescence

Skin autoflourescence of Advanced Glycation End products (AGE) was estimated in arbitrary units with an AGE Reader (DiagnOptics, Groningen, Netherlands). Ultraviolet light with a peak wavelength of 360-370 nm was radiated on the forearm, and the amount of light reflected and emitted measured by a spectrometer *(Fokkens, et al 2016)*.

Arterial stiffness, pulse wave velocity

Carotid-femoral (c-f) PWV was measured using arterial tonometry (*Laurent et al, 2006)* as the median of two measurements (three in case of large variations) in supine position with a Sphygmocor device (Atcor, Australia). Subjects rested for five minutes before the first measurement and then one minute between the first and the second measurement. Pulses were measured at the femoral artery and the carotid artery, respectively, and the distance between the two points multiplied by 0.8. The pulse transit time was divided by this length to estimate pulse wave velocity *(Butlin et al, 2016)*.

Biomarkers (Paper I-V)

Plasma biomarkers were measured from fasting plasma samples frozen at - 80° C immediately after collection *(Melander et al, 2009)*.

CRP was measured by a high-sensitivity Tina-quant® latex assay (Roche Diagnostics, Basel, Switzerland). The average coefficient of variation (CV) was 4.6% (15).

Lp-PLA_2 was expressed as enzymatic activity and mass (quantity) *(Persson et al, 2007)*. Lp-PLA_2 activity was measured in duplicate using [3H]-platelet activating factor as substrate. The range of detection was 8 – 150 nmol/min/ml, and average CV 5.8%.

Lp-PLA_2 mass measurements were performed using the commercially available second generation PLAQ™ test (diaDexus Inc., South San Francisco, CA, USA) enzyme-linked immunosorbent assay (ELISA) kit (16). Plasma-EDTA samples are stable for Lp-PLA_2 activity and mass measurements within seven days of collection for refrigerated samples and for more than 10 years from collection when stored at -70 °C *(Persson et al, 2007)*.

Proneurotensin was measured using a chemiluminometric sandwich immunoassay *(Melander et al, 2012)*.

Levels of MR-proADM were measured using immunoluminometric sandwich assays targeted against amino acids in the midregions of the peptide (BRAHMS AG, Henningsdorf, Germany) *(Morgenthaler et al, 2005)*. Lower and upper limits of detection were 0.08 and 25 nmol/L, respectively.

MR-proANP was measured using immunoluminometric sandwich assays targeted against amino acids in the midregion of the peptide (BRAHMS, Berlin, Germany).

NT pro-BNP was measured using the automated Dimension Vista Intelligent Lab System method (Siemens Diagnostics, Nürnberg, Germany) *(Melander et al, 2009)*. Mean inter-assay CVs were ≤10% for MR-proADM, ≤10% for MR-pro-ANP, and 2.7% for NT pro-BNP.

Copeptin was measured using a commercially available assay in the chemiluminescence/coated tube format (BRAHMS AG, Henningsdorf, Germany). Lower detection limit was 0.4 pmol/L and functional assay sensitivity (<20% interassay CV) was <1 pmol/L *(Fenske et al, 2009)*.

Cystatin C was measured using a particle-enhanced immune-nephelometric assay (N Latex Cystatin, Siemens Diagnostics, Dade Behring, Deerfield, IL) with a mean inter-assay CV of 4.3%.

Table 2. Overview of laboratory measurements of biomarkers.

Name	Assay	Manufacturer	CV (%)
CRP	High-sensitivity Tina-quant® latex	Roche Diagnostics	4.59
Lp-PLA_2 (mass)	Sandwich enzyme immuno-	diaDexus Inc	4.62
Lp-PLA_2 (activity)	Enzyme-linked immunosorbent	Non commercial	5.78
Proneurotensin	Chemiluminescence	Non commercial	4.1-6.2
MR-proADM	Immunoluminometric sandwich	Brahms AG	≤10
MR-proANP	Immunoluminometric sandwich	Brahms AG	≤10
NT pro-BNP	Automated Dimension Vista Intelligent Lab System	Siemens diagnostics	2.7
Copeptin	Chemiluminescence	Brahms AG	<20
Cystatin C	Particle-enhanced immune-nephelometric	Siemens diagnostics	4.3

CRP; C-reactive protein, Lp-PLA_2 (activity and mass); lipoprotein-associated phospholipase A2, MR-proADM; mid-regional proadrenomedullin, MR-proANP; mid-regional proatrial natriuretic peptide, CV; coefficient of variation.

Endpoint ascertainment (Paper I-V)

All subjects were followed up from the baseline examination until first event, emigration from Sweden, mortality, or end of follow-up at December 31, 2016. Individuals from the MDCS-CV with a first registered diagnosis of PAD (papers I-II), CAS (papers III-IV), atherosclerotic disease (paper V), and AAA (paper V) were identified from Swedish registers (the Inpatient and Outpatient Registries and the Cause of Death Register *(Ludvigsson et al, 2011; http://www.socialstyrelsen.se/register/halsodataregister/inenglish. Accessed Sept 11, 2018 and May 14, 2020)* by linkage of the 10-digit personal identification number unique to each Swedish resident. The Inpatient Register includes information on dates of admission and discharge as well as diagnostic and procedural codes from all hospitalizations in Sweden. The Cause of Death Register includes diagnoses of underlying and contributing causes of death from death certificates. In both validated *(Ludvigsson et al, 2011)* registers, diagnoses are coded using a Swedish revision of the International Classification of Diseases (ICD), version 8, 9, 10, and surgical procedures are coded using a Swedish classification system *(Landenhed et al, 2015)*.

Subjects with registred diagnoses of PAD (papers I-II), CAS (papers III-IV), atherosclerotic disease (paper V), and AAA (paper V) already at baseline were excluded from the analyses reported in the respective papers. In paper V, isolated atherosclerotic disease was defined as atherosclerosis without concomitant AAA, and isolated AAA as AAA without concomitant atherosclerotic disease.

Validation of PAD diagnosis (papers I-II)

One hundred patients from the original MDCS cohort *(Hedblad et al, 2000; Manjer et al, 2001; Manjer et al, 2002)* with diagnosis of PAD were randomly selected for the validation procedure of patient record data in digital and paper archives at Skåne University Hospital. Among 100 patients, 69 had CLTI, 13 had acute limb ischemia, 15 had IC and one had asymptomatic PAD. Two patients had venous insufficiency and were misdiagnosed. The diagnosis of PAD was thus confirmed in 98% of cases and symptomatic PAD in 97%.

Validation of carotid artery stenosis diagnosis (papers III-IV)

One hundred patients from the original MDCS cohort *(Hedblad et al, 2000; Manjer et al, 2001; Manjer et al, 2002)* with a diagnosis of CAS were randomly selected for the validation procedure of patient record data in digital and paper archives at Skåne University Hospital. A stenosis degree of ≥ 60% measured with ultrasound, CT-, or MR-angiography was considered as validation of the diagnosis. Among these 100 patients, 57 had symptomatic CAS and 42 asymptomatic CAS. The proportions of

operated patients with symptomatic and asymptomatic stenosis were 85.9% (49/57) and 14.3% (6/42), respectively. One patient had coronary artery disease and was misdiagnosed. The diagnosis of CAS could thus be confirmed in 99% of cases, and had been symptomatic in 57% of the patients.

Validation of atherosclerotic disease and AAA (paper V)

Extended validation was performed for paper V. Diagnoses obtained from the Swedish National Patient register were validated in digital and paper archives at Skåne University Hospital in 100 patients with coronary artery disease and 100 patients with ischemic stroke for the endpoint atherosclerotic disease. The above mentioned validations of 100 patients with PAD for papers I-II and 100 patients with CAS for papers III-IV were used also for for the endpoint atherosclerotic disease in paper V. Additionaly, 100 patients with a registered diagnosis of AAA were validated regarding this endpoint.

Coronary artery disease was confirmed in 96% of cases, ischemic stroke in 89%, PAD in 98%, CAS in 99%, and AAA in 95% of the patients. (This information is available in the supplemental material to paper V.)

Statistics (papers I-VI)

Quantitative normal and skewed distributed variables are presented as mean with standard deviation and median with interquartile range (IQR), respectively. Dichotomous variables are presented as count and proportion. Differences between groups were assessed with Mann-Whitney U test for continuous non-parametric variables, t-test for continuous parametric variables, and with Chi-Square test for nominal variables. In paper V, comparison between nominal variables was performed with Fisher´s exact test. Correlations between continuous variables were tested with the Pearson test.

In papers I-II individuals with a diagnosis of PAD at baseline were excluded from the studies and prospective analyses included only incident PAD. In papers III-IV individuals with a diagnosis of CAS at baseline were excluded from the studies and prospective analyses included only incident CAS. In paper V, participants with either prevalent atrial flutter or fibrillation (AF), coronary artery disease, ischemic stroke, carotid artery disease, PAD, or AAA at baseline were excluded.

Plasma biomarkers and confounders for incident PAD (papers I-II), CAS (papers III-IV), atherosclerotic disease (paper V), and AAA (paper V) were assessed using Cox regression models, and hazard ratios (HRs) were expressed per one standard deviation (SD) increment of each respective log transformed plasma biomarker (skewed distributed) in the Cox regression models. Cumulative incidences of PAD (papers I-II), CAS (papers III-IV), isolated atherosclerotic disease (paper V), and

isolated AAA (paper V) were analyzed using Kaplan-Meier method. In paper I, log-rank test was used in the comparison of quartiles for Lp-PLA_2 activity.

In paper VI, paired samples T-test was used to analyze the change in laboratory data between two different time points after OGTT. The mean change in p-glucose/s-insulin/p-glucagon from nadir value to 2-hour test was compared in men with and without AAA using univariate analysis of variance (ANOVA), adjusted for smoking, waist-hip ratio (WHR), and nadir values of p-glucose, s-insulin, or p-glucagon, respectively. Associations between hyperglycemia and odds for AAA were evaluated with multivariable logistic regression analysis, adjusted for smoking, WHR, and each hyperglycemia variable separately. Odds were expressed as odds ratios (OR) with 95 % confidence intervals (CI). Associations between plasma lipids and presence of AAA, adjusted for treatment with lipid-lowering agents, were evaluated with logistic regression. Associations between laboratory variables and abdominal aortic diameter, adjusted for smoking, treatment with lipid-lowering agents, and WHR were evaluated with linear regression analysis.

Analyses were performed using SPSS for Windows, versions 23.0 and 26 (SPSS Inc, Chicago, IL). P-values less than 0.05 were considered significant.

Ethical approvals

All scientific work was performed after appropriate approval of the following applications by the Ethics Committee of Lund University* or the Regional Ethical Reviw Board in Lund, Sweden:

Papers I-V

- Dnr LU 51-90, approved February 14, 1990*.
- Dnr 2007/166, approved April 12, 2007.
- Dnr 2009/633, approved November 19, 2009.
- Dnr 2013/566, approved August 27, 2013.

Paper VI

- Dnr LU 2010/239, approved July 2, 2010.
- Dnr LU 2014/643, approved October 30, 2014.

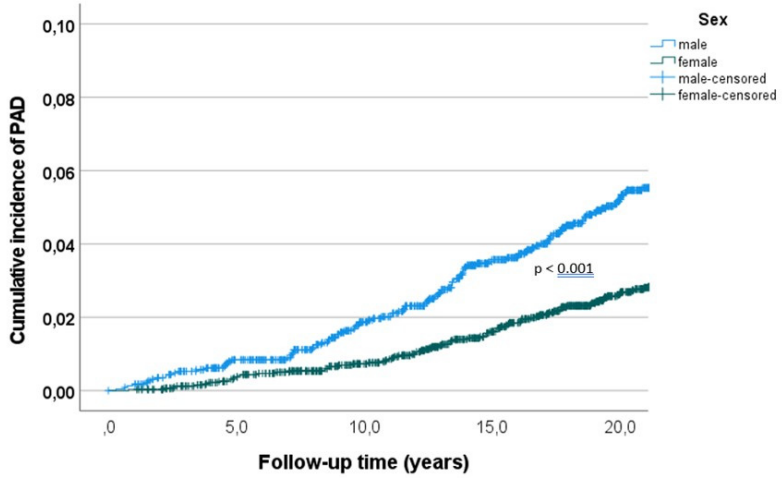
Extraction from the MDCS database of data used for papers I-V was approved May 20, 2015 (MKC 2015-03) and November 11, 2017 (MKC-2015-003 kompl 2) by the Steering Committé for the Malmö Preventive Project and MDCS databases.

Results

Paper I. Inflammatory plasma biomarkers and incident PAD

Cumulative incidence of PAD

After exclusion of 15 subjects with a diagnosis of PAD already at baseline, the cumulative incidence of PAD during a median follow up of 23.4 years (IQR 19.4–24.3) was 4.4% (244/5550); 5.9% (137/2307) for men and 3.3% (107/3243) for women ($p < 0.001$, Fig 6).



Persons at risk

Men	2295	2198	2046	1840	1543
Women	3241	3164	3026	2836	2531

Fig 6. Cumulative incidence of symptomatic PAD in relation to sex among participants in the Malmö Diet and Cancer cohort.

Main findings

In multi-variable analysis adjusted for conventional risk factors, Lp-PLA_2 activity (HR 1.33; 95% CI 1.17–1.52), Lp-PLA_2 mass (HR 1.20; 95% CI 1.05–1.37), and CRP (HR 1.55; 95% CI 1.36–1.76) were all independently associated with incident PAD during follow-up (Table 3). With CRP added as a covariate together with Lp-PLA_2 (activity or mass), age, current smoking, BMI, sex, arterial hypertension, and diabetes mellitus in an extended multi-variable analysis, Lp-PLA_2 activity (HR 1.33; 95% CI 1.17–1.52; $p < 0.001$), Lp-PLA_2 mass (HR 1.16; 95% CI 1.01–1.32; $p = 0.038$) and CRP (HR 1.36; 95% CI 1.18–1.58; $p < 0.001$) were still associated with occurrence of PAD.

Table 3. Multi-variable adjusted hazards ratios for incident PAD in relation to plasma biomarkers.

Plasma inflammatory biomarkers	**PAD** N=244. HR* (95% CI)	p-value
CRP (n=5300)	1.55 (1.36 – 1.76)	< 0.001
Proneurotensin (n=4627)	0.94 (0.80 – 1.09)	0.41
Lp-$PLAS_2$ (mass) (n=5390)	1.20 (1.05 – 1.37)	0.008
Lp-$PLAS_2$ (activity) (n=5395)	1.33 (1.17 – 1.52)	< 0.001

The following variables were entered in the multivariable analysis besides each respective plasma biomarker: Age at study entry, BMI, sex, current smoking, diabetes mellitus, and hypertension. *Hazard ratios (HR) were expressed per one SD increment of each respective log transformed plasma biomarker PAD; peripheral artery disease, CRP; C-reactive protein, Lp-PLA_2 (activity and mass); lipoprotein-associated phospholipase A2, HR; Hazard Ratio, CI; Confidence interval

Paper II. Plasma hemodynamic biomarkers and incident PAD

Main findings

Copeptin, (HR 1.46; 95% CI 1.19–1.80), N-BNP (HR 1.28; 95% CI 1.11–1.48), and cystatin C (HR 1.19; 95% CI 1.10–1.29) were independently associated with incident PAD in multivariable adjusted analysis, whereas we were not able to detect any such associations regarding MR-proANP, (HR 1.13; 95% CI 0.98–1.31) or MR-proADM (HR 1.16; 95% CI 1.00–1.34) (Table 4).

Table 4. Multivariable analysis of plasma hemodynamic biomarkers at baseline in subjects later developing PAD.

Plasma hemodynamic biomarkers	**PAD** N=244. HR* (95% CI)	p-value
Cystatin C (n=5150)	1.19 (1.10 – 1.29)	< 0.001
Copeptin (n=5248)	1.46 (1.19 – 1.80)	< 0.001
N-BNP (n=5156)	1.28 (1.11 – 1.48)	0.001
MR-proANP (n=5255)	1.13 (0.98 – 1.31)	0.010
MR-proADM (n=5254)	1.16 (1.00 – 1.34)	0.050

The following variables were entered in the multivariable analysis besides each respective plasma biomarker: Age at study entry, BMI, sex, current smoking, diabetes mellitus, hypertension, total cholesterol *Hazard ratios (HR) were expressed per one SD increment of each respective log transformed plasma biomarker PAD; peripheral arterial disease, N-BNP; N-terminal pro-B-type natriuretic peptide, MR-proANP; midregional proatrial natriuretic peptide, mid-regional proadrenomedullin (MR-proADM), HR; Hazard Ratio, CI; Confidence interval

A score taking levels of all the three above mentioned predictive biomarkers into account also had predictive potential regarding PAD development during follow-up. Hazard ratios were 1, 1.31, 2.25, and 3.29 for subjects having 0, 1, 2, and 3 biomarkers in the highest quartiles of the respective markers.

Paper III. Plasma biomarkers, incident CAS, and incident carotid surgery

Cumulative incidence of carotid artery stenosis

Seven individuals with either CAS already at the baseline ultrasound examination of the right carotid or with a previously registered CAS diagnosis were excluded. The cumulative incidence of CAS was 2.3% (125/5543), 3.4% (75/2227) in men and 1.5% (50/3316) in women ($p < 0.001$), during median follow up of 23.4 years (IQR 19.5-24.3) (Fig 7).

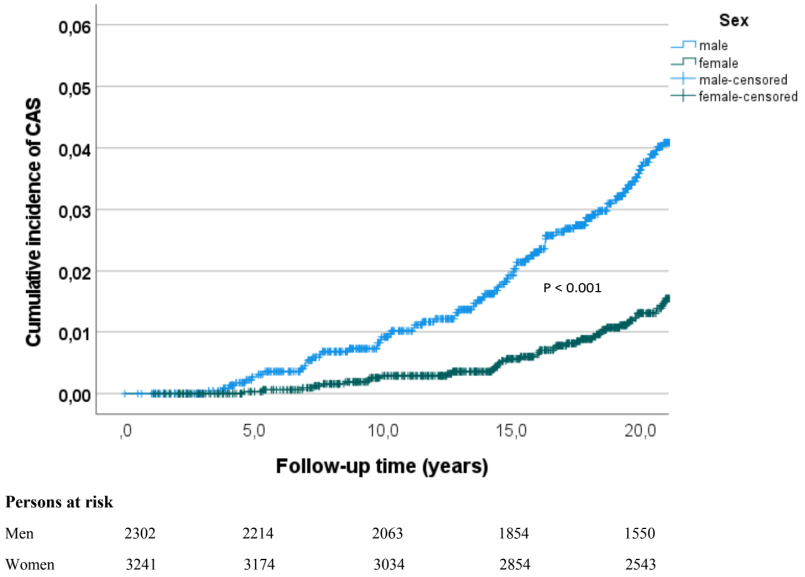


Fig 7. Cumulative incidence of CAS in relation to sex among participants in the Malmö Diet and Cancer cohort.

Main findings

NT pro-BNP (HR 1.36; 95% CI 1.12-1.65; p = 0.002) was independently associated with incident CAS in the Cox regression analysis (Table 5), whereas such associations nearly reached significance for CRP (HR 1.20; 95% CI 0.98-1.48; p = 0.071) and MRproADM (HR 1.21; 95% CI 0.99-1.47; p = 0.061). In a separate analysis of the the 55 patients undergoing either open surgery or stenting for CAS, MRproADM (HR 1.30; 95% CI 1.03-1.65; p = 0.029) was independently associated with incident intervention for CAS, whereas the association for NT pro-BNP narrowly failed to reach significancy (HR 1.31; 95% CI 1.00-1.72; p = 0.052).

Table 5. Multi-variable adjusted hazards ratios for incident CAS in relation to plasma biomarkers.

Plasma biomarkers	Carotid stenosis N=125. HR* (95% CI)	p-value
C-reactive protein	1.20 (0.98 – 1.48)	0.071
Lipoprotein-associated phospholipase A2 (mass)	1.01 (0.83-1.23)	0.92
Lipoprotein-associated phospholipase A2 (activity)	1.10 (0.89-1.37)	0.37
Proneurotensin	0.94 (0.76-1.17)	0.57
Mid-regional proadrenomedullin	1.21 (0.99-1.47)	0.061
Mid-regional proatrial natriuretic peptide	1.03 (0.84-1.27)	0.77
N-terminal pro-B-type natriuretic peptide	1.36 (1.12-1.65)	0.002
Copeptin	1.21 (0.93-1.58)	0.16
Cystatin C	1.05 (0.86-1.28)	0.64

The following variables measured at baseline were entered in the multivariable analysis besides each respective plasma biomarker: age, BMI, sex, current smoking, diabetes mellitus, hypertension, cholesterol. *Hazard ratios (HR) were expressed per one SD increment of each respective log transformed plasma biomarker.

Paper IV. Plasma biomarkers and incident symptomatic and asymptomatic CAS

Identifying symptomatic and asymptomatic CAS

Among 125 patients registered as having incident CAS, four patients were misdiagnosed, of which three had other atherosclerotic manifestations and one had carotid artery dissection. In 11 patients, the diagnosis of CAS could not be confirmed as records were incomplete. One hundred and ten patients had confirmed CAS; 56 had been judged as having symptomatic stenosis by a multidisciplinary round comprising a neurologist, a vascular physician, and a vascular surgeon whereas 54 had been judged as having asymptomatic CAS.

Main findings

In the Cox regression analysis, NT pro-BNP (HR 1.59; CI: 1.20–2.11), MR-proADM (HR 1.40; CI: 1.13–1.73), cystatin C (HR 1.21; CI: 1.02–1.43), and CRP (HR 1.53; CI: 1.13–1.73) at baseline were independently associated with incident symptomatic CAS during follow-up (Table 6), whereas the corresponding associations were not significant for Lp-PLA_2 mass (HR 1.28; 95% CI: 0.97–1.69) and Lp-PLA_2 activity (HR 1.34; 95% CI: 0.99–1.81). Regarding prediction of

incident asymptomatic CAS, on the other hand, no relationships with baseline plasma biomarker pattern could be demonstrated (Table 6).

Table 6. Multi-variable adjusted hazards ratios for plasma biomarkers in relation to incident symptomatic and asymptomatic CAS

Plasma biomarkers	Symptomatic CAS N=56. HR* (95% CI)	p-value	Asymptomatic CAS N=54. HR* (95% CI)	p-value
Lipoprotein-associated phospholipase A2 (mass)	1.28 (0.97 – 1.69)	0.080	0.87 (0.64 – 1.18)	0.36
Lipoprotein-associated phospholipase A2 (activity)	1.34 (0.99 – 1.81)	0.061	0.95 (0.68 – 1.33)	0.77
Proneurotensin	0.90 (0.63 – 1.28)	0.55	0.96 (0.70 – 1.30)	0.77
Mid-regional proadrenomedullin	1.40 (1.13 – 1.73)	0.002	0.98 (0.69 – 1.40)	0.92
Mid-regional proatrial natriuretic peptide	1.12 (0.83 – 1.52)	0.47	0.79 (0.58 – 1.09)	0.15
N-terminal pro-B-type natriuretic peptide	1.59 (1.20 – 2.11)	0.001	1.08 (0.80 – 1.47)	0.61
Copeptin	1.35 (0.88 – 2.06)	0.17	1.16 (0.80 – 1.67)	0.43
Cystatin C	1.21 (1.02 – 1.43)	0.030	0.75 (0.50 – 1.10)	0.14
C-reactive protein	1.53 (1.13 – 2.05)	0.005	1.08 (0.80 – 1.46)	0.63

The following variables analysed at baseline were entered in the multivariable analysis besides each respective plasma biomarker: Age, sex, BMI, current smoking, diabetes mellitus, hypertension, and cholesterol. Asymptomatic patients were excluded when assessing symptomatic CAS, and symptomatic patients were excluded when assessing asymptomatic CAS. *Hazard ratios (HR) were expressed per one SD increment of each respective log transformed plasma biomarker.

Paper V. Plasma biomarkers for incident isolated atherosclerotic disease and incident isolated AAA

Main findings

During median follow- up of 23.1 years (IQR16.3–24.2), the cumulative incidence of isolated atherosclerotic disease (i.e. without concomitant AAA) was 22.2% (1,196/5,381); 28.6% (622/2,178) in men and 17.9% (574/3,203) in women. The cumulative incidence of isolated AAA (i.e. without concomitant atherosclerotic disease) was 1.6% (88/5,381), and 68 (77.3%) of study subjects diagnosed with AAA were men.

Lp-PLA_2 (activity) and MR-proADM were both associated with incident isolated atherosclerotic disease and incident isolated AAA during follow-up. NT pro-BNP,

copeptin, cystatin C, proneurotensin, and CRP were associated only with incident isolated atherosclerotic disease, whereas Lp-PLA_2 (mass) was associated only with incident isolated AAA (Table 7). Adjusted HR for Lp-PLA_2 (mass) (HR 1.53, 95% CI 1.14–2.04 vs. HR 1.05, 95% CI 0.99–1.12) was higher for incident isolated AAA compared to incident isolated AD, respectively.

Table 7. Adjusted hazard ratios for plasma biomarkers in relation to incident isolated AD and incident isolated AAA

Plasma biomarker	Incident AD (free from incident AAA). HR* (95% CI)	p-value	Incident AAA (free from incident AD). HR* (95% CI)	p-value
Lipoprotein-associated phospholipase A2 (activity)	1.12 (1.04 – 1.19)	0.001	1.53 (1.11 – 2.11)	0.009
Lipoprotein-associated phospholipase A2 (mass)	1.05 (0.99 – 1.12)	0.096	1.53 (1.14 – 2.04)	0.004
Copeptin	1.09 (1.01 – 1.17)	0.018	0.98 (0.70 – 1.39)	0.92
Mid-regional proadrenomedullin	1.17 (1.10 – 1.25)	<0.001	1.47 (1.15 – 1.88)	0.002
Mid-regional proatrial natriuretic peptide	1.03 (0.97 – 1.11)	0.31	1.01 (0.71 – 1.43)	0.97
N-terminal pro-B-type natriuretic peptide	1.16 (1.08 – 1.24)	<0.001	1.13 (0.80 – 1.60)	0.49
Cystatin C	1.17 (1.11 – 1.23)	<0.001	1.13 (0.82 – 1.55)	0.47
Proneurotensin	1.07 (1.02 – 1.13)	0.010	1.09 (0.85 – 1.40)	0.49
C-reactive protein	1.17 (1.10 – 1.25)	<0.001	1.22 (0.88 – 1.68)	0.24

AD = atherosclerotic disease, AAA = abdominal aortic aneurysm. Adjusted for age, sex, BMI, current smoking, hypertension and total cholesterol and each respective plasma biomarker. * HR were expressed per 1 SD increment. Participants with incident AAA were excluded when assessing participants with incident AD, and participants with incident AD were excluded when assessing participants with incident AAA.

Papers I-V. Overview of predictive biomarkers

The utility of the different studied biomarkers for prediction of different types of incident arterial disease during follow-up are summarized in table 8 below.

Table 8. Summary of properties of the biomarkers for prediction of different arterial diseases. Utility for prediction shown as +.

	CRP	Lp-PLA_2 (mass)	Lp-PLA_2 (activity)	PNT	MR-proADM	MR-proANP	NT pro-BNP	Copeptin	Cystatin C
PAD	+	+	+				+	+	+
CAS							+		
Operation for CAS					+				
Symptomatic CAS	+				+		+		+
Asymptomatic CAS									
Isolated atherosclerotic disease	+	+		+	+		+	+	+
Isolated AAA		+	+		+				

PAD; peripheral arterial disease, CAS; carotid artery stenosis, AAA; abdominal aortic aneurysm, CRP; C-reactive protein, Lp-PLA_2 (activity and mass); lipoprotein-associated phospholipase A2, PNT; proneurotensin, MR-proADM; mid-regional proadrenomedullin, MR-proANP; mid-regional proatrial natriuretic peptide.

Paper V. Traditional risk factor profiles for incident isolated atherosclerotic disease and incident isolated AAA

Main findings

Hypertension (HR 1.57, 95% CI 1.36–1.80), DM (HR 2.57, 95% CI 2.08–3.18), and total cholesterol (HR 1.10/SD increment, 95% CI 1.04–1.17) were associated with incident isolated atherosclerotic disease in the adjusted Cox regression analysis, whereas BMI (HR 1.43/SD increment, 95% CI 1.02–2.00) was associated with incident isolated AAA (Table 9). Adjusted HR for male sex (HR 4.8, 95% CI 2.42–9.48, vs. HR 1.76, 95% CI 1.56–1.98) and current smoking (HR 4.79, 95% CI 2.42–9.47 vs. HR 1.97, 95% CI 1.73–2.23) were higher in subjects with incident isolated

AAA compared to those with incident isolated atherosclerotic disease. When substituting hypertension for systolic blood pressure in the Cox regression model, systolic blood pressure was associated with incident isolated atherosclerotic disease (HR 1.33/SD increment, 95% CI 1.25–1.42) but not with incident isolated AAA (HR 1.026/SD increment, 95% CI 0.73–1.44).

Table 9. Adjusted hazards ratios for traditional risk factors in relation to incident isolated AD and incident isolated AAA.

Plasma biomarker	Incident AD (free from incident AAA). HR* (95% CI)	p-value	Incident AAA (free from incident AD). HR* (95% CI)	p-value
Age years, median (IQR)	1.66[a] (1.55 – 1.78)	<0.001	1.82[a] (1.30 – 2.54)	<0.001
Male sex, %	1.76 (1.56 – 1.98)	<0.001	4.80 (2.42 – 9.48)	<0.001
Body mass index, kg/m^2, median (IQR)	1.04[a] (0.98 – 1.11)	0.17	1.43[a] (1.02 – 2.00)	0.038
History of hypertension (%)	1.57 (1.36 – 1.80)	<0.001	0.81 (0.42 – 1.56)	0.53
History of diabetes (%)	2.57 (2.08 – 3.18)	<0.001	b	b
Current smoking (%)	1.97 (1.73 – 2.23)	<0.001	4.79 (2.42 – 9.47)	<0.001
Total cholesterol, mmol/L, median (IQR)	1.10[a] (1.04 – 1.17)	0.002	1.12[a] (0.81 – 1.55)	0.51

AD = atherosclerotic disease, AAA = abdominal aortic aneurysm. Adjusted for variables in the table. [a] HR were expressed per one SD increment. Participants with incident AAA were excluded when assessing participants with incident atherosclerotic disease, and participants with incident atherosclerotic disease were excluded when assessing participants with incident AAA. [b] No participant with DM developed AAA without concomitant atherosclerotic disease so diabetes was excluded from the Cox regression model when analyzing independent risk factors associated with incident AAA.

Paper VI. AAA in relation to glucose metabolism and insulin resistance

Main findings

Men with and without AAA

Men with AAA had higher BMI, abdominal height, WHR, smoking exposure, and more often hypertension, peripheral arterial disease, and lipid lowering treatment than those without AAA. Total cholesterol, LDL-, and HDL cholesterol were lower in men with AAA despite adjustment of their higher use of lipid lowering agents, whereas triglycerides, leukocytes, and homocysteine were higher in men with AAA compared to those with normal aortic diameter. There were no differences between groups in blood pressure, c-f-PWV, or skin autofluorescence of AGE.

Glucose metabolism in men with and without AAA

Both the frequency of diabetes mellitus, mean HbA_{1c} values, and HOMA-IR were higher in men with screening-detected AAA compared to those without AAA. During OGTT, s-insulin was higher at 0, 60, and 120 minutes in men with AAA compared to those without. There was also a significantly higher increase in s-insulin in men with AAA compared to those with normal aortic diameter after OGTT, adjusted for smoking, WHR, and nadir value of s-insulin.

Glucose metabolism and AAA

When adjusting for smoking and WHR there were no associations between hyperglycemia variables and the odds for AAA. When entering DM or prediabetes, smoking, and WHR as covariates, only smoking (OR 3.3, 95% CI 1.3 – 8.3; p=0.013) and WHR (OR 4.2, 95% CI 1.7 – 10.2; p=0.002) were independently associated with AAA.

Glucose metabolism and abdominal aortic diameter

In analyses adjusted for smoking habits, lipid-lowering treatment, and WHR, the increase in p-insulin at 2-hours (p=0.006) after OGTT and p-homocysteine were associated with abdominal aortic diameter, whereas there were no associations between any variable reflecting hyperglycemia and abdominal aortic diameter. B-leukocytes and p-homocysteine were positively associated, and p-total and LDL-cholesterol were inversely associated with abdominal aortic diameter, adjusted for smoking and WHR.

Discussion

Methodological considerations

Limitations of the studies

All research results reported, and conclusions drawn in this thesis are derived from research based on the research subjects´ voluntary participation. As population-based cohorts were invited to the studies reported in papers I-V, there is no sampling bias which needs to be taken into account, but as in other screening situations *(Zarrouk et al, 2013)* subjects complying with the invitation can be expected to have better health status than those who chose not to participate. Follow-up of participants and non-participants in MDCS confirmed that this was the case also in the present study (*Manjer et al, 2001).* On the other hand, no specific analysis has been performed to evaluate whether those choosing to volunteer for MDCS-CV differed from the average of MDCS participants with regard to social background or health status. The loss of subjects for analysis in relation to the original MDCS sample undoubtedly consists a limitation of the studies, however, and despite the poulation based study design the conclusions drawn regarding associations between biomarkers, PAD, CAS, other atherosclerotic disease, or AAA might still not reflect associations in the entire background population.

Other limitations of the studies reported in papers I-V were the storage of samples before analysis and the lack of baseline assessment of PAD, CAS, and AAA. As the MDCS was originally planned as a study of relationships between dietary intake and cancer, no ABI measurement or aortic ultrasound was performed at baseline, and only the right carotid artery was assessed by ultrasound. Exclusion of subjects with prevalent disease already at baseline therefore had to be made through scrutinization of registry data and patient files. Re-invitation of study participant survivors for objective detection and verification of development of vascular manifestations would also have been valuable, as this would have captured both asymptomatic atherosclerosis and changes in risk factor status and medication. The present study design only allows us to report incident disease of enough clinical importance to cause an episode of hospitalization to be registered in national registries. Neither were any interventional studies included in the thesis. Assessment of potential therapeutic interventions, both upon biomarker levels and the conditions studied,

would also have strengthened the reported associations between biomarkers, PAD, CAS, and AAA.

Furthermore, it must be kept in mind that only a few of the many biomarkers with potential importance for prediction of PAD, CAS, and AAA have been assessed, and no conclusions can be drawn regarding potential relevance of other different biomarkers. And as always when relating biomarkers to the occurrence of disease manfestations, causality of the reported can be questioned. The reported studies certainly fulfill the Bradford Hill criteria *(Hill, 1965)* of temporality and plausibility, but many of the other criteria for causality: strength, consistency, specificity, biological gradient, coherence, experimental evidence, and analogy *(Hill, 1965)* are not always met.

Several important limitations apply to the study reported in paper VI. As screening for AAA is recommended *(Wanhainen et al, 2019)* and conducted *(Zarrouk et al, 2013)* only in 65-year old men, the results are only applicable to this particular group. Furthermore, the study was cross-sectional with a small sample. In analogy with the MDCS *(Manjer et al, 2001),* subjects complying with the invitation to ultrasound screening for AAA *(Zarrouk et al, 2013)* and in particular with the extended investigations reported in the paper can also be expected to have better health status than those who chose not to participate.

Strengths of the studies

Major strengths of the studies reported in papers I-V, on the other hand, were the longitudinal study design, the inclusion of healthy middle-aged individuals, and the long follow-up of 23.4 years. Additionaly, thourough validation of patient files from 100 patients in each of the different disease groups confirmed that hospital diagnoses were accurate in the vast majority of cases. It must also be emphasized that the associations between different biomarkers and manifestations of vascular disease were independent of conventional well established risk factors, such as smoking, blood pressure and lipid levels, and glycaemic status. On the other hand, we did not adjust for other risk markers of potential importance such as nutritional factors *(Kulezic et al, 2019)*, psychosocial stress *(Sara et al, 2022)* and family history *(Banerjee, 2012)*. Extraction of registry data regarding hospitalisation for atherosclerotic disease in first degree relatives to MDCS subjects has recently been ethically approved (Dnr 2020/01652 and 2021/01814), however, potentially enabling such analysis in the future.

The major strength of paper VI is the thourough investigation performed, particularily the the fact that sampling was performed at three different occasions during the OGTT; 0, 60, and 120 minutes.

Ethical considerations

As evident both from the study acronym and the above listed multitude of different ethical applications, the MDCS was originally planned as a study of relationships between dietary intake and cancer. Extension of the study aims to allow also assessment of the collected data in relation to incident cardiovascular disease as reported in both this thesis and many other publications therefore had to be separately sought and approved in the following supplementary applications 2007, 2009, and 2013.

A PubMed search for ”Malmo diet and cancer study” performed August 17, 2022 retreived 631 results, highlighting the question whether separate ethical approval should be mandatory for each individual publication or if several publications can be written on atherosclerotic disease as has been performed in this thesis.

Another ethical issue relevant for all biomarker research is whether study subjects should be informed about the results of biomarker analysis. As levels of the different biomarkers investigated and reported in this thesis do not presently constitute clinical treatment indications, such information was not given in this case.

For an individual study participant, the knowledge of the prescence of a potential but not yet treatable risk factor or disease might perhaps also have negative psychosocial consequences *(Ericsson et al, 2017)*. This has been shown to be relevant *(Ericsson et al, 2017)* for the AAA screening project *(Zarrouk et al, 2013)* from which study subjects were recruited for paper VI. The extended examination regarding glucose metabolism and arterial stiffness, on the other hand, is hardly controversial from an ethical point of view. The ethical application specified that potential newly discovered disturbances in glucose metabolism were to be properly managed and treated by the study team.

Aspects on the validation of endpoints

The validation procedure of different cardiovascular endpoints helped ascertain that participants hospitalized for clinically relevant PAD, CAS, and AAA had been identified from the registries, whereas individuals with subclinical disease were few. Almost all participants with incident PAD were symptomatic and 82% had advanced disease, either CLTI or acute lower limb ischaemia, almost half of participants with incident CAS had an asymptomatic moderate to severe CAS, and 70% of participants with incident AAA had a medium to large-sized asymptomatic AAA incidentally found at imaging or abdominal palpation. The studied plasma biomarkers therefore reflect long-term exposure to pathophysiological mechanisms promoting development of clinically relevant PAD, CAS, and AAA.

Comparison of plasma biomarker profile at baseline regarding incident symptomatic and asymptomatic disease manifestations was possible only for CAS (in paper IV). Participants with ≥60% asymptomatic CAS as judged by absence of neurological symptoms or deficits, or cerebrovascular accidents as reported in paper IV should not be confused with MDCS participants with subclinical mild carotid plaques defined as focal intima-media thickness > 1.2 mm on carotid ultrasound *(Rosvall et al, 2006)*. The incidences of moderate (>50%) and severe (>70%) asymptomatic CAS in the MDCS by systematic evaluation of the right carotid artery at baseline were 0.6% and 0.1% *(de Weerd et al, 2014)*, resulting in 43 cases with right-sided CAS. This means that clinically relevant asymptomatic CAS might have been present at baseline in around 70 participants if they would have been examined for CAS with bilateral carotid ultrasound, as 10% could be expected to have bilateral CAS *(Johnston et al, 2004; Khan et al, 2021)*. The proportion of participants with asymptomatic moderate to severe CAS at end of follow-up in paper IV (when the median age of the MDCS participants was around 80 years) can therefore roughly be predicted to have been around 5% if they would have been systematically screened with ultrasound at this stage *(de Weerd et al, 2010)*, a figure substantially higher than the 1% rate of asymptomatic CAS reported at end of follow-up in paper IV. Despite this, the low number of participants in each group, and the resulting low statistical power, it was possible to show elevated expressions of several plasma biomarker levels in incident symptomatic compared to asymptomatic CAS.

Utility of biomarkers for prediction of PAD, CAS, and AAA

Biomarkers could potentially be used for several different purposes in PAD, CAS and AAA (Table 10). The studies reported in papers I-V in this thesis have all been focused on prediction of incident disease, and subjects with prevalent disease at baseline had been excluded from analysis.

Table 10. Potential use of biomarkers in PAD, CAS, and AAA.

Prediction of incident disease
Diagnosis of prevalent disease
Marker of disease severity
Prediction of prognosis
For choice of, or monitoring of efficacy of currently available treatment
For choice of, or monitoring of efficacy of future potentially available treatment

PAD

Results reported in papers I-II suggest that elevated plasma levels of inflammatory biomarkers such as Lp-PLA2 activity and mass, CRP, and copeptin could all be considered markers of subclinical disease long before diagnosis of PAD advanced enough to cause severe symptoms and hospitalisation. In addition to prediction of PAD, CRP is a marker of both prevalent PAD *(Pande et al, 2015)* and a more ominous of prognosis and future CV events *(Vainas et al, 2005;* S*ingh et al, 2015)* in PAD patients. Additionaly, both CRP and other inflammatory markers such as TNF alpha and interleukin-6 have been shown to be markers of PAD severity *(Pande et al, 2015)*. In summary, inflammatory biomarkers are therefore of relevance in several of the situations listed in table 10 above.

In our studies, it is important to note that 82% of patients in whom the diagnosis of PAD was validated had either acute or chronic limb threatening ischaemia, however, and no conclusions can therefore be drawn from the present results regarding prediction of less severe forms of PAD not necessitating hospitalisation, such as asymptomatic PAD and IC. Inflammatory markers have been previously related to IC, however *(McDermott et al, 2003; Pande et al 2015)*. Neither can we draw any conclusions from our findings regarding the potential importance of elevated biomarker levels for limb prognosis, nor regarding mortality in patients in whom PAD has already been diagnosed.

Despite the fact that many drugs currently used in PAD and other forms of cardiovascular disease, such as antiplatelet agents, statins, and PCSK9 inhibitors have anti-inflammatory properties, we cannot draw any conclusions regarding the utility of the studied biomarkers for monitoring of treatment effects. Antiinflammatory features are even more pronounced in some drugs currently under investigation for their role in CVD colchicine, methotrexate, anti-TNF-alpha agents and monoclonal antibodies *(Antonopoulos et al, 2018)*.

Cardiac and renal markers such as NT pro-BNP and cystatin C were also predictors of future PAD in the MDCS subjects. But again, we cannot draw any conclusions regarding the utility of these markers for detection of prevalent PAD or the prognosis of PAD in our patients. Relationships with mortality are known to exist at least for NT-pro BNP *(Clemens et al, 2019)* in PAD patients, however.

When evaluating a combination of the two cardiac biomarkers troponin T and NT-pro BNP for prediction of incident PAD and especially CLTI (defined as hospitalization and leg revascularization) in 12,288 middle-aged subjects during 22 years of follow-up, Matsushita and colleagues reported that the risk was increased in subjects with elevated levels of both markers *(Matsushita et al, 2018)*. Subjects in the highest categories of both troponin T and NT-proBNP had up to 15 times increased risk for incident CLTI and 5 times increased risk for incident PAD *(Matsushita et al, 2018),* corroborating the notion *(Engelberger et al, 2015*) that multiple biomarker testing increases the utility of sampling. The latter was

confirmed also in our studies, in which a score taking high levels of biomarkers reflecting different pathophysiological mechanisms, copeptin, NT pro-BNP, and cystatin C into account could be used to predict PAD.

The concept of broader testing can be even further expanded, however. Studies using the Proseek 96 Multiplex CVD III 96^{x}96 proximity extension assay enabling plasma profiling of 92 CVD related proteins have confirmed that several novel protein biomarkers are associated with prevalent PAD in patients with other forms of arterial disease, such as myocardial infarction *(Jönelid et al, 2021)* and AAA *(Dakhel et al, 2022)*. This technique was not available, however, when biomarker testing was performed in the MDCS patients in our studies.

CAS

Only one biomarker, NT pro-BNP could be used to independently predict incident carotid artery stenosis during the same 23.4 years of follow up reported in paper III, implying that this marker apparently has the utility to predict occurrence of atherosclerosis at multiple locations in the arterial tree *(Wang et al, 2004)*.

The marker has previously been shown both to be related to surrogate markers such as measurements of the carotid artery *(Sasaki et al, 2020)* and hard clinical endpoints such as cardiovascular events and death *(Wang et al, 2004)*. This concept was further emphasised by the findings in paper V; NT pro-BNP was an independent relevant predictor also when subjects were followed up regarding a more widely defined outcome, a composite of atherosclerotic disease without concomitant AAA.

Subjects developing symptomatic CAS in paper IV underwent sampling several years before detection of CAS, but inflammatory biomarkers such as CRP, TNF-alpha, and Il-6 have also been associated with prevalent CAS *(Kigka et al, 2021)*. As outlined in table 10 biomarkers might also be used for prediction of prognosis in patients with prevalent disease, however, which might be of large relevance for CAS patients as their stroke incidence is low *(Abbott, 2009; Naylor et al, 2009; Marquardt et al, 2010)*. Selection of proper candidates for carotid artery surgery among patients in whom a CAS has so far remained asymptomatic is therefore important. The utility of the biomarkers tested in this thesis for prediction of neurological symptoms within the near future therefore needs to be further investigated together with other available methods such as ultrasound *(Goncalves et al, 2003; Gronholdt et al, 2001; Kölbel et al, 2010; Stenudd et al, 2020)* or radiological *(Kwee et al 2008; Kwee et al, 2009, Kwee et al, 2010)* plaque features in a cohort undergoing modern pharmacological treatment *(Naylor et al, 2018*) for established asymptomatic CAS. CRP, Il-6, and TNF-alpha have all been associated with carotid plaque vulnerability *(Kigka et al, 2021)*, and a recent study on malondialdehyde, a biomarker for oxidative stress, indicated that increased levels

of this marker could be another indicator of increased stroke risk *(Svoboda et al, 2023)*.

When restricting the analysis to prediction of incident symptomatic carotid artery stenosis in paper IV, relationships with more biomarkers were detectable. Apart from NT-pro BNP, also MR-proADM, cystatin C, and CRP were independently associated with the occurrence of symptomatic carotid stenosis, in addition to their previously documented associations with coronary artery disease and congestive heart failure *(Melander et al, 2009)*. These results corroborate that there are complex and significant differences between the pathophysiology in symptomatic CAS featuring active atherosclerotic embolization and cerebral events, and asymptomatic CAS with dormant atherosclerotic plaques *(Saam et al, 2006; Shaalan et al, 2004)*. That different plasma biomarker profiles could be demonstrated long before diagnosis of CAS might also indicate that individuals with incident symptomatic CAS have a more generalized and potentially progressive subclinical atherosclerosis already at baseline compared to those developing incident asymptomatic stenosis.

AAA

Follow-up regarding incident AAA in paper V revealed that both plasma biomarker and traditional risk factor profiles for incident isolated atherosclerotic disease and incident isolated AAA had several important differences, despite their many similarities.

As shown in table 8, seven different biomarkers predicted isolated atherosclerotic disease whereas incident isolated AAA was only predicted by three markers. Among the biomarkers, both the inflammatory marker Lp-PLA_2 activity, and the vasoactive marker MR-proADM were associated with both incident isolated atherosclerotic disease and incident isolated AAA, and could therefore be regarded as general indicators of increased risk for future arterial disease. The fact that Lp-PLA_2 mass was more elevated in those developing isolated AAA than in those developing isolated atherosclerotic disease might be interpreted as suggesting that the development of AAA has a more distinct component of arterial inflammation.

When different atherosclerotic diseases were analysed as a composite in paper V, the differences regarding Lp-PLA_2 patterns in atherosclerosis and AAA was different than when speculating on Lp-PLA_2 patterns in PAD and AAA in paper I. Both Lp-PLA_2 activity and mass *(Acosta et al, 2017)* and MR-proADM *(Acosta et al, 2017)* have previously been established as biomarkers of future AAA hospitalization in MDCS subjects, but in these previous reports AAA incidence was not evaluated as isolated AAA without concomitant atherosclerotic disease. Interestingly, however, there might apparently be differences in biomarker patterns regarding prediction of either small AAA or large AAA requiring hospitalization or surgery. The same markers predicted neither aortic dilatation nor asymptomatic

aneurysm when male MDCS participants underwent ultrasonic screening for AAA at age 65 *(Taimour et al, 2017),* suggesting that they might either not be relevant until closer to AAA diagnosis, or only for prediction of larger AAA. On the other hand, our results do not allow us to draw any conclusion regarding the proposed relationships *(Memon et al, 2020; Nana et al, 2021)* between biomarkers and AAA growth.

NT pro-BNP, copeptin, cystatin C, proneurotensin, and CRP, on the other hand, predicted only the outcome variable incident isolated atherosclerotic disease, i.e. a more wide set of cardiovascular manifestations than the outcomes incident PAD used in papers I-II and incident CAS evaluated in papers III-IV. This finding regarding CRP is surprising, since this marker has previously been cross-sectionally linked to occurrence of large AAA *(Badger et al, 2009).*

Apart from prediction of incident AAA disease, biomarkers could also be used to evaluate the prognosis in patients with an already existing AAA. Such analysis has shown that CRP is a reliable predictor also of major cardiovascular events *(Golledge et al, 2023),* and matrix metalloproteinase-9 has been proposed as an indicator of an increased risk of AAA rupture *(Yang et al, 2022)*. A broader approach with simultaneous investigation of several different protein biomarkers for detection and growth of prevalent AAA *(Memon et al, 2020)*, or markers such as global DNA methylation and homocysteine for detection of prevalent AAA *(Vats et al, 2020)* has also been proven useful.

To summarize, the differences in plasma biomarker profile long before diagnosis of either atherosclerotic disease or AAA might be interpreted as suggesting that these are different disease entities with at least partly different pathophysiologies.

Utility of different biomarkers, summary

During the 23.4 years of follow-up, we were able to document 244 cases of incident PAD, 125 cases of incident CAS, and 1,196 cases of incident isolated atherosclerotic disease. As expected, and shown in table 8, the highest number of predictive biomarkers could be demonstrated for the more commonly occurring conditions. Isolated atherosclerotic disease and PAD were predicted by seven and six different markers, respectively, out of which CRP, Lp-PLA_2 (mass), NT pro-BNP, copeptin, and cystatin C predicted both conditions. A combination of these different markers reflecting both inflammatory, cardiac, and renal mechanisms might therefore be relevant to evaluate prospectively in relation to PAD.

On the other hand, no predictive utility in relation to any condition could be documented for MR-proANP in the studies in this thesis, discouraging the use of this marker in future studies of prediction of arterial disease. In this context, it should be noted that the prior study of this marker in relation to PAD *(Kollerits et al, 2013)* was cross-sectional, evaluated the marker in relation to prevalent PAD, and that the

association between MR-proANP and PAD disappeared after adjustment for other biomarkers.

For proneurotensin, we were only able to demonstrate a relationship with the most broadly defined outcome, incident isolated atherosclerotic disease. This corroborates previous research on this marker *(Motiwala et al, 2014),* establishing the predictive ability of the marker for a broad composite endpoint of coronary events and stroke. Sex differences like those previously presented *(Motiwala et al, 2014)* were not assessed in our study.

Conventional risk factors for prediction of PAD, CAS, and AAA

The relevance of well established risk factors for atherosclerotic (*Mach et al, 2019; Cosentino et al, 2020; Mancia et al, 2023*) and aneurysmal *(Wanhainen et al, 2019)* arterial disease was confirmed in the thesis. Male gender, smoking, hypertension, lipid levels, and glycaemic status were related to the occurence of PAD in papers I-II, CAS in papers III-IV, and isolated atherosclerotic disease in paper V. When comparing the relation of conventional risk factors to either isolated atherosclerotic disease or isolated AAA in paper V it was interesting to note that no participant with DM at baseline developed isolated AAA, whereas diabetes was associated with an increased risk of isolated atherosclerotic disease as expected. This corroborates the notion of an inverse relationship between diabetes and AAA occurrence *(Avdic et al, 2018; Raffort et al, 2018; Xiao et al, 2021)*. As expected *(Wanhainen et al, 2019),* relationships with male sex and current smoking were also more evident among subjects developing isolated AAA than among those developing isolated atherosclerotic disease.

AAA in relation to glucose metabolism and insulin resistance

The above mentioned observation that no MDCS participant with DM at baseline developed isolated AAA during follow-up highlighted the need for the deeper investigation of glucose homeostasis reported in paper VI. This analysis revealed that AAA was related to hyperinsulinemia, whereas none of previously reported negative associations between AAA and diabetes *(Avdic et al, 2018; Raffort et al, 2018; Xiao et al, 2021)* could be confirmed.

On the other hand, we found relative hyperinsulinemia in 65-year old men with AAA. There are not much previous data available in this field, but hyperinsulinaemia and higher HOMA-IR were demonstrated when a small group of AAA patients with larger aortic diameters > 55 mm were compared to control patients with smaller aneurysms *(Lareyre et al, 2018)*, and retrospective associations between the metabolic syndrome and AAA size have been reported *(Lee et al, 2022)* suggesting a potential relationship between aortic diameter and insulin resistance. These studies were relatively small, however, a limitation which is highly relevant also regarding our study.

The influence of glucose metabolism might also be different upon formation and growth of AAA. One might speculate that factors related to AAA in this study such as insulin resistance and hyperinsulinaemia might promote AAA development, whereas hyperglycaemia and overt type 2 diabetes in later stages might counterbalance this trophic effect, explaining previous findings *(Avdic et al, 2018; Raffort et al, 2018; Xiao et al, 2021)*. In another screening study for AAA in 65-year old men *(Rabben et al, 2021)*, BMI > 30, hypertension, and smoking were independently associated with increased risk for small AAA, whereas overt diabetes mellitus was an independent protective factor against AAA. This supports the notion that hyperinsulinemia may be a trigger factor for AAA development as suggested in paper VI. But as we conducted a cross-sectional study, such hypotheses remain to be further evaluated.

The most common drug for treatment of type 2 DM, metformin, increases insulin sensitivity but has also been shown to effect pathophysiological mechanisms relevant for AAA growth *(Raffort et al, 2020; Unosson et al, 2021)*, which might partly explain the negative relationships between AAA and DM *(Raffort et al, 2020)*. The awaited results of ongoing randomized clinical trials evaluating whether metformin reduces growth of AAA in non-diabetic patients *(Wanhainen et al, 2021)* might therefore perhaps increase our understanding of these mechanisms.

We were not able to corroborate the increased aortic PWV previously reported in patients with aneurysmal disease *(Durmus et al, 2014)*. This might have been due to a comparably smaller aneurysm size in our patients. Aortic stiffness might increase locally in an AAA *(Xiong et al, 2008)* and focal measurements of stiffness as suggested in guidelines *(Laurent et al, 2006)* might have helped clarify this.

Future considerations

Presently, the clinical use of biomarkers for prediction, diagnosis, or prognosis of PAD, CAS, or AAA is limited, as it is relatively cheap and easy for trained operators to diagnose PAD with ABI and CAS and AAA with ultrasound. Guidelines for the respective diseases *(Aboyans et al, 2017; Naylor et al, 2018; Wanhainen et al, 2019)* therefore do not recommend routine biomarker testing. In primary care, however, where sometimes both the necessary equipment and skills for the above mentioned investigations might be lacking, investigation of circulating biomarkers related to incident atherosclerotic or aneurysmal disease might potentially become a feasible screening alternative to select individuals for more thorough investigation and preventive pharmacological measures to counteract cardiovascular risk factors at an early stage before development of manifest disease. CRP, Lp-PLA_2 (mass), NT pro-BNP, copeptin, and cystatin C predicted both PAD and incident atherosclerotic disease in the studies in this thesis. A combination of these markers might therefore be the most relevant for prospective evaluation.

Preventive medication for patients with PAD, CAS, or AAA consists of drugs targeting traditional cardiovascular risk factors *(Aboyans et al, 2017; Naylor et al, 2018; Wanhainen et al, 2019).* In patients with more advanced disease stages, imaging could potentially be combined with organ- or disease specific prognostic biomarkers both to stratify risk and evaluate indications for intensified medical treatment or interventional endovascular or surgical procedures, for example through surveillance of AAA growth *(Nana et al, 2021)*.

Furthermore, biomarker levels could be used to monitor treatment effects. Modern treatment can also be directed towards inflammatory processes *(Antonopoulos et al, 2018; Bhattacharya et al, 2022)* reflected by biomarker levels. For example inhibition of the IL1β-IL6-CRP pathway with canakinumab was associated with lower progression rate of PAD in a recent clinical trial *(Russell et al, 2019).*The concept of biomarker analysis as a monitor of treatment effects will potentially be even more relevant if and when future treatment can be directed more directly against inflammatory mechanisms *(Antonopoulos et al, 2018).* The potential utility of biomarker use for evaluation of effects of currently evaluated novel non-interventional revascularization methods such as gene- and cell therapy *(Nordanstig et al, 2023)* also remains to be established.

As the pathophysiology of both atherosclerotic and aneurysmal disease is multifactorial, and levels of many biomarkers are increased in several different inflammatory conditions, the interpretation of a certain level of a single marker might be complicated. Both the utility of a composite of three biomarkers in paper in paper II and the different biomarker patterns in relation to isolated AAA and

isolated atherosclerotic disease reported in paper V suggest both that broader multiple-biomarker approaches might be needed and that such approaches should be tailored with repect to the particular group of diseases one is aiming to investigate. In such analyses, the relative activation of different pathophysiological pathways such as for example inflammation, cardiac, and renal function, reflected by different sets or combinations of biomarkers *(Matsushita et al, 2018; Engelberger et al, 2015; Jönelid et al, 2021, Dakhel et al, 2022)* might be even more valuable for risk stratification and appropriate choice of treatment in the individual patient. Furthermore, many biomarkers not investigated in this thesis might of course prove to have large diagnostic and preventive potential, either alone or as adjuncts to either the presently tested biomarkers or conventional diagnostic tools such as imaging.

Laboratory methodology is undergoing continuous development. Plasma profiling with multiplex immunoassays allowing simultaneous measurement of a multitude of different proteins related to cardiovascular disease *(Doran et al, 2021)* has recently been established as a method for prediction of both peripheral atherosclerotic *(Jönelid et al, 2021; Dakhel et al, 2022)* and aneurysmal *(Memon et al, 2020)* disease. This type of analyses might enable detection of new and unexpected relationships, but require both large cohorts to yield enough statistical power and extensive resources to process the large output of data resulting from such analyses.

Analysis of disease-specific circulating endogenous single-stranded micro-RNAs regulating gene expression at the post-transcriptional level as diagnostic and prognostic biomarkers for PAD *(Stather et al, 2013; Li et al 2011; Stather et al, 2015)*, CAS *(Carballo-Perich et al, 2022)* and AAA *(Stather et al, 2015)* is another promising method.

Research on the role of biomarkers as adjuncts to traditional diagnostic tools in PAD *(Ziegler et al, 2022),* CAS *(Carballo-Perich et al, 2022), and* AAA *(Nana et al, 2021)* is rapidly expanding. Many promising substances are currently under investigation as either potential future markers of diagnosis and prognosis or targets for treatment.

The negative association between AAA and DM has been well established by now *(Avdic et al, 2018; Raffort et al, 2018; Xiao et al, 2021)*, whereas the concept of insulin resistance and hyperinsulinaemia promoting AAA development and hyperglycaemia and overt type 2 diabetes potentially counterbalancing this effect at later stages remains to be confirmed. Ongoing interventional studies regarding the effects of metformin upon AAA growth *(Wanhainen et al, 2021)*, might help shed more light on these mechanisms.

Conclusions

- CRP and Lp-PLA_2 activity and mass are independently associated with incident PAD during long-term follow-up when adjusted for conventional risk factors (Paper I).
- NT pro-BNP, cystatin C, and copeptin are independently associated with incident PAD during long-term follow-up when adjusted for conventional risk factors. (Paper II).
- NT pro-BNP and MR-proADM are independently associated with incident CAS during long-term follow-up when adjusted for conventional risk factors (Paper III).
- CRP, NT pro-BNP, cystatin C, and MR-proADM are independently associated with incident symptomatic CAS during long-term follow-up when adjusted for conventional risk factors (Paper IV).
- CRP, Lp-PLA_2 (mass), NT pro-BNP, copeptin, and cystatin C predicted both PAD and incident atherosclerotic disease. A combination of these markers might therefore be relevant for prospective evaluation.
- Different biomarker patterns predict incident isolated AAA and incident isolated atherosclerotic disease during long-term follow-up (Paper V).
- AAA was related to hyperinsulinemia, whereas none of previously reported negative associations between AAA and diabetes or any relations between AAA and c-f-PWV could be confirmed (Paper VI).

Populärvetenskaplig sammanfattning på svenska

Kärlsjukdom orsakad av åderförkalkning är en av våra viktigaste och vanligaste folksjukdomar. Trots att vi har kännedom om flera viktiga riskmarkörer som manligt kön, rökning, blodtrycksförhöjning, höga blodfetter och diabetes vore det av stort värde att hitta indikatorer som mer exakt talar om vilka individer som löper extra hög risk att drabbas, i syfte att erbjuda dessa aktiv förebyggande behandling.

En så kallad ”biomarkör” kan utgöras av ett blodprov som indikerar ökad eller minskad risk för att drabbas av ett visst sjukdomstillstånd. I avhandlingen har vi värderat nivåerna av flera sådana biomarkörer mätta hos deltagare i en befolkningsstudie i Malmö (Malmö Kost Cancerstudien [MDCS]) 1991-94 i relation till risken att utveckla av åderförkalkning i olika blodkärl respektive bråck på stora kroppspulsådern (abdominellt aortaaneurysm [AAA]) under 23,4 års uppföljning fram t.o.m. 2016.

Delarbete I utgörs av en långtidsuppföljning av insjuknande i åderförkalkning i nedre extremiteternas kärl (perifer artärsjukdom [PAD]) hos 5550 medelålders individer utan PAD vid baslinjeundersökningen 1991-94. Efter justering för ålder, kön, rökning, kroppsmått, blodtrycksförhöjning och diabetes mellitus innebar förhöjda nivåer av biomarkörerna lipoprotein-associated phospholipase A2 (Lp-PLA_2) och C-reaktivt protein (CRP) vid baslinjeundersökningen ökad risk för insjuknande i PAD under 23,4 års uppföljning.

I delabete II visade sig i samma material även förhöjda nivåer av biomarkörerna copeptin, N-terminal pro-B-typ natriuretisk peptid (NT pro-BNP) och cystatin C på motsvarande sätt innebära ökad risk för insjuknande i PAD under 23,4 års uppföljning, efter justering för ålder, kön, rökning, kroppsmått, blodtrycksförhöjning, blodfettnivå och diabetes.

I delarbete III visade sig i samma material förhöjda nivåer av biomarkörerna CRP, Lp-PLA_2, proneurotensin, midregional proatrial natriuretisk peptid (MR-proANP) och mid-regionalt proadrenomedullin (MR-proADM), copeptin och cystatin C vid baslinjeundersökningen även innebära ökad risk för uppkomst av åderförkalkning i halsblodkärlen under de 23,4 uppföljningsåren.

Delarbete IV bekräftade att förhöjda nivåer av NT pro-BNP, MR-proADM, cystatin C och CRP vara oberoende prediktorer av de fall av åderförkalkning i halskärlen som varit symptomgivande, och därför möjligen skulle kunna användas som hjälpmedel i urval av patienter som bör erbjudas en förebyggande operation.

Jämförelse i delarbete V mellan deltagare i MDCS som under de 23,4 årens uppföljning utvecklade åderförkalkningssjukdom med de som utvecklade AAA avseende nivåer av biomarkörer vid baslinjeundersökningen 1991-94. Förhöjda nivåer Lp-PLA_2 var mer tydligt förknippat med risk för AAA än för åderförkalkning, medan förhöjda nivåer av MR-proADM innebar ökad risk för både åderförkalkning och AAA. Ingen deltagare med diabetes vid baslinjeundersökningen utvecklade AAA, vilket skulle kunna stämma med tidigare studier som indikerar att diabetes skyddar mot AAA. Diabetes innebar däremot ökad risk för åderförkalkning. Manligt kön och rökning medförde en mer tydlig riskökning för AAA än för åderförkalkning.

I delarbete VI undersöktes 65-åriga män med och utan AAA avseende blodsocker- och insulinbalans. Höga insulinnivåer var kopplade till kroppspulsåderns diameter, men vi kunde inte belägga tidigare presenterade skyddseffekter av diabetes på utveckling av AAA.

Sammanfattningsvis visas i avhandlingen att förhöjda nivåer av flera olika biomarkörer innebär ökad risk att utveckla åderförkalkningssjukdom i halsens och nedre extremiteternas blodkärl eller AAA under långtidsuppföljning av medelålders personer utan tidigare kärlsjukdom, oberoende av tidigare kända riskfaktorer. Riskfaktorbilden avseende biomarkörer för utveckling av AAA avviker delvis från mönstret vid utveckling av åderförkalkning, och relationerna mellan AAA, insulinnivåer och diabetes behöver kartläggas bättre.

Comprehensive summary in Farsi

خلاصه علمی ساده به زبان فارسی

بیماري عروقي ناشي از آترو□کلروز)گرفتگی عروق یا تصلب شرائین (یکی از بیماري هاي رایج و مهم مي باشد .علیرغم اینکه آگاهی ن□بت به چندین عامل خطر یا ری□ک فاکتور مهم مثل جن□یت مذکر، کشیدن □یگار، فشار خون بالا، چربی خون بالا و دیابت وجود دارد اما یافتن شاخص هایی که بتواند بطور دقیق ،تر نشان دهد که کدام افراد در معرض خطر بالاتری قرار دارند با هدف ارائه درمانهای پیشگیرانه فعال .ارزشمند می باشد

بیومارکر یا نشانگر زی□تی، یک فاکتور موجود در خون ا□ت که میتواند افزایش یا کاهش خطر ابتلا به یک بیماری بخصوص را نشان دهد. در این پایان نامه □طح خوني چند نمونه از این بیومارکر ها که در و ۹۴-۱۹۹۱ (Malmö Diet and Cancer Study [MDCS]) یک مطالعه جمعیتی در شهر مالمو رابطه آن ها با ری□ک گ□ترش و ابتلا به آترو□کلروز در عروق خونی مختلف یا ان□اع شریان بزرگ طی ۲۳،۴ □ال پیگیری تا □ال ۲۰۱۶ انجام شده، اندازه گیری (AAA آنوری□م آئورت شکمی یا) بدن گردیده ا□ت.

مقاله تحقیقاتی اول شامل پیگیری طولانی مدت بروز بیماری آترو□کلروز در عروق اندامهای تحتانی شناخته (PAD) در ۵۵۵۰ فرد میان□ال بدون بیماری عروق محیطی ،PAD ،یا بیماری عروق محیطی ،شده در برر□ی پایه ۱۹۹۱-۹۴ می باشد .پس از هماهنگ□ازي از نظر□ن، جن□یت، کشیدن□یگار جثه بدنی، فشار خون بالا و دیابت شیرین، افزایش □طح بیومارکرهای ق□فولیپاز مرتبط با در برر□ی پایه به معنای افزایش CRP یا C- Reaktiv protein و LP-PLA2 یا A2 لیپوپروتئین ری□ک بیماری در عروق محیطی طی ۲۳،۴ □ال پیگیری می باشد.

Co peptin, N-terminal pro- در مقاله تحقیقاتی دوم در همان شرایط افزایش میزان بیومارکرهای به معنی افزایش ری□ک بیماری Cystatin C و (NT Pro- BNP) یا B- typ natriuretisk Peptid ،در عروق محیطی طی ۲۳،۴ □ال پیگیری و پس از هماهنگ□ازي□ن، جنس، کشیدن□یگار، جثه بدنی .افزایش فشار خون و چربی خون و دیابت نشان داده شد

CRP، LP- در مقاله تحقیقاتی □وم در همان شرایط افزایش میزان بیومارکرهای PLA2، Proneurotensin، midregional proatrial natriuretisk peptid(MR-proANP)، midregionalt proadrenomedullin(MR-Pro ADM)، Co peptin, Cystatin C در برر□ی پایه به معنای ری□ک بالای بوجود آمدن آترو□کلروزیس در عروق کاروتید طی .این ۲۳،۴ □ال پیگیری بود

مقاله تحقیقاتی چهارم تایید کرد که افزایش میزان NT Pro-BNP، MR-ProADM، Cystatin C و CRP هستند دار علامت که میباشند عروق در کلروزیس آترو متقل های کننده بینی پیش و

71/89

بنابراین احتمالا میتوان از آنها به عنوان ابزار کمکی جهت انتخاب بیمارانی که بهتر است جراحی با هدف پیشگیری به آنها پیشنهاد گردد استفاده کرد.

شرکت کردند و MDCS در مقاله تحقیقاتی پنجم مقایسه بین شرکت کنندگانی صورت گرفت که در شدند .مقایسه ي AAA طی ۲۳،۴ سال پیگیری به بیماری آترواسکلروزیس مبتلا شدند با کسانی که دچار به وضوح LP-PLA2 انجام شده در مورد میزان بیومارکرها در بررسی پایه بود ۱۹۹۱-۹۴ .میزان بالای به MR-ProADM نسبت به آترواسکلروزیس مرتبط بود در حالی که میزان بالایAAA به خطر ابتلا به و آترواسکلروزیس بود .هیچ شرکت کننده مبتلا به دیابتی AAA معنی ریسک بالای ابتلا به هر دو بیماری .نشد در حالی که دیابت به معنای افزایش ریسک آترواسکلروزیس می باشد AAA در بررسی پایه دچار در AAA از سوی دیگر جنسیت مذکر و کشیدن سیگار بطور واضح باعث افزایش بیشتر ریسک ابتلا به مقایسه با آترواسکلروزیس می شدند.

بطور خلاصه در این پایان نامه نشان داده می شود که میزان بالای چندین بیومارکر مختلف به معنای در طول AAA افزایش ریسک ابتلا به بیماری آترواسکلروزیس در عروق خونی گردن و اندام تحتانی یا پیگیری طولانی مدت در افراد میانسال بدون سابقه قبلی بیماری عروقی و متقل از عوامل خطر شناخته شده می باشد.

از لحاظ بیومارکرها تا حدودي متفاوت از الگوی ابتلا AAA الگوي ریسک فاکتورهاي ابتلا به سطح انسولین و دیابت نیاز به بررسي بیشتر دارد AAA به آترواسکلروز است و ارتباط بین.

Acknowledgements

I would like to express my gratitude and appreciation to all those who gave me the possibility to complete this thesis, without their tremendous support and collaboration this dissertation wouldn't be possible.

I would like to thank in particular:

Professor Anders Gottsäter, principal supervisor, who has always been there for guidance and support. It is very difficult to put in words my gratefulness for all the countless of hours and hard work you have dedicated to make this happen. Your support and advice carried me through all the stages of my PhD studies. Thank you!

Professor Stefan Acosta, my co-supervisor, you have been a collegue and friend who encouraged me to through the whole process of being a better researcher. Im grateful for all the invaluable experience and knowledge which you have shared without hesitation. Thank you.

PhD MD Moncef Zarzouk, my co-supervisor, you have been a priceless collegue and helped me with crucial decision throughout my PhD studies. Thank you for all the support and exquisite input and hard work.

My co-authors and colleagues **Ardwan Dakhel, Gunnar Engström, Olle Melander**, **Peter M. Nilsson,** and **Margaretha Persson.**

Sophia Ågren-Witteschus, Cecilia Kennbäck and her staff at the Clinical Research Unit, Skåne University Hospital, Malmö, Sweden for expert technical assistance in the studies reported in paper VI.

I would also like to give special thanks to my **family**, for their continuous support and understanding. Thanks to **Fredrik Von Wowern** who introduced me to research and science. Thanks to **Marcus Ohlsson,** my former clinical supervisor, who always backed me up when needed. Special thanks to **Adam Hviid**, a true friend, collegue and a life coach.

Special thanks to Kärlcentrum Malmö, **Per Kjellin**, Head of Department, **Kjerstin Ädel Malmborg** for administrative assistance, and all the nurses and assistant nurses which make my workplace gratifying.

I would also like to express my gratitude to **Trelleborg Hospital** and all my colleagues for their support.

Finally, I would like to thank **God**, for letting me through all the difficulties. I have experienced your guidance day by day. You are the one who let me finish my degree. I will keep on trusting you for my future.

References

Abbott AL. Medical (nonsurgical) intervention alone is now best treatment for prevention of stroke associated with asymptomatic severe carotid stenosis. Results of a systematic review and analysis. Stroke 2009;40:e573-83.

Abbruzzese TA, Havens J, Belkin M, Donaldson MC, Whittemore AD, Liao JK, et al. Statin therapy is associated with improved patency of autogenous infrainguinal bypass grafts. J Vascu Surg 2004;39:1178-85.

Aboyans V, Thomas D, Lacroix P. The cardiologist and smoking cessation. Curr Opin Cardiol 2010;25:469-77.

Aboyans V, Ricco J-B, Bartelink M-LEL, Björck M, Brodmann M, Cohnert T, et al. 2017 ESC Guidelines on the Diagnosis and Treatment of Peripheral Arterial Diseases, in collaboration with the European Society for Vascular Surgery (ESVS): Document covering atherosclerotic disease of extracranial carotid and vertebral, mesenteric, renal, upper and lower extremity arteries. Endorsed by: the European Stroke Organization (ESO)The Task Force for the Diagnosis and Treatment of Peripheral Arterial Diseases of the European Society of Cardiology (ESC) and of the European Society for Vascular Surgery (ESVS). Eur Heart J 2017;39:763-816.

ACES Investigators. The Asymptomatic Carotid Emboli Study: study design and baseline results. Int J Stroke 2009;4:398-405.

Acosta S, Taimour S, Gottsäter A, Persson M, Engström G, Melander O, et al. Lp-PLA 2 activity and mass for prediction of incident abdominal aortic aneurysms: A prospective longitudinal cohort study. Atherosclerosis 2017;262:14-8.

Acosta S, Gottsäter A, Engström G, Melander O, Zarrouk M, Nilsson PM, et al. Circulating Midregional Proadrenomedullin and Risk of Incident Abdominal Aortic Aneurysm: A Prospective Longitudinal Cohort Study. Angiology 2018;69:333-8.

Adler AI, Stevens RJ, Neil A, Stratton IM, Boulton AJM, Holman RR. UKPDS 59: Hyperglycemia and Other Potentially Modifiable Risk Factors for Peripheral Vascular Disease in Type 2 Diabetes. Diabetes Care 2002;25:894-9.

Alexandrova NA, Gibson WC, Norris JW, Maggisano R. Carotid artery stenosis in peripheral vascular disease. J Vasc Surg1996;23:645-9.

Anand SS, Bosch J, Eikelboom JW, *Connolly SJ, Diaz R, Widimsky P,* et al on behalf of the COMPASS Investigators. Rivaroxaban with or without aspirin in patients with stable peripheral or carotid artery disease: an international, randomised, double-blind, placebo-controlled trial. Lancet 2018;391:219-29.

Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ 2002;324:71-86.

Antonopoulos AS, Papanikolaou E, Vogiatzi G, Oikonomou E, Tousoulis D. Anti-inflammatory agents in peripheral arterial disease. Curr Opin Pharmacol 2018;39:1–8.

Aronow WS, Nayak D, Woodworth S, Ahn C. Effect of simvastatin versus placebo on treadmill exercise time until the onset of intermittent claudication in older patients with peripheral arterial disease at six months and at one year after treatment. Am J Cardiol 2003;92:711-2.

Avdic T, Franzén S, Zarrouk M, Acosta S, Nilsson PM, Gottsäter A, et al. Reduced long term risk of aortic aneurysm and aortic dissection among individuals with type 2 diabetes: a nationwide observational study. J Am Heart Assoc 2018;7:e007618.

Badger SA, Soong CV, O' Donnell ME, Mercer C, Young IS, Hughes AE. C-reactive protein (CRP) elevation in patients with abdominal aortic aneurysm is independent of the most important CRP genetic polymorphism. J Vasc Surg 2009;49:178-84.

Bahia SS, Vidal-Diez A, Seshasai SR, Shpitser I, Brownrigg JR, Patterson BO, et al. Cardiovascular risk prevention and all-cause mortality in primary care patients with an abdominal aortic aneurysm. Br J Surg 2016;103:1626e33.

Banerjee A. A review of family history of cardiovascular disease: risk factor and research tool. Int J Clin Pract 2012;66:536-43

Barnett HJM, Taylor DW, Eliasziw M, Fox AJ, Ferguson GG, Haynes B, et al for the North American Symptomatic Carotid Endarterectomy Trial Collaborators. Benefit of carotid endarterectomy in patients with symptomatic moderate or severe stenosis. N Engl J Med 1998;339:1415-25.

Belch J, MacCuish A, Campbell I, Cobbe S, Taylor R, Prescott R, et al. The prevention of progression of arterial disease and diabetes (POPADAD) trial: factorial randomised placebo controlled trial of aspirin and antioxidants in patients with diabetes and asymptomatic peripheral arterial disease. BMJ 2008;337:a1840.

Belch JJ; Dormandy J, Biasi GM, Cairols M, Diehm C, Eikelboom B, et al, CASPAR Writing Committee. Results of the randomized, placebo-controlled clopidogrel and acetylsalicylic acid in bypass surgery for peripheral arterial disease. J Vasc Surg 2010;52:825-33.

Bhattacharya P, Kanagasooriyan R, Subramanian M. Tackling inflammation in atherosclerosis: Are we there yet and what lies beyond? Curr Opin Pharmacol 2022;66:102283.

Bonaca MP, Bauersachs RM, Anand SS, *Debus ES, Nehler MR, Patel MR,* et al. Rivaroxaban in peripheral artery disease after revascularization. N Engl J Med 2020;382:1994-2000.

Butlin M, Qasem A. Large artery stiffness assessment using SphygmoCor Technology. Pulse 2016;4:180-192.

Bux F, Favale S, Bhuva A, Scicchitano Pietro C, Caragnano Vito G, Lavopa G, et al. Anteroposterior diameter of the infrarenal abdominal aorta is higher in women with polycystic ovary syndrome. Vasc Health Risk Manag 2009;5:561-6.

Camm AJ, Lüscher TF, Maurer G, Serruys PW. The ESC textbook of cardiovascular medicine. Third edition. ed. Oxford ; New York, NY: Oxford University Press/European Society of Cardiology; 2019.

Cannon CP, Blazing MA, Giugliano RP, McCagg A, White JA, Theroux P, et al. Ezetimibe Added to Statin Therapy after Acute Coronary Syndromes. N Engl J Med 2015;372:2387-97.

CAPRIE Steering Committee. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). Lancet 1996;348:1329-39.

Carballo-Perich L, Puigoriol-Illamola D, Bashisr S, Terceno M, Silva M, Gubern-Merida C, et al. Clinical parameters and epigenetic biomarkers of plaque vulnerability in patients with carotid stenosis. Int J Mol Sci 2022;23:5149.

Ceasovschih A, Sorodoc V, Onofrei Aursulesei V, Tesloianu D, Tuchilus C, Anisie E, et al. Biomarker Utility for Peripheral Artery Disease Diagnosis in Real Clinical Practice: A Prospective Study. Diagnostics (Basel) 2020;10:723.

Charniot JC, Khani-Bittar R, Albertini JP, Giral P, Cherfils C, Cosson C, et al. Interpretation of lipoprotein-associated phospholipase A2 levels is influenced by cardiac disease, comorbidities, extension of atherosclerosis and treatments. Int J. Cardiol 2013;168:132-8.

Chien DK, Chang WH, Yeh YH. Radiographic findings of a ruptured abdominal aortic aneurysm. Circulation 2010;122:1880.

Classification and Diagnosis of Diabetes: Standards of medical care in diabetes—2020. Diabetes Care 2020;43(Suppl 1):S14-31.

Clemens RK, Annema W, Baumann F, Roth-Zetzsche S, Seifert B, von Eckardstein A, et al. Cardiac biomarkers but not measures of vascular atherosclerosis predict mortality in patients with peripheral artery disease. Clin Chim Acta 2019;495:215–20

Collins R. for the Heart Protection Study Collaborative Group. Effects of cholesterol-lowering with simvastatin on stroke and other major vascular events in 20536 people with cerebrovascular disease or other high risk conditions. Lancet 2004;63:757-67.

Conte MS, Bradbury AW, Kolh P, White JV, Dick F, Fitridge R, et al: GVG Writing Group for the Joint Guidelines of the Society for Vascular Surgery (SVS), European Society for Vascular Surgery (ESVS), and World Federation of Vascular Societies (WFVS). Global Vascular Guidelines on the Management of Chronic Limb-Threatening Ischemia. Eur J Vasc Endovasc Surg 2019;58:S1eS109.

Cosentino F, Grant PJ, Aboyans V, Bailey CJ, Ceriello A, Delgado V, et al, ESC Scientific Document Group. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EAS. Eur Heart J 2020;41:255-323.

Crisby M, Nordin-Fredriksson G, Shah PK, Yano J, Zhu J, Nilsson J. Pravastatin treatment increases collagen content and decreases lipid content, inflammation, metalloproteinases, and cell death in human carotid plaques: implications for plaque stabilization. *Circulation* 2001;103:926-33.

Crouse JR, Byington RP, Hoen HM, Furberg CD. *Reductase inhibitor monotherapy and stroke prevention.* Arch Intern Med. 1997;157:1305-10.

Dakhel A, Memon AA, Zarrouk M, Ågren-Witteschus S, Sundquist J, Sundquist K, et al. Novel cardiovascular biomarkers associated with peripheral arterial disease in men screened for abdominal aortic aneurysm. Vasa 2022;51:167-73.

DeFronzo RA, Ferrannini E, Groop L, Henry RR, Herman WH, Holst JJ, et al. Nat Rev Dis Primers 2015;1:15019

de Weerd M, Greving JP, Hedblad B, Lorenz MW, Mathiesen EB, O´Leary DH, et al. Prevalence of asymptomatic carotid artery stenosis in the general population: Stroke 2010; 41:1294-7.

de Weerd M, Greving JP, Hedblad B, Lorenz MW, Mathiesen EB, O´Leary DH, et al. Prediction of asymptomatic carotid artery stenosis in the general population: identification of high-risk groups. Stroke 2014;45:2366-71.

Doran S, Arif M, Lam S, Bayraktar A, Turkez H, Uhlen M, et al. Multi-omics approaches for revealing the complexity of cardiovascular disease. Briefings Bioinf 2021;22: bbab061.

Dowsett L, Higgins E, Alanazi S, Alshuwayer NA, Leiper FC, Leiper J. ADMA: A Key Player in the Relationship between Vascular Dysfunction and Inflammation in Atherosclerosis. J Clin Med 2020;9:3026.

Durmus I, Kazaz Z, Altun G, Cansu A. Augmentation index and aortic pulse wave velocity in patients with abdominal aortic aneurysms. Int J Clin Exp Med 2014;7:421-5.

Edlinger C, Lichtenauer M, Wernly B, Pistulli R, Paar V, Prodinger C, et al. Disease-specific characteristics of vascular cell adhesion molecule-1 levels in patients with peripheral artery disease. Heart Vessels 2019;34:976-83.

Eikelboom JW, Connolly SJ, Bosch J, *Dagenais GR, Hart RG, Shestakovska O,* et al; COMPASS Investigators. *Rivaroxaban with or without aspirin in stable cardiovascular disease. N Engl J Med* 2017;377:1319-30.

El Barghouty N, Geroulakos G, Nicolaides A, Androulakis A, Bahal V. *Computer assisted carotid plaque characterization.* Eur J Vasc Endovasc Surg 1995;9:389-93.

Elewa U, Fernandez-Fernandez B, Alegre R, Sanchez-Niño MD, Mahillo-Fernández I, Perez-Gomez MV, et al. Modifiable risk factors for increased arterial stiffness in outpatient nephrology. PLoS One 2015;10:e0123903:1-14.

Engelberger RP, Limacher A, Kucher N, Baumann F, Silbernagel G, Benghozi R, et al. Biological variation of established and novel biomarkers for atherosclerosis: Results from a prospective, parallel-group cohort study. Clin Chim Acta 2015;447:16-22.

Ericsson A, Holst J, Gottsäter A, Zarrouk M, Kumlien C. Psychosocial consequences in men taking part in a national screening program for abdominal aortic aneurysm. J Vasc Nurs 2017;35:211-20.

Executive committee for the Asymptomatic Carotid Atherosclerosis Study (ACAS). Endarterectomy for asymptomatic carotid artery stenosis. JAMA 1995;273:1421-8.

European Carotid Surgery Trialists Collaborative Group. MRC European Carotid Surgery Trial: interim results for symtomatic patients with severe (70-99%) or with mild (0-29%) carotid stenosis. Lancet 1991;337:1235-43.

European Carotid Surgery Trialists Collaborative Group. Randomised trial of endarterectomy for recently symptomatic carotid stenosis: final results of the MRC European Carotid Surgery Trial (ECST). Lancet 1998;351:1379-87.

Fakhry F, Spronk S, van der Laan L, Wever JJ, Teijink JA, Hoffmann WH, et al. Endovascular Revascularization and Supervised Exercise for Peripheral Artery Disease and Intermittent Claudication: A Randomized Clinical Trial. JAMA 2015;314:1936-44.

Falkensammer J, Frech A, Duschek N, Gasteiger S, Stojakovic T, Scharnagl H, et al. Prognostic relevance of ischemia-modified albumin and NT-proBNP in patients with peripheral arterial occlusive disease. Clin Chim Acta 2015;438:255-60.

Fenske W, Störk S, Blechschmidt A, Maier SGK, Morgenthaler NG, Allolio B. Copeptin in the differential diagnosis of hyponatremia. J Clin Endocrinol Metab 2009;94:123-9.

Findley CM, Mitchell RG, Duscha BD, Annex BH, Kontos CD. Plasma levels of soluble Tie2 and vascular endothelial growth factor distinguish critical limb ischemia from intermittent claudication in patients with peripheral arterial disease. J Am Coll Cardiol 2008;52:387-93.

Flondell-Sité D, Lindblad B, Kölbel T, Gottsäter A. Cytokines and systemic biomarkers are related to the size of abdominal aortic aneurysms. Cytokine 2009;46:211-5.

F-NBW Group. BEST (Biomarkers, EndpointS, and other Tools) Resource. Silver Spring (MD) Bethesda (MD): Food and Drug Administration (US) National Institutes of Health (US); 2016.

Fokkens BT, Smit AJ. Skin fluorescence as a clinical tool for non-invasive assessment of advanced glycation and long-term complications of diabetes. Glycoconj J 2016; 4:527-35.

Fowkes FG, Price JF, Stewart MC, Butcher I, Leng GC, Pell AC, et al. Aspirin for prevention of cardiovascular events in a general population screened for a low ankle brachial index: a randomized controlled trial. JAMA 2010;303:841-8.

Fowkes FGR, Rudan D, Rudan I, Aboyans V, Denerberg JO, McDermott MM, et al. Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. Lancet 2013;382:1329-40.

Frank U, Nikol S, Belch J, Boc V, Brodmann M, Carpentier PH, et al. European Society of Vascular Medicine (ESVM). Guideline on peripheral arterial disease. Vasa 2019;48 Suppl 102:1-79.

Gæde P, Vedel P, Larsen N, Jensen GVH, Parving H-H, Pedersen O. Multifactorial Intervention and Cardiovascular Disease in Patients with Type 2 Diabetes. N Engl J Med 2003;348:383-93.

Gardner AW, Parker DE, Montgomery PS, Sosnowska D, Casanegra AI, Esponda OL, et al. Impaired vascular endothelial growth factor A and inflammation in patients with peripheral artery disease. Angiology 2014;65:683-90.

Garg PK, Arnold AM, Hinckley Stukovsky KD, Koro C, Jenny NS, Mukamal KJ, et al. Lipoprotein-Associated Phospholipase A2 and Incident Peripheral Arterial Disease in Older Adults: The Cardiovascular Health Study. Arterioscler Thromb Vasc Biol 2016;36:750-6.

Garg PK, Jorgensen NW, McClelland RL, Jenny NS, Criqui MH, Allison MA, et al. Lipoprotein-associated phospholipase A(2) and risk of incident peripheral arterial disease in a multi-ethnic cohort: The Multi-Ethnic Study of Atherosclerosis. Vasc Med 2017;22:5-12.

Garg PK, Norby FL, Polfus LM, Boerwinkle E, Gibbs RA, Grove ML, et al. Lipoprotein-associated phospholipase A(2) and risk of incident peripheral arterial disease: Findings from The Atherosclerosis Risk in Communities study (ARIC). Atherosclerosis. 2018;268:12-8.

Ghilardi G. Carotid stenotic-obliterative lesions. Distribution in 16,379 subjects 45-75 years of age. Minerva Cardioangiol 1994;42:345-50.

Golledge J, Velu R, Quigley F, Jenkins J, Singh TP. *The predictive value of four serum biomarkers for major adverse events in patients with small abdominal aortic aneurysm.* J Vasc Surg 2023;77:1037-44.

Goncalves I, Moses J, Dias N, Pedro LM, Fernandes e Fernandes J, Nilsson J, et al. Changes related to age and cerebrovascular symptoms in the extracellular matrix of human carotid plaques. Stroke 2003;34:616–22.

Gottsäter A, Flondell-Site D, Kölbel T, Lindblad B. Associations Between Statin Treatment and Markers of Inflammation, Vasoconstriction, and Coagulation in Patients With Abdominal Aortic Aneurysm. Vasc Endovasc Surg 2009;42:567-73.

Grønholdt ML, Nordestgaard BG, Schroeder TV, Vorstrup S, Sillesen H. Ultrasonic echolucent carotid plaques predict future strokes. Circulation 2001;104:68–73.

Halliday A, Bulbulia R, Bonati LH, Chester J, Cradduck-Bamford A, Peto R, et al for the ACST-2 Collaborative Group. Second asymptomatic carotid surgery trial (ACST-2): a randomised comparison of carotid artery stenting versus carotid endarterectomy. Lancet 2021;*398:1065-1073*.

Heald CL, Fowkes FGR, Murray GD, Price JF, Ankle Brachial Index Collaboration. Risk of mortality and cardiovascular disease associated with the ankle-brachial index: Systematic review. Atherosclerosis *2006;189:61-9.*

Hedblad B, Nilsson P, Janzon L, Berglund G. Relation between insulin resistance and carotid intima-media thickness and stenosis in non-diabetic subjects. Results from a cross-sectional study in Malmo, Sweden. Diabet Med 2000;17:299-307.

Hegland O, Kurz MW, Munk PS, Larsen JP. The effect of statin therapy on the progression of carotid artery stenosis in relation to stenosis severity. Acta Neurol Scand 2010;121:11-5.

Hennrikus D, Joseph AM, Lando HA, Duval S, Ukestad L, Kodl M, et al. Effectiveness of a Smoking Cessation Program for Peripheral Artery Disease Patients. A Randomized Controlled Trial. J Am Coll Cardiol 2010;56:2105-12.

Herget-Rosenthal, S. Cystatin C. In: Vincent JL, Hall, JB (ed). Encyclopedia of Intensive Care Medicine. 2012, Springer.

Hiatt WR, Bonaca MP, Patel MR, Nehler MR, Debus ES, Anand SS, et al. Rivaroxaban and aspirin in peripheral artery disease lower extremity revascularization. Impact of concomitant clopidogrel on efficacy and safety. Circulation 2020);142:2219-30.

Hiatt WR, Goldstone J, Smith SC, McDermott M, Moneta G, Oka R, et al. Atherosclerotic Peripheral Vascular Disease Symposium II. Nomenclature for Vascular Diseases. Circulation 2008;118:2826-9.

Hill AB. The Environment and Disease: Association or Causation? Proc Royal Soc Med 1965;58:295.

Hsu C-Y, Chen Y-T, Su Y-W, Chang C-C, Huang P-H, Lin S-J. Statin Therapy Reduces Future Risk of Lower-Limb Amputation in Patients With Diabetes and Peripheral Artery Disease. J Clin Endocrinol Metab2017;102:2373-81.

Jansen SCP, Hoorweg BBN, Hoeks SE, van den Houten MML, Scheltinga MRM, Teijink JAW, et al. A systematic review and meta-analysis of the effects of supervised exercise therapy on modifiable cardiovascular risk factors in intermittent claudication. J Vasc Surg 2019;69:1293-1308.

Johansson M, Ricci F, Di Martino G, Rogmark C, Sutton R Hamrefors V, et al. Cardiovascular biomarkers predict fragility fractures in older adults. Heart 2019;105:449-54.

Johnston SC, O´Meara ES, Manolio TA, Lefkowitz D, O´Leary DH, Goldstein S, et al. Cognitive impairment and decline are associated with carotid artery disease in patients without clinically evident cerebrovascular disease. Ann Intern Med 2004;140:237-47.

Jönelid B, Christersson C, Hedberg P, et al. Screening of biomarkers for prediction of multisite artery disease in patients with recent myocardial infarction. Scand J Clin Lab Invest 2021;81:353-60.

Jorenby DE, Leischow SJ, Nides MA, Rennard SI, Johnston JA, Hughes AR, et al. A Controlled Trial of Sustained-Release Bupropion, a Nicotine Patch, or Both for Smoking Cessation. N Engl J Med1999;340:685-91.

Jukema JW, Szarek M, Zijlstra LE, de Silva HA, Bhatt DL, Bittner VA, et al; ODYSSEY OUTCOMES Committees and Investigators. Alirocumab in Patients With Polyvascular Disease and Recent Acute Coronary Syndrome: ODYSSEY OUTCOMES Trial. J Am Coll Cardiol 2019;74):1167-1176.

Juvonen J, Surcel H, Satta J, Teppo A, Bloigu A, Syrjälä H, et al. Elevated Circulating Levels of Inflammatory Cytokines in Patients With Abdominal Aortic Aneurysm. Atheroscler Thromb Vasc Biol 1997;17:2843-7.

Katsanos K, Spiliopoulos S, Saha P, Diamantopoulos A, Karunanithy N, Krokidis M, et al. Comparative efficacy and safety of different antiplatelet agents for prevention of major cardiovascular events and leg amputations in patients with peripheral arterial disease: a systematic review and network meta-analysis. PLoS One 2015;10:e0135692.

Khan AA, Patel J, Desikan S, Chrencik M, Martinez-Delcid J, Caraballo B, et al. Asymptomatic carotid artery stenosis is associated with cerebral hyoperfusion. J Vasc Surg 2021;73:1611-21.

Kiechl S, Willeit J, Rungger G, Egger G, Oberhollenzer F. Quantitative assessment of carotid atherosclerosis in a healthy population. Neuroepidemiology 1994;13:314-7.

Kigka VI, Potsika V, Mantzaris M, Tsakanikas V, Koncar I, Fotiadis DI. Serum biomarkers in carotid artery disease. Diagnostics 2021;11:2143.

Kölbel T, Goncalves I, Dias N, Strandberg K, Acosta S, Gottsäter A. Coagulation activation and ultrasound characteristics in patients with carotid artery disease. *Thromb Res. 2010;125:171-7.*

Kollerits B, Sturm G, Lamina C, Hammerer-Lercher A, Rantner B, Stadler M, et al. Comparison and evaluation of cardiac biomarkers in patients with intermittent claudication: results from the CAVASIC study. Clin Chem 2013;59:692-702.

Kremers B, Wübbeke L, Mees B, Ten Cate H, Spronk H, Ten Cate-Hoek A. Plasma Biomarkers to Predict Cardiovascular Outcome in Patients With Peripheral Artery Disease: A Systematic Review and Meta-Analysis. Arterioscler Thromb Vasc Biol 2020;40:2018-32.

Kulezic A, Bergwall S, Fatemi S, Sonestedt E, Zarrouk M, Gottsäter A, et al. Healthy diet and fibre intake are associated with decreased risk of incident symptomatic peripheral arterial disease - a prospective cohort study. Vasc Med 2019;24:511-8.

Kwee RM, van Oostenbrugge J, Hofstra L Teule GJ, van Engelshoven JMA, Mess WH, et al. Identifying vulnerable carotid plaques by non-invasive imaging. Neurology 2008;70:2401-9.

Kwee RM, Teule GJ, van Oostenbrugge J, Mess WH, Prins MH, van der Geest RJ, et al. Multimodality imaging of carotid artery plaques: 18F-fluoro-2-deoxyglucose positron emission tomography, computed tomography, and magnetic resonance imaging. Stroke 2009;40:3718-24.

Kwee RM, van Oostenbrugge J, Prins MH, Ter Berg JW, Franke CL, Korten AG, et al. Symptomatic patients with mild and moderate carotid stenosis. Plaque features at MRI and association with cardiovascular risk factors and statin use. Stroke 2010;41:1389-93.

Landenhed M, Engström G, Gottsäter A, Caulfield M, Hedblad B, Newton-Cheh C, et al. Risk profiles for aortic dissection and ruptured or surgically treated aneurysms: a prospective cohort study. J Am Heart Assoc 2015;4:e001513.

Lareyre F, Moratal C, Zereg E, Carboni J, Panaïa-Ferrari P, Bayer P, et al. Association of abdominal aortic aneurysm diameter with insulin resistance index. Biochem Med (Zagreb) 2018;28:030702.

Larsson E, Granath F, Swedenborg J, Hultgren R. A population-based case-control study of the familial risk of abdominal aortic aneurysm. J Vasc Surg 2009;49:47-50.

Laurent S, Cockcroft J, van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J 2006;27:2588-605.

Lee AJ, MacGregor AS, Hau CM, Price JF, Rumley A, Lowe GD, et al. The role of haematological factors in diabetic peripheral arterial disease: the Edinburgh artery study. Brit J Haematol 1999;105:648-54.

Lee JS, Park SC, Kim SD. The relation between metabolic syndrome and aspects of abdominal aortic aneurysm. Asian J Surg 2022;45:307-314.

Li T, Cao H, Zhuang J, Wan J, Guan M, Yu B, et al. Identification of miR-130a, miR-27b and miR-210 as serum biomarkers for atherosclerosis obliterans. Clin Chim Acta. 2011;412:66-70.

Li SB, Yang F, Jing L, Ma J, Jia YD, Dong SY, et al. Correlation between plasma lipoprotein-associated phospholipase A(2) and peripheral arterial disease. Exp Therap Med 2013;5:1451-5.

Li Y, Yang D, Zheng Y. Challenges of applying circulating biomarkers for abdominal aortic aneurysm progression. Exp Biol Med (Maywood) 2021;246:1054-9

Li Y, Zhong X, Cheng G, Zhao C, Zhang L, Hong Y, et al. Hs-CRP and all-cause, cardiovascular, and cancer mortality risk: A meta-analysis. Atherosclerosis 2017;259:75–82.

Liarakos AL, Tentolouris A, Kokkinos A, Eleftheriadou I, Tentolouris N. Impact of Glucagon-like peptide 1 receptor agonists on peripheral arterial disease in people with diabetes mellitus: A narrative review. J Diabetes Complications 2023;37:108390.

Libby P, Buring JE, Badimon L, Hansson GK, Deanfield J, Bittencourt MS, et al. Atherosclerosis. Nat Rev Dis Prim 2019;5:56.

Lindberg S, Zarrouk M, Holst J, Gottsäter A. Inflammatory markers associated with abdominal aortic aneurysm. Eur *Cytokine* Netw 2016;27:75-80.

Lindholt JS, Erlandsen EJ, Henneberg EW. Cystatin C deficiency is associated with the progression of small abdominal aortic aneurysms. Br J Surg 2001;88:1472-5.

Lindholt JS, Vammen S, Juul S, Henneberg EW, Fasting H. The validity of ultrasonographic scanning as screening method for abdominal aortic aneurysm. Eur J Vasc Endovasc Surg 1999;17:472e5.

Liu H, Yao Y, Wang Y, Ji L, Zhu K, Hu H, et al. Association between high sensitivity C-reactive protein, lipoprotein-associated phospholipase A2 and carotid atherosclerosis: a crosssectional study. J Cell Mol Med 2018;22:5145-50

Lorey MB, Öörni K, Kovanen T. Modified Lipoproteins Induce Arterial Wall Inflammation During Atherogenesis. Front Cardiovasc Med *2022;9:841545.*

Ludvigsson JF, Andersson E, Ekbom A, Feychting M, Kim JL, Reuterwall C, et al. External review and validation of the Swedish national inpatient register. BMC Public Health 2011;11:450.

Mach F, Baigent C, Catapano AL, *Koskinas KC, Casula M, Badimon L,* et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS). Eur Heart J 2019;41:111-88.

Mancia G, Kreutz R, Brunstrom M, Burnier M, Grassi G, Januszewicz A, et al. 2023 ESH Guidelines for the management of arterial hypertension The Task Force for the management of arterial hypertension of the European Society of Hypertension. J Hypertens. 2023;41:1874-2071.

Manjer J, Carlsson S, Elmståhl S, Gullberg B, Janzon L, Lindström M, et al. The Malmö diet and cancer study: representativity, cancer incidence and mortality in participants and non-participants. Eur J *Cancer* Prev 2001;10:489-99.

Manjer J, Elmståhl S, Janzon L, Berglund G. Invitation to a population-based cohort study: differences between subjects recruited using various strategies. Scand J Public Health 2002;30:103-12.

Marquardt L, Olivia CG, Mehta Z, Rothwell PM. Low risk of ipsilateral stroke in patients with asymptomatic carotid stenosis on best medical treatment. A prospective, poulation-based study. Stroke 2010;41:e11-7.

Marulanda-Londoño E, Chaturvedi S. Stroke due to large vessel atherosclerosis. Neurol Clin Pract. 2016;6:252-8.

Mathiesen EB, Joakimsen O, Bønaa KH. Prevalence of and risk factors associated with carotid artery stenosis: the Tromsø Study. Cerebrovasc Dis 2001;12:44-51.

Mathiesen EB, Bønaa KH, Joakimsen O. Echolucent plaques are associated with high risk of ischemic cerebrovascular events in carotid stenosis: the Tromsø study. Circulation 2001;103:2171-5.

Matsushita K, Kwak L, Yang C, Pang Y, Ballew SH, Sang Y, et al. High-sensitivity cardiac troponin and natriuretic peptide with risk of lower-extremity peripheral artery disease: the Atherosclerosis Risk in Communities (ARIC) Study. Eur Heart J 2018;39:2412-9.

Mayberg MR, Wilson E, Yatsu F, Weiss DG, Messina L, Hershey LA, et al for the Veterans Affairs Cooperative Studies Program 309 Trialist Group. Carotid endarterectomy and prevention of cerebral ischaemia in symptomatic carotid stenosis. JAMA 1991;266:3289-94.

Mazari FA, Khan JA, Samuel N, Smith G, Carradice D, McCollum PC, et al. Long-term outcomes of a randomized clinical trial of supervised exercise, percutaneous transluminal angioplasty or combined treatment for patients with intermittent claudication due to femoropopliteal disease. Br J Surg 2017;104:76-83.

McDermott MM, Greenland P, Green D, Guralnik JM, Criqui MH, Liu K, et al. D-Dimer, inflammatory markers, and lower extremity functioning in patients with and without peripheral arterial disease. Circulation 2003;107:3191–8.

McGuire DK, Shih WJ, Cosentino F, Charbonnel B, Cherney DZI, Dagogo-Jack S, et al. Association of SGLT2 Inhibitors With Cardiovascular and Kidney Outcomes in Patients With Type 2 Diabetes. A Meta-analysis. JAMA Cardiol 2021;6:148-158.

McIntyre C, Crowley, L. Avoidance and treatment of cardiovascular disease in dialysis. In Nissenson, AR and Fine, RN (ed). Handbook of Dialysis Therapy, 5th edition. 2017, Elsevier.

Melander O, Maisel A, Almgren P, Manjer J, Belting M, et al. Plasma proneurotensin and incidence of diabetes, cardiovascular disease, breast cancer, and mortality. JAMA 2012;308:1469-75.

Melander O, Newton-Chec C, Almgren P, Hedblad B, Berglund G, Engström G, et al. Novel and conventional biomarkers for prediction of incident cardiovascular events in the community. JAMA 2009;302:49-57.

Memon AA, Zarrouk M, Agren-Witteschus S, Sundquist J, Gottsäter A, Sundquist K. Identification of novel diagnostic and prognostic biomarkers for abdominal aortic aneurysm. Eur J Prev Cardiol 2020;27:132-42.

Mohler ER, 3rd, Hiatt WR, Creager MA. Cholesterol reduction with atorvastatin improves walking distance in patients with peripheral arterial disease. Circulation 2003;108:1481-6.

Morgenthaler NG,Struck J, Alonso C, Bergmann A. Measurement of midregional proadrenomedullin in plasma with an immunoluminometric assay. Clin Chem 2005;51:1823-9.

Motiwala SR, Sarma A, Januzzi JL, O'Donoghue ML. Biomarkers in ACS and Heart Failure: Should Men and Women Be Interpreted Differently? Clin Chem 2014;60:35-43.

MRC Asymptomatic Carotid Surgery Trial (ACST) Collaborative Group. Prevention of disabling and fatal strokes by successful carotid endarterectomy in patients without recent neurological symptoms: randomised controlled trial. Lancet 2004;363:1491-502.

Mueller C, Möckel M, Giannitsis E, Huber K, Mair J, Plebani M, et al; ESC Study Group on Biomarkers in Cardiology of the Acute Cardiovascular Care Association. Use of copeptin for rapid rule-out of acute myocardial infarction. Eur Heart J Acute Cardiovasc Care 2018;7:570-6

Mustonen P, Lepäntalo M, Lassila R. Physical exertion induces thrombin formation and fibrin degradation in patients with peripheral atherosclerosis. Arterioscler Thromb Vasc Biol 1998;18:244-9.

Nana P, Dakis K, Brodis A, Spanos A, Kouvelos G. Circulating biomarkers for the prediction of abdominal aortic aneurysm growth. J Clin Med 2021;10:1718.

Naylor AR, Gaines PA, Rothwell PM. Who benefits most from intervention for asymptomatic carotid stenosis: patients or professional? Eur J Vasc Endovasc Surg 2009;37:625-32.

Naylor AR, Ricco JB, de Borst GJ, Debus S, de Haro J, Halliday A, et al. Management of Atherosclerotic Carotid and Vertebral Artery Disease: 2017 Clinical Practice Guidelines of the European Society for Vascular Surgery (ESVS). Eur J Vasc Endovasc Surg 2018;55:3-81.

Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, et al, CANVAS Program Collaborative Group. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. N Engl J Med 2017;377:644e57.

Nordanstig J, Behrendt CA, Bradbury AW, deBorst GJ, Fowkes FGR, Golledge J, et al. Peripheral arterial disease (PAD) - A challenging manifestation of atherosclerosis. Prev Med 2023;171:107489.

Norman P, Le M, Pearce C, Jamrozik K. Infrarenal Aortic Diameter Predicts All-Cause Mortality. Atheroscler Thromb Vasc Biol 2004;24:1278-82.

North American Symptomatic Carotid Endarterectomy Trial Collaborators. Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. N Engl J Med 1991;325:445-53.

O'Holleran LW, Kennelly MM, McClurken M, Johnson JM. *Natural history of asymptomatic carotid plaque.* Am J Surg1987;154:659-62.

Öner Ö, Deveci F, Telo S, Kuluöztürk M, Balin M. MR-proADM and MR-proANP levels in patients with acute pulmonary embolism. J Med Biochem 2019 Nov;38:1-8.

Öörni K, Kovanen PT. *Aggregation susceptibility of low-density lipoproteins-A novel modifiable biomarker of cardiovascular risk*. *J Clin Med* 2021:1*0*:1769.

Oyama K, Giugliano RP, Tang M, Bonaca MP, Saver JL, Murphy SA, et al. Effect of evolocumab on acute arterial events across all vascular territories: results from the FOURIER trial. Eur Heart J *2021;42:4821-9.*

Pande RL, Brown J, Buck S, Redline W, Doyle J, Plutzky J, et al. Association of monocyte tumor necrosis factor α expression and serum inflammatory biomarkers with walking impairment in peripheral artery disease. J Vasc Surg 2015;61:155-61.

Peeters Weem SMO, van Haelst STW, den Ruijter HM, Moll FL, de Borst GJ. *Lack of Evidence for Dual Antiplatelet Therapy after Endovascular Arterial Procedures: A Meta-analysis*. Eur J Vasc Endovasc Surg 2016;52:253-62.

Pentikäinen MO, Öörni K, Ala-Korpela M, Kovanen PT. *Modified LDL – trigger of atherosclerosis and inflammation in the arterial intima*. *J Intern Med* 2000;*247*:359-70.

Persson M, Nilsson J-Å, Nelson J, Hedblad B, Berglund G. The epidemiology of Lp-PLA_2: Distribution and correlation with cardiovascular risk factors in a population-based cohort. Atherosclerosis 2007;190:388-96.

Petersen MJ, Cambria RP, Kaufman JA, LaMuraglia GM, Gertler JP, Brewster DC, et al. Magnetic resonance angiography in the preoperative evaluation of abdominal aortic aneurysms. J Vasc Surg 1995;21:891e8.

Rabben T, Mansoor SM, Bay D, Sundhagen JO, Guevara C, Jorgensen JJ. Screening for Abdominal Aortic Aneurysms and Risk Factors in 65-Year-Old Men in Oslo, Norway. Vascular Health and Risk Management 2021:17: 561–70.

Raffort J, Hassen-Khodja R, Jean-Baptiste E, Lareyre F. Relationship between metformin and abdominal aortic aneurysm. J Vasc Surg 2020;71:1056-62.

Raffort J, Lareyre F, Clément M, Hassen-Khodja R, Chinetti G, Mallat Z. Diabetes and *aortic aneurysm: current state of the art.* Cardiovasc Res 2018;114:1702-13.

Ramos R, García-Gil M, Comas-Cufí M, Quesada M, Marrugat J, Elosua R, et al. Statins for Prevention of Cardiovascular Events in a Low-Risk Population With Low Ankle Brachial Index. J Am Coll Cardiol *2016;67:630-640.*

Risum Ø, Sandven I, Jon O. Sundhagen JO, et al. Editor's choice - Effect of statins on total mortality in abdominal aortic aneurysm repair: A systematic review and meta-analysis. Eur J Vasc Endovasc Surg 2021;61:114e120.

Rosvall M, Östergren P-O, Hedblad B, Isacsson S-O, Janzon L, Berglund G. Socioeconomic differences in the progression of carotid atherosclerosis in middle-aged men and women with subclinical atherosclerosis in Sweden. Soc Sci Med 2006;62:1785-98.

Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, et al. Global Burden of Cardiovascular Diseases and Risk Factors, 1990–2019: Update From the GBD 2019 Study. J Am Coll Cardiol 2020;76:2982-3021.

Russell KS, Yates DP, Kramer CM, Feller A, Mahling P, Colin L, et al. A randomized, placebo-controlled trial of canakinumab in patients with peripheral artery disease. Vasc Med 201924:414-21.

Saam T, Cai J, Ma L, Cai YQ, Ferguson MS, Polissar NL, et al. Comparison of symptomatic and asymptomatic atherosclerotic carotid plaque features with in vivo MR imaging. Radiology 2006;240:464-72.

Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al; IDF Diabetes Atlas Committee. *Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition.* Diabetes Res Clin Pract 2019;157:107843.

Safar ME, Czernichow S, Blacher J. Obesity, arterial stiffness, and cardiovascular risk. J Am Soc Nephrol 2006;17(suppl 2):S109-11.

Sara JDS, Toya T, Ahmad A, Clark MM, Gilliam WP, Lerman LO, et al. Mental Stress and Its Effects on Vascular Health. Mayo Clin Proc 2022;97:951-90.

Sasaki N, Yamamoto H, Ozono R, Maeda R, Kihara Y. Association of common carotid artery measurements with N-terminal pro B-type natriuretic peptide in elderly participants. Intern Med 2020;59:917-25.

Sattar N, Lee MMY, Kristensen SL, Branch KRH, Del Prato S, Khurmi NS, et al. *Cardiovascular, mortality, and kidney outcomes with GLP-1 receptor agonists in patients with type 2 diabetes: a systematic review and meta-analysis of randomised trials.* Lancet Diab Endocrinol 2021;9:653-62

Schnurr TM, Jakupović H, Carrasquilla GD, Ängquist L, Grarup N, Sørensen TIA, et al. *Obesity, unfavourable lifestyle and genetic risk of type 2 diabetes: a case-cohort study.* Diabetologia. 2020;63:1324-32.

Shalaan WE, Cheng H, Gewertz B, McKinsey JF, Schwartz LB, Katz D, et al. Degree of carotid plaque calcification in relation to symptomatic outcome and plaque inflammation. J Vasc Surg 2004;40:262-9.

Signorelli SS, Mazzarino MC, Di Pino L, Malaponte G, Porto C, Pennisi G, et al. High circulating levels of cytokines (IL-6 and TNFalpha), adhesion molecules (VCAM-1 and ICAM-1) and selectins in patients with peripheral arterial disease at rest and after a treadmill test. Vasc Med 2003;8:15-9.

Signorelli SS, Anzaldi M, Fiore V, Simili M, Puccia G, Libra M, et al. Patients with unrecognized peripheral arterial disease (PAD) assessed by ankle-brachial index (ABI) present a defined profile of proinflammatory markers compared to healthy subjects. Cytokine 2012;59:294-8.

Signorelli SS, Anzaldi M, Fiore V, Candido S, Di Marco R, Mangano K, et al. Neopterin: a potential marker in chronic peripheral arterial disease. Mol Med Rep 2013;7:1855-8.

Signorelli SS, Anzaldi M, Libra M, Navolanic PM, Malaponte G, Mangano K, et al. Plasma Levels of Inflammatory Biomarkers in Peripheral Arterial Disease: Results of a Cohort Study. Angiology 2016;67:870-4.

Singh TP, Morris DR, Smith S, Moxon JV, Golledge, J. Systematic Review and Meta-Analysis of the Association Between C-Reactive Protein and Major Cardiovascular Events in Patients with Peripheral Artery Disease. Eur J. Vasc Endovasc Surg 2017;54:220-3.

Siong Chan DC, Cao TH, Ng LL. Proenkephalin in Heart Failure. Heart Fail Clin. 2018;14:1-11.

Sohn M-W, Meadows JL, Oh EH, Budiman-Mak E, Lee TA, Stone NJ, et al. Statin use and lower extremity amputation risk in nonelderly diabetic patients. J Vasc Surg 2013;58:1578-85.

Stehr A, Töpel I, Müller S, Unverdorben K, Geissler EK, Kasprzak PM, et al. VEGF: a surrogate marker for peripheral vascular disease. Eur J Vasc Endovasc Surg 2010;39:330-2.

Spence JD, Coates V, Li H, Tamayo A, Munoz C, Hackam DG, et al. Effects of intensive medical therapy on microemboli and cardiovascular risk in asymptomatic carotid stenosis. Arch Neurol 2010;67:180-6.

Sproston NR, Ashworth JJ. Role of C-Reactive Protein at Sites of Inflammation and Infection. Front Immunol 2018;9:754.

Starck J, Aaltonen L, Björses K, Lundgren F, Gottsäter A, Sonesson B, et al. A significant correlation between body surface area and infrarenal aortic diameter is detected in a large screening population with possibly clinical implication. Int Angiol 2019; 38:395-401.

Stather PW, Sylvius N, Wild JB, Choke E, Sayers RD, Bown MJ. Differential microRNA expression profiles in peripheral arterial disease. Circulation Cardiovasc Genet 2013;6:490-7.

Stather PW, Sylvius N, Sidloff DA, Dattani N, Verissimo A, Wild JB, et al. Identification of microRNAs associated with abdominal aortic aneurysms and peripheral arterial disease. Br J Surg 2015;102:755-66.

Steg PG, Bhatt DL, Wilson PWF, D´Agostino Sr R, Ohman EM, Röther J, et al. One-year cardiovascular event rates in outpatients with atherothrombosis. JAMA 2007;297:1197-206.

Steinberg MB, Greenhaus S, Schmelzer AC, Bover MT, Foulds J, Hoover DR, et al. Triple-combination pharmacotherapy for medically ill smokers: a randomized trial. Ann Intern Med 2009;150:447-54.

Stenudd I, Sjödin I, Nyman E, Wester P, Johansson E, Grönlund C. Ultrasound risk marker variability in symptomatic carotid plaque: impact on risk reclassification and association with temporal variation pattern. Int J Cardiovasc Imag *2020;36:1061-8*.

Sugawara J, Hayashi K, Yokoi T, Tanaka H. Age-associated elongation of the ascending aorta in adults. JACC Cardiovasc Imaging 2008;1:739-48.

Svoboda N, Kocí K, Seidlová A, Mandys V, Suttnar J, Hlavácková A, et al.

Oxidative stress as a reliable biomarker of carotid plaque instability: A pilot study. Antioxidants 2023;12:506.

Sweeting MJ, Thompson SG, Brown LC, Powell JT, RESCAN collaborators. Meta-analysis of individual patient data to examine factors affecting the growth and rupture of abdominal aortic aneurysms. Br J Surg 2012;99:655e65.

Taimour S, Zarrouk M, Holst J, Melander O, Engström G, Smith JG, et al. No relation between biomarkers at age 47–49 and aortic diameter after 14–19 years of follow-up – a population-based study. Vasa 2017;46:291-5.

Tunstall-Pedoe H, Peters SAE, Woodward M, Struthers AD, Belch JJF. Twenty-Year Predictors of Peripheral Arterial Disease Compared With Coronary Heart Disease in the Scottish Heart Health Extended Cohort (SHHEC). JAMA 2017;6:e005967.

Twine CP, Kakkos SK, Aboyans V, Baumgartner I, Behrendt CA, Bellmunt-Montoya S, et al. European Society for Vascular Surgery (ESVS) 2023 Clinical Practice Guidelines on Antithrombotic Therapy for Vascular Diseases. Eur J Vasc Endovasc Surg 2023;65:627-89.

Umemura T, Kawamura T, Mashita S, Kameyama T, Sobue G. Higher levels of cystatin C are associated with extracranial carotid artery steno-occlusive disease in patients with noncardioembolic ischemic stroke. Cerebrovasc Dis Extra 2016;6:1-11.

Unosson J, Wågsäter D, Bjarnegård N, de Basso R, Welander M, Gottsäter A, et al. Metformin prescription is associated with reduced AAA growth rate and reduced chemokine expression in a Swedish cohort. Ann Vasc Surg 2021;70:425-33.

Unlü Y, Karapolat S, Karaca Y, Kiziltunç A. Comparison of levels of inflammatory markers and hemostatic factors in the patients with and without peripheral arterial disease. Thromb Res 2006;117:357-64.

Urbonaviciene G, Shi GP, Urbonavicius S, Henneberg EW, Lindholt JS. Higher cystatin C level predicts long-term mortality in patients with peripheral arterial disease. Atherosclerosis 2011;216:440-5.

Vainas T, Stassen FR, de Graaf R, Twiss ELL, Herngreen SB, Welten RJTJ, et al. C-Reactive protein in peripheral arterial disease: relation to severity of the disease and to future cardiovascular events. J Vasc Surg 2005;42:243-51.

van Lie B, Ong SC, Belgi A, Delaine C, Andrikkopoulus S, Haworth NL. Insulin in motion: The A6-A11 disulfide bond allosterically modulates structural transitions required for insulin activity. Sci Rep 2017;7:17239.

Vats S, Sundquist K, Wang X, Zarrouk M, Ågren-Witteschus S, Sundquist J, et al. Associations of global DNA methylation and homocysteine levels with abdominal aortic aneurysm: a cohort study from a population-based screening program in Sweden. Int J Cardiol 2020;321:137-42.

Villard C, Wågsäter D, Swedenborg J, Eriksson P, Hultgren R. Biomarkers for Abdominal Aortic Aneurysms From a Sex Perspective. Gend Med 2012;9:259-66.e2.

Visseren FLJ, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M, et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J. 2021;42:3227-37.

Wang TJ, Larson MG, Levy D, Benjamin EJ, Leip EP, Omland T, et al. Plasma natriuretic peptide levels and the risk of cardiovascular events and death. N Engl J Med 2004;350:655-63.

Wanhainen A, Unosson J, Mani K, Gottsäter A, on behalf of the MAAAGI Trial Investigators. The Metformin for Abdominal Aortic Aneurysm Growth Inhibition (MAAAGI) trial. Eur J Vasc Endovasc Surg 2021;61:710-1.

Wanhainen A, Verzini F, Van Herzeele I, Allaire E, Bown M, Cohnert T, et al. Editor's choice - European Society for Vascular Surgery (ESVS) 2019 Clinical practice guidelines on the management of abdominal aorto-iliac artery aneurysms. Eur J Vasc Endovasc Surg 2019;57:8e93.

Weber T, Ammer M, Rammer M, Adji A, O'Rourke MF, Wassertheurer S, et al. Noninvasive determination of carotid–femoral pulse wave velocity depends critically on assessment of travel distance: a comparison with invasive measurement. J Hypertens 2009;27:1624-30.

Wieczór R, Kulwas A, Rość D. Implications of Hemostasis Disorders in Patients with Critical Limb Ischemia-An In-Depth Comparison of Selected Factors. J Clin Med. 2020;9(3).

Wieczór R, Rość D, Wieczór AM, Kulwas A. VASCULAR-1 and VASCULAR-2 as a New Potential Angiogenesis and Endothelial Dysfunction Markers in Peripheral Arterial Disease. Clin Appl Thromb Hemost 2019;25:1076029619877440.

Wilkes S, Stansby G, Sims A, Haining S, Allen J. Peripheral arterial disease: diagnostic challenges and how photoplethysmography may help. Brit J Gen Pract 2015;65:323-4.

Willigendael EM, Teijink JAW, Bartelink M-L, Kuiken BW, Boiten J, Moll FL, et al. Influence of smoking on incidence and prevalence of peripheral arterial disease. J Vasc Surg 2004;40:1158-65.

Wirfält E, Mattisson I, Johansson U, Gullberg B, Wallström P, Berglund G. A methodological report from the Malmö Diet and Cancer study: development and evaluation of altered routines in dietary data processing. Nutr J 2002;1:3.

Weir-McCall J, Duce SL, Gandy SJ, Matthew SZ, Martin P, Cassidy DB, et al. Whole body cardiovascular magnetic resonance imaging to stratify symptomatic and asymptomatic atherosclerotic burden in patients with isolated cardiovascular disease. BMC Medical Imaging 2016;16:18.

Wong KS, Chen C, Fu J, Chang HM, Suwanwela NC, Huang YN, et al; CLAIR study investigators. Clopidogrel plus aspirin versus aspirin alone for reducing embolisation in patients with acute symptomatic cerebral or carotid artery stenosis (CLAIR study): a randomised, open-label, blinded-endpoint trial. Lancet Neurol 2010;9:489-97.

Xiao J, Borné Y, Bao X, Persson M, Gottsäter A, Acosta S, Engström G. Comparisons of risk factors for abdominal aortic aneurysm and coronary heart disease: A prospective cohort study. *Angiology 2021;72:24-31.*

Xiong J, Wang SM, Zhou W, Wu JG. Measurement and analysis of ultimate mechanical properties, stress-strain curve fit, and elastic modulus formula of human abdominal aortic aneurysm and nonaneurysmal abdominal aorta. J Vasc Surg 2008;48:189-95.

Yagi H, Nishigori M, Murakami Y, Osaki T, Muto S, Iba Y, et al. Discovery of novel biomarkers for atherosclerotic aortic aneurysm through proteomics-based assessment of disease progression. Sci Rep 2020;10:6429.

Yang C, Kwak L, Ballew SH, Garimella PS, Jaar BG, Folsom AR, et al. Kidney function, bone-mineral metabolism markers, and future risk of peripheral artery disease. Atherosclerosis 2017;267:167-74.

Yang L, Sui HG, Wang MM, Li JY, He XF, Li JY, et al. *MiR-30c-1-3p targets matrix metalloproteinase 9 involved in the rupture of abdominal aortic aneurysms.* J Mol Med 2022;100:1209-21.

Zarrouk M, Holst J, Malina M, Lindblad B, Wann-Hansson C, Rosvall M, et al. The importance of socioeconomic factors for compliance and outcome at screening for abdominal aortic aneurysm in 65-year-old men. J Vasc Surg 2013;58:50-5.

Ziegler L, Hedin U, Gottsäter A. Biomarkers in lower extremity peripheral artery disease. Eur Cardiol Rev 2022;17:e09.

Printed by Media-Tryck, Lund 2024 NORDIC SWAN ECOLABEL 3041 0903

Aboute the author

Shahab Fatemi was born in Isfahan, Iran, in 1982. He earned his degree from Wrocław Medical University in Poland in 2013. Currently, he serves as a specialist in Internal Medicine at Trelleborg Hospital.

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Clinical Sciences, Malmö

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2024:75
ISBN 978-91-8021-570-1
ISSN 1652-8220