Peripheral arterial disease, diabetes, and biomarkers

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2023

Document Version:
Publisher's PDF, also known as Version of record

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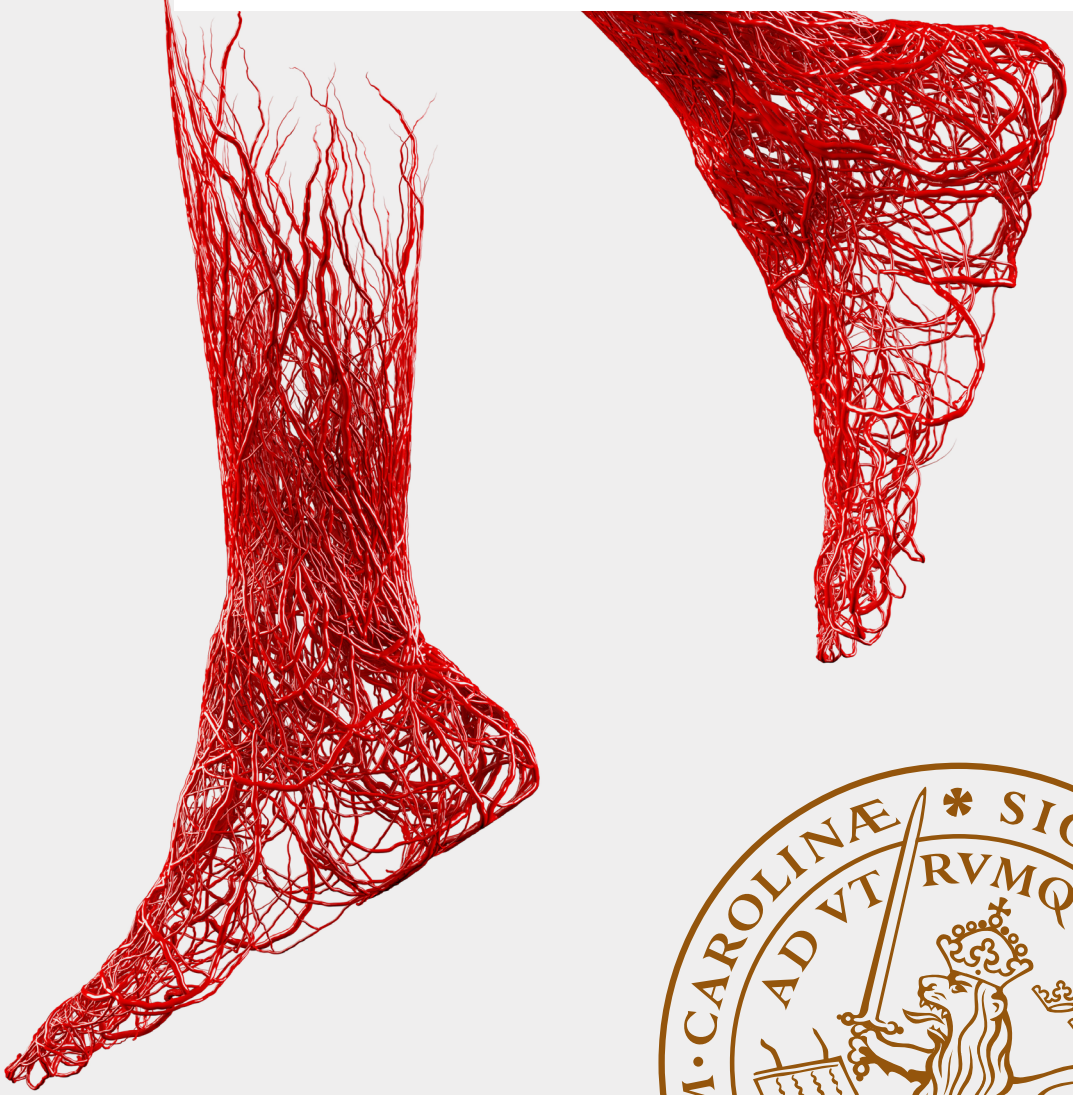

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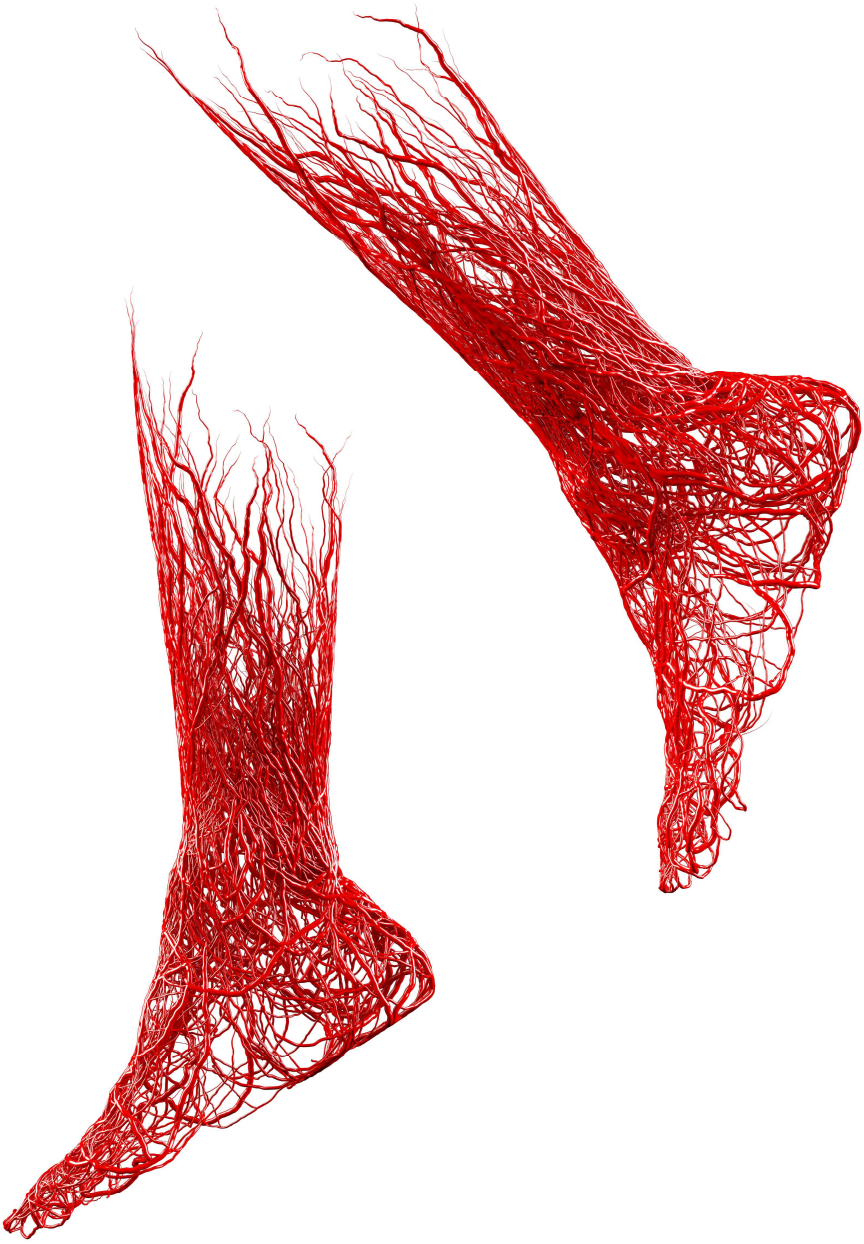

Peripheral arterial disease, diabetes, and biomarkers

Ardwan Dakhel

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 at

Hoodsalen, Ruth Lundskogsgata 3, Malmö. November 29th, 2023, at 9 AM.

Faculty opponent
Professor Bo Carlberg

Department of Public Health and Clinical Medicine, Umeå University

Organization	Document name
LUND UNIVERSITY Faculty of medicine	DOCTORAL DISSERTATION
Department of Clinical sciences, Malmö	**Date of issue** 2023-11-29
Author Ardwan Dakhel	Sponsoring organization

Title and subtitle
Peripheral arterial disease, diabetes, and biomarkers

Abstract
Background: Diabetes mellitus (DM) is an established risk factor for peripheral arterial disease (PAD) and its possible manifestation intermittent claudication (IC). Indications for surgery in infrainguinal IC are debated, especially among IC patlents with DM. PAD diagnosis relies on ankle brachial index (ABI) measurement which has limitations in patients with arterial calcification. Prior publications have shown potential of biomarkers for identification and prediction of PAD.

Aims: The aims of this thesis was to evaluate short- and long-term effects of DM upon cardiovascular morbidity and mortality in patients after infra-inguinal endovascular and open surgery for IC. We also aimed at identifiying novel biomarkers that could be used for prediction and identification of PAD.

Methods: In papers I and II, data from the Swedish Vascular Registry and the National Diabetes Register were compiled and patients with and without DM underwent propensity score adjusted long-term follow-up regarding cardiovascular morbidity and mortality. Paper III was a proscpective 11.2 year follow-up analysis of incident PAD in a prospective cohort (Malmö Preventive Project) in relation to biomarkers analyzed at baseline, whereas paper IV was a cross sectional study evaluation of biomarkers and PAD in 65-year old men participating in abdominal aortic aneurysm screening.

Results: Long-term (5 year) follow-up of 626 patients with and 1,112 patients without DM after endovascular surgery in paper I showed higher rates of major adverse cardiovascular events (MACE, hazard ratio [HR] 1.26, 95% confidence interval [CI] 1.07-1.48; p<0.01), acute myocardial infarction (AMI, HR 1.48, CI 1.09-2.00; p=0.01), and major amputation (HR 2.31, CI 1.24-4.32; p<0.01) in those with DM. During 5 years of follow-up after open surgery in paper II, 323 patients with DM showed higher rates of MACE (HR 1.33, CI 1.08–1.62; p<0.01) and AMI (HR 2.21, CI 1.46–3.35; p<0.01) than 679 patients without DM. In paper III, higher levels of C-terminal endothelin-1 (CT-proET-1, HR 1.8; 95% CI 1.4-2.3), N-Terminal prosomatostatin (NT-proSST, (HR 1.5;95% CI 1.2-2.0), and midregional proatrial natriuretic peptide (MR-proANP, HR 1.7; 95% CI 1.3-2.3) in 5,160 individuals at baseline examination were independently associated with incident PAD during 11.2 years of follow-up. Furhtermore, higher levels of CT-proET-1 (HR 1.3; 95% CI 1.2-1.5), NT-proSST (HR 1.2;95% CI 1.1-1.3), MR-proANP (HR 1.4; 95% CI 1.3-1.6), procalcitonin (HR 1.1;95% CI 1.0-1.2), and copeptin (HR 1.2; 95% CI 1.1-1.4) at baseline were independently associated with mortality during follow-up. In cross-sectional analysis of 267 65-year old men in paper IV, those with PAD had significantly higher levels of secretoglobin family 3A member 2, osteoprotegerin, urokinase-type plasminogen activator surface receptor, serum macrophage chemokine ligand 16, matrix metalloproteinase 9, p-selectin, growth differentiation factor 15, elafin, cystatin B, trefoil factor 3, and fatty acid-binding protein 4.

Conclusions: DM patients undergoing endovascular surgery, however, have increased risk of major adverse cardiovascular events, acute myocardial infarction and major amputation in the long-term. Furthermore, DM patients undergoing open surgery have increased risk of MACE and AMI. Elevated levels of CT-proET-1, NT-proSST, and MR-proANP independently predicted the risk of future PAD. Elevated plasma levels of Secretoglobin Family 3A Member 2, Osteoprotegerin, Urokinase-type plasminogen activator surface receptor, Serum macrophage chemokine ligand 16, Matrix metalloproteinase 9, P-selectin, Growth differentiation factor 15, Elafin, Cystatin B, Trefoil factor 3, and Fatty acid-binding protein 4 can potentially be used to identify PAD in men.

Key words Peripheral arterial disease, intermittent claudication, diabetes mellitus, biomarkers, vascular surgery, atherosclerosis.

Classification system and/or index terms (if any)

Supplementary bibliographical information		**Language** English
ISSN and key title: 1652-8220		**ISBN:** 978-91-8021-478-0
Recipient's notes	**Number of pages** 89	Price
	Security classification	

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Coverphoto by Chawalit Banpot

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ISBN 978-91-8021-478-0

ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2023

To my parents

Table of Contents

Abbreviations

AAA - Abdominal aortic aneurysm

ACEi – Angiotensin converting enzyme inhibitors

AI – Artificial intelligence

AMI - Acute myocardial infarction

ARB – Angiotensin receptor blockers

AUC - Area under the curve

BMI - Body mass index

BMT- Best medical treatment

BP - Blood pressure

CAD - Coronary artery disease

CI - Confidence interval

CKD - Chronic kidney disease

CLTI - Chronic limb threatening ischemia

COMPASS - Cardiovascular Outcomes for People Using Anticoagulation Strategies

CSTB - Cystatin B

CT-proET-1 - C-terminal endothelin-1

CTA - Computed tomography angiography

CV - Cardiovascular

CVD - Cardiovascular disease

CXCL16 - Serum macrophage chemokine ligand 16

DM - Diabetes mellitus

ESVS - European Society for Vascular Surgery

FABP4 - Fatty acid-binding protein 4

GDF-15 - Growth differentiation factor 15

GLP1-RA - Glucagon-like peptide-1 receptor agonists

HR - Hazard ratio

HRQoL - Health-related quality of life

IC - Intermittent claudication

IPTW - Inverse probability of treatment weighting

IQR - Interquartile range

ISTH - International Society on Thrombosis and Haemostasis

LDL-C - Low-density lipoprotein cholesterol

MACE - Major adverse cardiovascular events

MALE - Major adverse limb events

MMP-9 - Matrix metalloproteinase 9

MR-proANP - Midregional proatrial natriuretic peptide

NDR - National Diabetes Register

NT-proSST - N-Terminal prosomatostatin

OPG - Osteoprotegerin

PAD - Peripheral arterial disease

PCT – Procalcitonin

PI3 - Elafin

RCT - Randomized controlled trial

ROC - Receiver-operating characteristic

SCGB3A2 - Secretoglobin Family 3A Member 2

SD - Standard deviation

SELP - P-selectin

SFA - Superficial femoral artery

SGLT2 - Sodium-glucose transport protein 2

T1DM - Type 1 diabetes mellitus

T2DM - Type 2 diabetes mellitus

TIMI - Thrombolysis in Myocardial Infarction

TFF3 - Trefoil factor 3

U-PAR - Urokinase-type plasminogen activator surface receptor

VOYAGER PAD - Efficacy and Safety of Rivaroxaban in Reducing the Risk of Major Thrombotic Vascular Events in Subjects With Symptomatic Peripheral Artery Disease Undergoing Peripheral Revascularization Procedures of the Lower Extremities

List of publications

- Paper I: Dakhel A, Zarrouk M, Ekelund J, Acosta S, Nilsson P, Miftaraj M, Eliasson B, Svensson AM, Gottsäter A. Worse cardiovascular prognosis after endovascular surgery for intermittent claudication caused by infrainguinal atherosclerotic disease in patients with diabetes. Ther Adv Endocrinol Metab 2020;11:2042018820960294.

- Paper II: Dakhel A, Zarrouk M, Ekelund J, Acosta S, Miftaraj M, Eliasson B, Svensson AM, Gottsäter A. Higher long-term cardiovascular morbidity after open surgery for intermittent claudication caused by infrainguinal atherosclerotic disease in patients with diabetes – a nationwide observational cohort study. Vasa 2021;50:224-230.

- Paper III: Dakhel A, Engström G, Melander O, Acosta S, Fatemi S, Gottsäter A, Zarrouk M. Vasoactive biomarkers associated with long-term incidence of symptomatic peripheral arterial disease and mortality. Angiology 2021;72:550-555.

- Paper IV: Dakhel A, Memon AA, Zarrouk M, Ågren-Witteschus S, Sundquist J, Sundquist K, Gottsäter A. Novel cardiovascular biomarkers associated with peripheral arterial disease. Vasa 2022;51:167-173.

Thesis at a glance

Paper, title	Aim	Methods	Main results
I. Worse cardiovascular prognosis after endovascular surgery for intermittent claudication caused by infrainguinal atherosclerotic disease in patients with diabetes	Compare short- and long-term total and cardiovascular (CV) mortality, and morbidity after elective endovascular surgery for infrainguinal intermittent claudication (IC) in patients with and without diabetes mellitus (DM), and potential relationships between glycaemic control and outcomes in patients with DM.	Propensity score adjusted nationwide registry study of 626 patients with DM and 1,112 patients without DM undergoing endovascular surgery for IC. Follow up after 30 days and 5 years.	No significant difference between groups in morbidity or mortality during short-term follow-up. Patients with DM showed higher rates of major adverse cardiovascular events (MACE), acute myocardial infarction (AMI), and major amputations during long-term follow-up. Higher glycated haemoglobin (HbA1c) in diabetic patients was associated with higher total mortality.
II. Higher long-term cardiovascular morbidity after open surgery for intermittent claudication caused by infrainguinal atherosclerotic disease in patients with diabetes – a nationwide observational cohort study	Compare short- and long-term total and CV mortality, and morbidity after elective open surgery for infrainguinal IC in patients with and without DM, and potential relationships between glycaemic control and outcomes in patients with DM.	Propensity score adjusted nationwide registry study of 323 patients with DM and 679 patients without DM undergoing open surgery for IC. Follow up after 30 days and 5 years.	No significant difference between groups in morbidity or mortality during short-term follow-up. Patients with DM showed higher rates of MACE and AMI during long-term follow-up. Higher HbA1c in diabetic patients was associated with higher rates of MACE, stroke, and total mortality.
III Vasoactive biomarkers associated with long-term incidence of symptomatic peripheral arterial disease and mortality	Evaluate if biomarkers associated with other forms of CV disease can predict incident peripheral arterial disease (PAD) and mortality during long-term follow-up.	Retrospective analysis of 5,160 participants from the Malmö Preventive Project, regarding incidence of PAD in participants with elevated levels of biomarkers during 11.2 years of follow-up.	Participants with elevated levels of the CT-proET-1, NT-proSST, and MR-proANP had a significantly higher risk of developing PAD. Elevated levels of all analyzed biomarkers were associated with higher mortality.
IV Novel cardiovascular biomarkers associated with peripheral arterial disease in men screened for abdominal aortic aneurysm	Identify novel biomarkers associated with PAD and identify their diagnostic potential.	Cross sectional study of 91 CV specific proteins in a cohort of 267 65-year-old men recruited from a screening program for abdominal aortic aneurysm. PAD was defined as ankle brachial index <0.9.	Elevated levels of 11 different biomarkers were associated with higher risk of PAD

Introduction

Peripheral Arterial Disease

Epidemiology

Peripheral arterial disease (PAD) refers to an atherosclerotic occlusive disease of the lower extremities [1], which can also be referred to as peripheral vascular disease, peripheral arterial occlusive disease, or lower extremity arterial disease [1].

PAD is the third most common manifestation of atherosclerosis, following coronary artery disease (CAD) and stroke [2]. Despite the high prevalence rate, PAD remains largely underdiagnosed and undertreated [2].

A systematic review and modelling analysis by Song et al. from 2015 estimated that 237 million individuals worldwide were affected by PAD [3], with a prevalence of 5.6% in individuals aged 25 years and older. Compared to an older systematic review and analysis of community-based studies between 2000 and 2010 showing a prevalence of 202 million individuals with PAD [4], the figures reported by Song et al. indicate a relative increase of 17.1% percent within 5 years [3]. The prevalence of PAD in young individuals is higher in countries with low- and middle-income compared to in high income countries [3]. After the age of 50 years, however, PAD prevalence in low- and middle-income countries decreases compared to in high income countries, possibly due to a relatively lower life expectancy in low- and middle-income countries [3].

PAD prevalence increases with age [3], and in Sweden almost a fifth of all elderly individuals are estimated to be affected with PAD [5]. The prevalence of PAD in Sweden in ages 60 to 90 years is 18%; 16.5% in men and 19.2% in women [5].

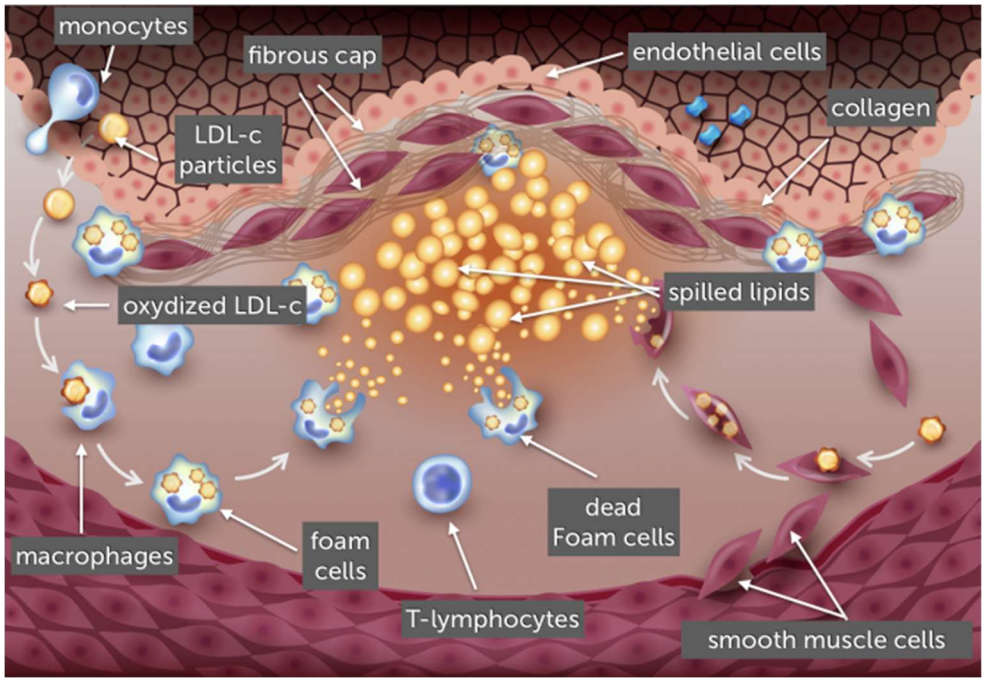

Figure I Illustration of atherosclerotic progression, modified image purchased from shutterstock.com

Atherosclerosis

The pathogenesis of atherosclerosis can be divided into three phases: initiation, progression, and complications [6].

Cumulative exposure of the vessel wall to concentrations of low-density lipoprotein cholesterol (LDL-C) in excess of physiological needs is a principal determinant of initiation and progression of atherosclerosis [6]. Impaired endothelial barrier function resulting from chronic inflammatory processes at sites with disturbed laminar flow [7] allows excess LDL-C particles to accumulate within the intima and undergo oxidation by reactive oxygen species. This leads to increased secretion of chemokines by endothelial cells and intimal immune cell infiltration [6]. Additionally, extravascular sites of inflammation resulting from the below mentioned atherosclerotic risk factors might affect the arterial walls by disturbing production of endogenous vasodilators and increasing the release of inflammatory mediators, leading to endothelial inflammation [6]. Oxidized LDL-C expresses scavenger receptors involved in transformation of macrophages into foam cells filled with lipids [6]. Dying foam cells and smooth

muscle cells constitute the necrotic core of the resulting atheroma or atherosclerotic plaque composed of collagen and smooth muscle cells. During the evolution of atherosclerotic plaques, some regions might develop calcification contributing to plaque instability and rupture potentially provoking thrombosis, resulting in vessel occlusion or embolization [6].

Early stages of atherosclerosis are often asymptomatic, but with progression of the disease, clinical manifestations might occur with symptoms depending on the localization of the lesions in the arterial tree. The three most common forms of atherosclerotic cardiovascular disease (CVD) are coronary heart disease including angina pectoris and acute myocardial infarction (AMI), precerebral and cerebral vascular disease including transitory ischaemic attacks and ischemic stroke, and PAD which will be discussed more in detail below [6].

Risk factors for PAD

As PAD is a manifestation of atherosclerosis, the established risk factors for initiation and progression of atherosclerosis have all been shown to increase the risk of PAD. The different risk factors listed in table I can be categorized into either modifiable or non-modifiable [1, 4, 8].

Modifiable risk factors	Non-modifiable risk factors
Smoking	Age
Arterial hypertension	Male gender
Diabetes mellitus	Genetic factors
Sedentary lifestyle	Chronic kidney disease
LDL cholesterol	Hyperhomocysteinemia*
Obesity	

Table I. Modifiable and non-modifiable risk factors for atherosclerosis. * Supplements with Vitamin-B or folate can lower homocysteine levels but high quality evidence for the benefits of treatment is lacking. As supplemental B vitamins and folate in patients with CAD showed no benefit and suggested possible harm, supplementation therapy is not recommended [8].

In addition to the above-mentioned well-established risk factors, it has been shown that environmental factors such as poverty, industrialization, and

infection may also affect the development of atherosclerosis and PAD particularly in low- and middle-income countries [9].

Smoking

Smoking is an important risk factor for development of CVD, and both active and passive smoking are related to development of PAD [9]. Cigarette smoking induces endothelial cell dysfunction, oxidative stress, vascular inflammation, platelet coagulation, and smooth muscle cell remodelling through multiple molecular mechanisms [9, 10]. Furthermore, the severity of PAD, risk of amputation, risk of postoperative peripheral graft occlusion, and mortality in PAD patients all increase with the number of cigarettes smoked. Heavy smokers have a four-fold higher risk of developing PAD manifesting as intermittent claudication ([IC], which will be described in detail below) when compared to non-smokers [8].

Moreover, pre- and post- operative smoking have also been shown to have a large negative influence on the postoperative prognosis after lower extremity revascularization [9].

Furthermore, smoking is the most important modifiable risk factor for PAD and other atherosclerotic diseases. Cessation reduces the risk of PAD development and may even reverse or delay disease progression [9].

Smoking cessation may also reduce the risk of development and progression of other forms of atherosclerosis and CVD and its associated morbidity and mortality, risk of atrial fibrillation, risk of venous thromboembolism, development of abdominal aortic aneurysm (AAA), chronic obstructive pulmonary disease, and various type of cancer [11]. Support for smoking cessation can be offered utilizing nicotine replacement therapy. Support for smoking cessation can be offered utilizing nicotine replacement therapy, varenicline, bupropion, and formal cessation support programs [12].

Between 2004 and 2022 daily smoking in Sweden decreased in the population aged 16–84 years. It was estimated that about six percent of both men and women smoked daily in 2022 [13]. It has also been shown that smoking rates vary depending on educational level. Individuals with a post high school degree have lower rates of smoking compared to individuals with only a high school degree, and those without [13]. In recent years usage of nicotine containing oral snuff (snus) has increased among young Swedish individuals, particularly in young women [13]. A recent community-based study of 14,344 participants showed that rates of PAD in individuals consuming smokeless tobacco (defined as chewing tobacco or taking snuff) had a twofold increased risk of developing PAD compared to individuals that

had never used tobacco products, furthermore, PAD rates were comparable to those of cigarette smokers [14].

Arterial hypertension

Arterial hypertension is associated with the development of atherosclerosis and with a two- to three-fold increase in the risk for PAD [8].

Hypertension is defined as office systolic blood pressure (BP) ≥140 mmHg and/or diastolic BP ≥90 mmHg [15]. Current European guidelines from 2023 recommend that blood pressure should be treated when >140/90 mmHg and lowered to <130/80 mmHg in patients 18-64 years old and < 140/80 mmHg in ages 65-79 years if the treatment is well tolerated, whereas systolic BP targets <120 mmHg should be avoided [15]. The same targets apply for patients with diabetes Mellitus (DM) [15].

Diabetes Mellitus

DM (covered in more detail later in this thesis) is strongly associated with atherosclerotic disease and increases the risk of PAD approximately three- to four-fold, and the risk of IC two-fold [8]. Furthermore, most diabetic patients have additional risk factors such as smoking, hypertension, and dyslipidemia further contributing to the development of CVD [8].

Manifestations and diagnosis of PAD

PAD can have different manifestations depending on disease severity. The main focus of this thesis is, however, on intermittent claudication (IC).

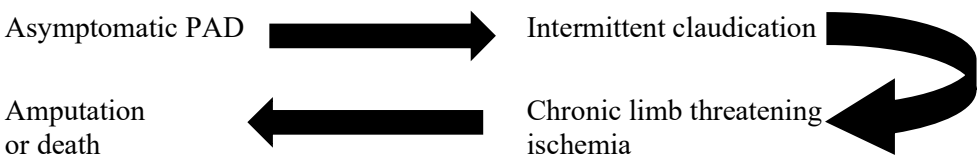

Figure II PAD progression from asymptomatic to amputation or death. PAD - Peripheral arterial disease

Asymptomatic PAD

It is estimated that approximately 20 – 50% of individuals with PAD lack symptoms [16]. However, one third of these individuals may develop leg pain during a 6-minute walk test. Thus, these individuals may be asymptomatic due to self-imposed reduction of walking pace or due to relative physical

inactivity in order to avoid leg pain [17, 18]. Furthermore, subjects with asymptomatic PAD experience progressive functional impairment and diminished walking capability compared to those without PAD [18].

Intermittent claudication

During ambulation the metabolic needs of the calf muscles increase, demanding greater oxygen supply [19]. This results in a physiological compensatory arterial vasodilation, increasing vital blood supply. However, due to narrow arterial lumina and impaired endothelial vasodilator responses, patients with PAD cannot achieve sufficient compensatory vasodilation [17, 18]. This supply-demand mismatch is a hallmark of intermittent claudication, presenting as temporary ischemia of the muscles distal to the atherosclerotic lesions; manifesting as pain, cramping, or fatigue, causing individuals with IC to stop walking to alleviate the supply-demand mismatch and its symptoms [17]. The temporary ischemic pain results in severe limitation of exercise performance and walking ability, leading to greatly impaired walking distance, walking speed, and overall function [8]. The reduction of walking capacity can be quantified with a graded treadmill test, in which onset of ischemic pain and peak walking time can be determined [8]. Additionally, patients with symptomatic PAD experience reduced health-related quality of life (HRQoL, discussed in greater details on page 29) and have higher prevalence of depression [18].

Chronic limb threatening ischemia

Chronic limb threatening ischemia (CLTI) is the end stage form of PAD, a condition with significantly higher risk of mortality and tissue-loss [20]. The hallmark of CLTI is ischemic rest pain or tissue loss affecting the forefoot, the pain is most severe when the leg is resting and is relieved by muscle activation [20]. As a result of the ischemic pains, patients often sleep with their legs in a position in which gravitational forces may improve the blood flow. At this point walking capacity is severely impaired, and in severe cases walking is almost or absolutely impossible [8]. Studies have shown that untreated CLTI has an all-cause mortality rate of 22% and a similar 22% risk of major amputation within one year of follow-up [21].

Diagnosis of PAD

PAD diagnosis is based on evaluation of patient history combined with clinical examination and measurement of the ankle-brachial index (ABI) [5]. An ABI <0.9 has 75% sensitivity and 86% specificity for detection of peripheral stenosis [22] and is the method of diagnosis most commonly used in both clinical practice and epidemiological research [1]. The sensitivity of ABI

is, however, poor in patients with heavily calcified arteries (media sclerosis), such as those with DM, renal insufficiency, or old age; in these patients the ABI can be falsely elevated resulting in false negative results [23,24].

ABI and prognosis

ABI is a reflection of generalised atherosclerotic disease and shows a nonlinear relationship with the occurrence of CVD [25]. A low ABI has been related to increased risk of both total- and CV-mortality, myocardial infarction, and stroke [25]. ABI > 1.4 can indicate medial calcification, however, which is common in diabetes and renal failure and associated with an increased CVD risk. ABI values between 0.9 and 1.4 are therefore considered optimal [25], in patients with ABI >1.4 toe-brachial index might be needed for a correct diagnosis [26]. It is, nevertheless, important to note that resting ABI is only a modest predictor of the degree of walking impairment [27].

Other diagnostic options

A treadmill test can be useful method of both determining IC severity and revealing stenotic arterial lesions not evident at rest [12]. Duplex ultrasound can be used for both screening and diagnosis of PAD [26]. Computed tomography angiography (CTA) is a relatively fast, non-invasive imaging method that provides high resolution images with the ability to visualise calcifications, stents, and aneurysms [26]. Magnetic resonance angiography (MRA) can be used both with and without contrast medium, ensuring a crucial role in diagnostics of patients with chronic kidney disease (CKD); but contraindications for MRA such as non-compatible pacemakers and claustrophobia must be acknowledged [26]. Digital subtraction angiography, an invasive imaging method which was previously considered as the standard reference for diagnosis of PAD has nowadays been replaced with non-invasive diagnostic methods [26].

Therapeutic options

Non-surgical treatment

Given that PAD patients have increased risk of systemic atherosclerotic complications such as myocardial infarction and stroke, treatment aims both to decrease the overall CV risk and improve limb function [28].

The recommended non-pharmacological measures are the same as those recommended for management of hypertension; smoking cessation, healthy diet, weight loss, and regular exercise (table II) [26].

Pharmacological treatment should be offered in accordance with European guidelines as a combination known as best medical therapy (BMT) [26]. Treatment should be tailored to fit the individual patient and consists of a combination of antihypertensive, lipid-lowering, and antiplatelet medications in patients with symptomatic PAD [26]. A meta-analysis has shown that the risk for a patient with IC to develop advanced ischemia ranges between 4% and 27% [29].

Management of hyperlipidemia

European guidelines recommend lipid-lowering drugs for all patients with PAD; with a LDL-C goal of <1.4 mmol/L, or a decrease by ≥50% if the initial LDL-C level is between 1.8 and 3.5 mmol/L [12, 26].

Statins are the most commonly used drug groups for reduction of lipid levels[28], owing to their potential in reducing cardiovascular morbidity and mortality, and improving IC symptoms [28]. In a study of symptomatic PAD patients, statin use reduced the risk adverse limb outcomes, including worsening symptoms, peripheral revascularization, and ischaemic amputations by approximately 18% [30]. If LDL-C targets are not reached with maximal tolerable statin dose alone, addition of ezetimibe or PCSK9 inhibitors may be considered [12]. The addition of PCSK9 inhibitors to statins have shown a potential pan-vascular impact of aggressive lipid-lowering therapy, decreasing CV events in patients with PAD [31].

Management of hypertension

European guidelines recommend initial treatment with non-pharmacological lifestyle changes listed in table II for hypertensive individuals who are not at high level of CV risk [15].

Lifestyle modification	Comment
Smoking cessation	Cigarette smoking is accompanied by activation of the sympathetic nervous system leading to a temporary BP increase [15].
Diet change	The optimal dietary interventions for hypertension are the DASH (Dietary Approaches to Stop Hypertension) and the Mediterranean diets which can lower the risk of all-cause and cause-specific mortality and BP irrespectively of hypertension status [15]. Furthermore, a low-caloric diet in adults with hypertension can reduce BP with 6.5/4.6 mmHg [15].
Physical activity	Physical activity can potentially prevent and treat hypertension while simultaneously lowering CV risk and mortality, with aerobic exercise reducing systolic BP with 5-8 mmHg in patients with hypertension [15].
Salt restriction	Restricting salt intake to approximately 5.8 g per day results in an average decrease in BP by 5/2 mmHg in patients with hypertension [15].
Reduced alcohol consumption	The risk of hypertension increases with daily alcoholic intake more than 10-20 g alcohol and alcohol reduction close to abstinence can lower BP by 3.3/2.0 mmHg with potential for 5.5/4.0 mmHg reduction in heavy drinkers [15].
Weight reduction	Overweight and obesity are associated with increased risk of CV death, all-cause mortality, and hypertension. A healthy body mass index (BMI) of 20 – 25 kg/m^2 is recommended to prevent hypertension in healthy people and to reduce BP in hypertensive patients [32]. Modest weight loss is recommended for patients with hypertention as it lowers all-cause mortality and for each kilogram of body weight loss BP can be lowered by 1/1 mmHg [15].

Table II Recommended lifestyle changes in hypertensive patients, also applicable for patients with PAD [32]. PAD – peripheral arterial disease, CV – cardiovascular, BP – blood pressure.

Pharmacological treatment can be offered in addition to lifestyle modifications to achieve optimal BP control [32]. Studies have shown similar efficiency in improving major CV outcomes and mortality across all major classes of antihypertensive drugs [32] but angiotensin converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB) are considered first-line therapy in PAD as they have been shown to reduce CV events in

this particular group of patients [8,33]. Furthermore, guidelines recommend treatment initiation with a combination of two drugs to improve speed, efficiency, and predictability of achieving BP control [32].

Antihypertensive medication	Comment
Angiotensin converting enzyme inhibitors (ACEi)/ angiotensin receptor blockers (ARB)	First-line therapy in patients with PAD, shown to reduce CV events in this group [12].
Calcium channel blockers and thiazide diuretics	Can be used as first additions to the first-line therapy [12].
Spironolactone	Can be added if BP is not optimal despite a combination of the abovementioned medications [12].
Beta blockers	Should be used with caution in patients with CLTI [12].

Table III Simplified algorithm for antihypertensive treatment in PAD [32]. PAD – peripheral arterial disease, CV – cardiovascular, BP – blood pressure, CLTI – chronic limb threatening ischemia.

Antithrombotic medication

Antiplatelet medication

No beneficial effects of antithrombotic treatment have been documented in patients with asymptomatic PAD [34-36]. Antiplatelet medication should therefore only be offered to patients with symptomatic PAD as studies have shown that they can reduce major adverse cardiovascular events (MACE) in this condition. [26] Either aspirin or clopidogrel are recommended as first line therapy [36, 37]. Studies have, however, shown that secondary preventive pharmacotherapy with antithrombotic medication is generally underused in patients with symptomatic PAD [36].

Antiplatelet medication in combination with low dose anticoagulation with rivaroxaban

The Cardiovascular Outcomes for People Using Anticoagulation Strategies (COMPASS) [38] showed better CV outcomes but also more major bleeding in PAD patients assigned to low dose of rivaroxaban (2.5mg twice daily) plus low-dose aspirin than those assigned to aspirin alone [38]. *Efficacy and Safety of Rivaroxaban in Reducing the Risk of Major Thrombotic Vascular Events in Subjects With Symptomatic Peripheral Artery Disease Undergoing Peripheral Revascularization Procedures of the Lower Extremities (VOYAGER PAD)* [39] study corroborated the evidence on efficacy of combination treatment by expanding these results to patients having undergone surgical revascularization due to symptomatic PAD. *VOYAGER*

PAD also showed that major bleeding as classified by the Thrombolysis in Myocardial Infarction (TIMI) classification did not differ significantly between the groups. The incidence of major bleeding according to the International Society on Thrombosis and Haemostasis (ISTH) was, however, significantly higher with the combination of rivaroxaban and aspirin than with aspirin alone [39].

Surgical treatment

Revascularization options are highly dependent on the anatomical location and extension of arterial lesions [26]. Additionally, lesions can be categorized into supra- or intralingual based on their anatomical location in relation to the inguinal ligament.

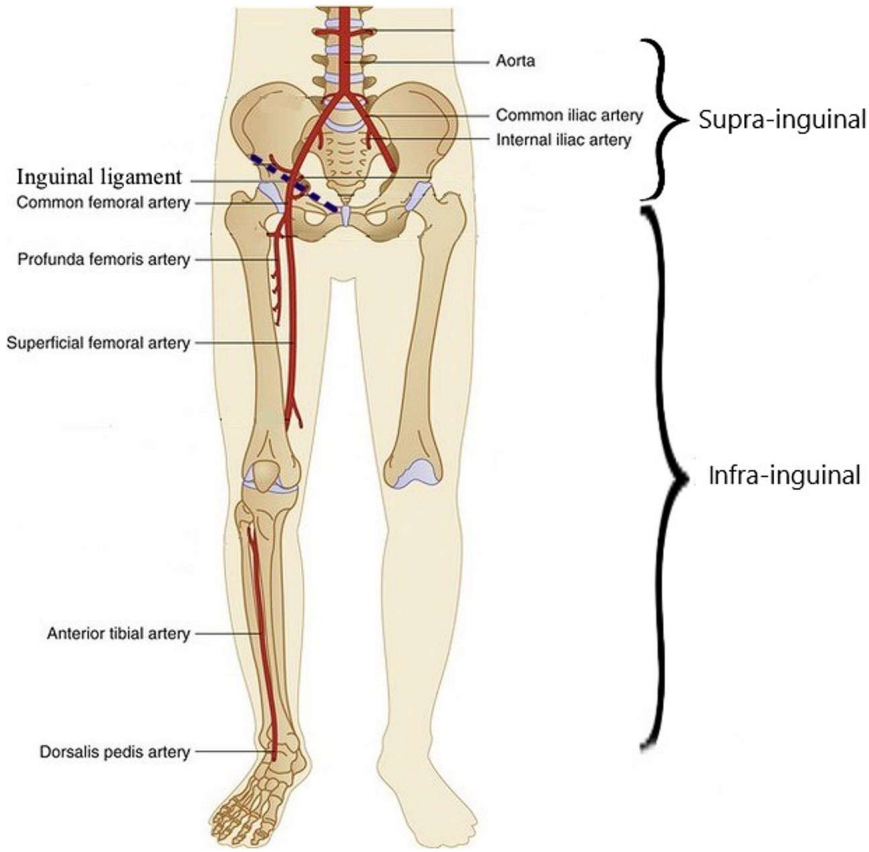

Figure III. Illustration of the supra- and infra-inguinal division used in this thesis Image modified with permission from Radiologykey.

As the durability of interventions for infrainguinal lesions is generally inferior compared to in suprainguinal interventions, most experienced clinicians have a higher threshold for invasive intervention for IC in patients with infrainguinal lesions [27]. The official recommendations regarding indications for surgical revascularization of infrainguinal atherosclerotic lesions in patients with IC are also conservative [26]. As surgical revascularization is associated with both morbidity and mortality [26]; it is only recommended in patients who have not responded favorably to BMT and supervised exercise training, or have disabling symptoms that substantially alter daily life activities [26]. Furthermore, an inability to perform an occupation or quality of life issues arising from the inability to provide care to a spouse or a family member may also be an indication for revascularization [27]. All patients with CLTI should, however, if possible, be offered surgical revascularization [26].

Infrainguinal atherosclerotic lesions can be operated by either endovascular- or open surgical approach [26].

Surgical complications –short & long term

Postoperative complications can be divided into short-term ≤30 days complications, commonly reflecting perioperative factors, and long term >30 days complications, reflecting pre- and postoperative factors such as natural progression of the disease [40].

Short-term complication	Long-term complication
Arterial dissection	Stent fracture
Arterial perforation*	Chronic arterial erosion
Pseudoaneurysm *	Arterial perforation*
Acute recoil with abrupt closure or restenosis	Restenosis with potential occlusion
Distal embolization	Loss of collateral branches
Arteriovenous fistula	Pseudoaneurysm formation*

Table IVa. The most common short- and long-term surgical complications following endovascular surgery [27]

*Complications which might occur both as short- and long term.

Short term complications	Long term complication
Incisional wound hematoma	Pseudoaneurysm formation
Ipsilateral lymphedema	Graft infection
Graft stenosis*	Graft stenosis*
Graft occlusion*	Graft occlusion*
Major amputation*	Major amputation*
Incisional wound infection	Graft infection

Table IVb. The most common short- and long-term surgical complications following open surgery [8, 41] * Complications which might occur both as short- and long term.

Endovascular surgery

Endovascular procedures are generally well tolerated, resulting in minimal complications and require relatively short hospitalization when compared to open surgical revascularization [27]. They are, on the other hand, considered to be less durable than surgical bypass operations and have greater need for reintervention, especially in cases with diffuse or long stenosis [27]. Furthermore, the durability of revascularization is further diminished with greater lesion length, occlusive, multiple, or diffuse lesions, DM, CKD, and smoking [42].

Patients undergoing surgical revascularization due to IC caused by infra-inguinal atherosclerotic lesions are recommended endovascular surgery as first choice for lesions up to 25cm in length [26].

Endovascular procedures involve a variety of devices and methods including balloon angioplasty, drug-coated balloons, stenting, drug-eluting stents, and atherectomy [42]. The operating method of choice is dependent on anatomic location, lesion length, degree of calcification, and operator experience [42].

Open surgery

Open surgical interventions have greater patency and durability, it is, however, also associated with greater risk of adverse perioperative events [42]. Bypass surgery is the mainstay of open surgery for IC but is being less frequently performed due to more modern revascularization methods such as endovascular surgery [27]. Stenosis or occlusion of the superficial femoral- and proximal popliteal arteries are the most probable cause of infrainguinal claudication, making femoral-popliteal bypass the most common open surgical procedure for claudication [42]. Furthermore, the success of surgical bypass is dependent on arterial inflow, outflow, target vessel, and quality of the bypass conduit [27]. Endarterectomy is a surgical method for direct removal of obstructive plaque that is best applied for focal lesions in vessels of large caliber, particularly at bifurcations [41]. Advantages of endarterectomy are due to the autogenous nature of the procedure; not requiring a conduit. Limitations, on the other hand, lies in the thrombogenicity of the resulting surface and subsequent arterial healing which may lead to recurrent stenosis [41].

Hybrid surgery

Open and endovascular surgical procedures can be combined, performing a common femoral artery endarterectomy and then angioplasty of proximal iliac artery lesion or distal superficial femoral artery (SFA) lesion [27].

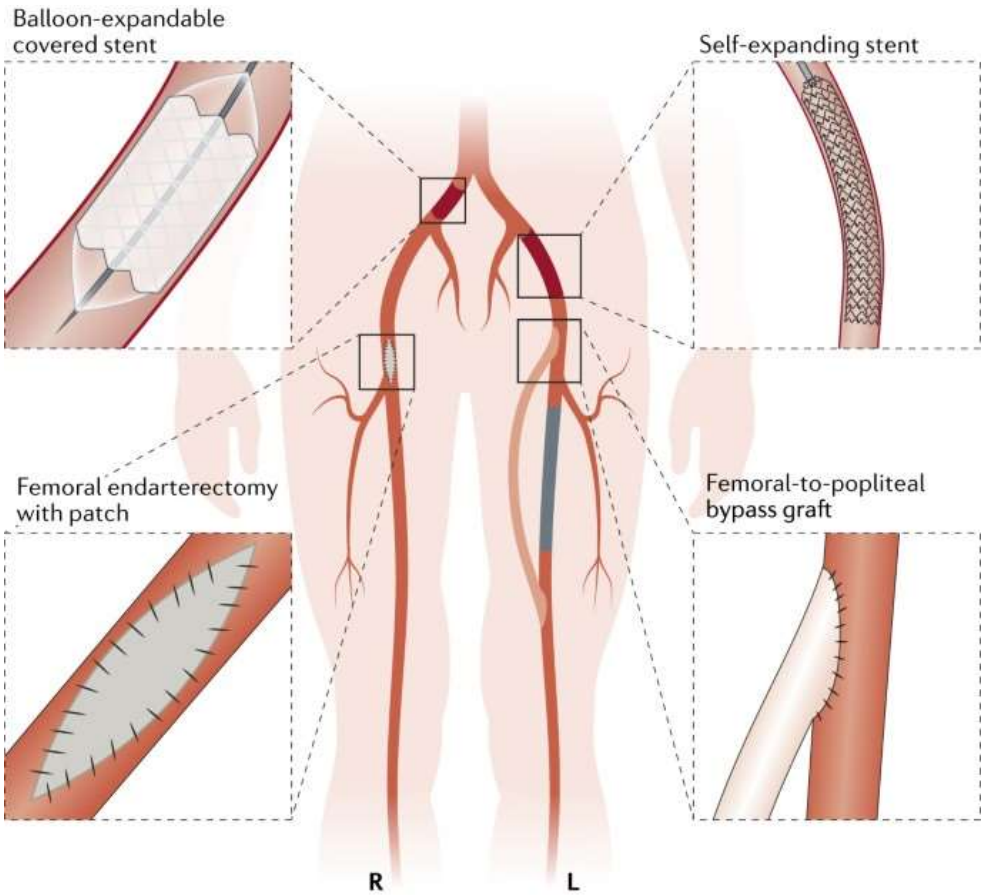

Figure IV. Illustration showing the endovascular interventional methods on the top and open surgical methods on the bottom. Image used with permission from Nature

Health related quality of life

Health-related quality of life (HRQoL) is a multidimensional assessment of how disease and treatment affect a patients sense of overall function and wellbeing; representing physical, psychological, and social aspects of life [43]. Patients with IC have significantly lower quality of life (QoL) and reduced functional status [27]; making HRQoL improvement an important treatment goal in management of IC [44].

Endovascular revascularization of the SFA in combination with BMT in patients with IC has shown to improve HRQoL and walking distance more than BMT alone at 12 and 24 months of follow-up after revascularization[45,46]. Extended follow-up has shown that HRQoL remains improved after 36 months but that the benefits were no longer detectable at 60 months [44].

Diabetes Mellitus

Epidemiology

Diabetes Mellitus (DM) is a chronic condition estimated to affect 463 million adults globally [47]. The main types of DM are type 1 DM (T1DM) which constitutes approximately 5% of all cases of DM, type 2 DM (T2DM) constituting 90% of all DM cases, and gestational and other types of DM 5%[48].

Obesity and insulin resistance commonly associated with DM [49] are thought to decrease nitric oxide activity and contribute to endothelial dysfunction [49]. In physiological conditions, insulin inhibits thrombosis and increases fibrinolysis [49], insulin resistance, however, has a prothrombotic effect [49]. Furthermore, relative lack of insulin due to insulin resistance leads to calcium accumulation in platelets, which enhances platelet aggregation and contributes to atherogenesis [49, 50]. Furthermore, hyperglycemia can through the formation advanced glycation end products contribute to CVD development by inducing inflammatory changes in the vascular endothelium [49].

If left untreated, long-term hyperglycemia may lead to both macro- and microvascular complications [47]. Macrovascular complications include peripheral arterial disease, stroke, heart failure, myocardial infarction, and angina pectoris [47, 51]. Microvascular complications include peripheral neuropathy, nephropathy eventually leading to chronic kidney disease (CKD), and retinopathy [47, 52].

T1DM is caused by insulin deficiency due to autoimmune-mediated loss of beta-cell function and is typically diagnosed in childhood or early adulthood, as the acute symptoms of T1DM makes it easier to diagnose [48].

T2DM is characterized by hyperglycemia due to progressive resistance to the action of insulin that eventually a loss of loss of beta- cell function as well. The gradual onset of symptoms caused by T2DM makes it harder to diagnose, as a result it is typically diagnosed in adulthood [48].

Atherosclerotic lesions of diabetic patients are more likely to be diffuse and more distal, and diabetic patients may present with atypical IC symptoms [53]. Furthermore, diabetic patients also experience worse lower extremity function than individuals with PAD alone [53].

Treatment of T2DM

As the risk of complications is highly correlated with glycaemic control, glucose-lowering and other medication specifically addressing the prevention or therapy of diabetes-related complications are the cornerstone of diabetes management [54]. The optimal and most common method of assessing glycaemic control is done by measuring Hemoglobin A1c (HbA1c) and both Swedish and European guidelines recommend a near normal HbA1c goal in diabetic patients [55, 56](42 -52 mmol/mol in patients with DMT2 in the first five to ten years and 53 – 69 mmol/mol in patients with longer DM duration [55]). Lowering HbA1c confers a reduction in microvascular complications, whereas evidence is less compelling regarding reduction of macrovascular complications [56].

The most commonly used oral glucose-lowering medication is Metformin, it has effective glucose lowering abilities, is cheap and has a relatively good overall safety profile [57]. Studies have also shown that metformin can reduce the risk of MI, coronary death, and stroke in overweight patients with newly diagnosed T2DM without previous CVD [56], suggesting that metformin improves CV prognosis [56]. Furthermore, metformin is considered as the first choice of treatment in patients with DM unable to achieve glycemic targets by diet- and lifestyle interventions [56].

A relatively new class of antidiabetic medication are sodium-glucose transport protein 2 (SGLT2) inhibitors acting on the SGLT-2 proteins expressed in the renal proximal convoluted tubules to reduce the reabsorption of filtered glucose and promote urinary glucose excretion [58]. Dapagliflozin is a SGLT2 inhibitor which has been shown to improve the control of blood glucose levels, decrease the risk of CV adverse events in T2DM subjects with underlying CV illness, and minimize the risk of CV mortality and heart failure hospitalization in adult subjects with underlying heart failure and decreased ejection fraction [58]. Although PAD patients have been included in trials and observational research on SGLT2 inhibitors, the heterogeneity of methods and patient selection makes it difficult to draw concrete conclusions regarding the utility of this drug class in PAD [59].

Another relatively new class of antidiabetic medication are the glucagon-like peptide-1 receptor agonists (GLP1-RA) which act by stimulating insulin synthesis and secretion [60]. Recent studies have shown that GLP1-RA have beneficial effects on CV events in patients with T2DM [12, 60].

A meta-analysis consisting of eight randomized placebo-control CV outcome trials with data of 60,800 individuals reported lower rates of MACE (defined as CV mortality, stroke, or MI), all-cause mortality, hospitalization due to heart failure, and improved renal outcomes [61]. Data regarding the effects of

GLP-1 RAs in PAD are mainly derived from retrospective studies comparing them to other antidiabetic drugs such as SGLT-2 inhibitors [60]. These comparisons show lower rates of major adverse limb event in diabetic patients treated with GLP1-RA compared to those treated with SGLT2 inhibitors [60]. Furthermore, GLP1-RA conferred a significant reduction in hospitalization due to PAD and lower limb complications when compared to other antidiabetic medications, including SGLT2 inhibitors [60]. There are currently no randomized controlled trials (RCTs) focusing on diabetic patients with PAD. The highly anticipated RCT *the Effects of Semaglutide on Functional Capacity in Patients With Type 2 Diabetes and Peripheral Arterial Disease study* is ongoing, however, and aims to examine the impact of semaglutide on walking ability in diabetic people with PAD [60]. It is, however, important to note that data regarding the impact of GLP1-RA in PAD are scarce and there are no RCTs directly comparing effects of GLP1-RAs with SGLT2 inhibitors. Therefore, clear recommendations cannot be issued regarding the choice of treatment [60].

Abdominal aortic aneurysm

Abdominal aortic aneurysm (AAA) is a dilation of the abdominal aorta most commonly defined as maximum infrarenal abdominal aortic diameter of ≥30mm on ultrasonography or CT imaging [62]. AAA development is traditionally considered to be a result of weakening of the arterial wall caused by inflammatory activity in the aortic media and vascular smooth muscle cell apoptosis [62]. Another theory is that AAA development is a compensatory arterial response to atherosclerotic plaque growth and expansion causing remodelling stimulated by hemodynamic changes [62].

AAA is usually asymptomatic until potentially lethal aortic rupture occurs [62]. Risk factors for AAA development are similar to those of PAD mentioned on pages 18-20; with the exception of DM which has shown to be associated with reduced prevalence of AAA [62]. Best medical treatment of AAA includes antihypertensive, antiplatelet and lipid lowering medications, and smoking cessation and aims to lower the overall CV risk profile [63]. Early detection of AAA by ultrasound screening and prophylactic surgery has been shown to reduce AAA-related mortality and all-cause mortality [64, 65]. All 65-year-old men in Sweden and Great Britain are offered screening for AAA as an effective preventive health measure [65, 66].

Plasma biomarkers

The definition of a biomarkers is "A defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention." [67] In medicine, biomarkers can be used for several different purposes of which the most important are listed in table V.

Biomarker type	Purpose
Diagnostic	Detection or confirmation of a disease or condition
Monitoring	Assessment of disease status or efficacy of treatment
Pharmacodynamic	Evaluation of response to medical therapy
Predictive	Prediction of individuals at risk of unfavourable effects of exposure to a medical or environmental agent
Prognostic	Identification of risk for a clinical event or disease
Safety	Measurement before and after an exposure or intervention to evaluate likelihood of adverse event.
Susceptibility/risk	Indication of risk for disease development in an otherwise healthy individual

Table V. Different types and purpouses of biomarkers [67]

Plasma biomarkers of relevance in PAD

Many biomarkers have been proposed for prediction, diagnosis, monitoring, prognosis, and other aspects of PAD [68]. In papers III-IV in this thesis, a total of 96 different biomarkers were evaluated. The below markers were those for which a relevant association with PAD could be demonstrated.

C-terminal endothelin-1 (CT-proET-1)

CT-proET-1 is a precursor peptide of endothelin-1 (ET-1) [69]. ET-1 was initially discovered as a powerful vasoconstrictor and has thus been implicated in the pathogenesis of arterial hypertension [69]. Furthermore, renal vasculature is particularly sensitive to ET-1, strengthening the evidence for a role of this peptide in arterial hypertension [69]. ET-1 is synthesized by almost every cell type but has higher expression in vascular endothelial and smooth muscle cells [69]. Its secretion is activated in response to major CV risk factors [69].

CT-proET-1 can be used as a diagnostic and prognostic biomarker for CVD [69]. Increased levels have been reported in individuals with arterial hypertension, cigarette smoking, DM, and obesity, leading to impaired endothelial function and vasodilation [69]. Studies have shown a causal involvement of endothelin in atherogenesis due to endothelial overexpression of ET-1 causing endothelial injury and aggravating atherosclerotic progression and lesion severity [69].

N-Terminal prosomatostatin (NT-proSST)

NT-proSST is a fragment of the somatostatin precursor [70]. Somatostatin is also known as growth hormone inhibiting hormone [71]. It was first discovered in the hypothalamus but is also produced in the gastrointestinal tract, pancreas, and the central nervous system (CNS) [71]. Somatostatin is involved in the exocrine, endocrine, and CNS systems and its actions are mentioned in table VI [71]. To avoid spreading actions of somatostatin, it is rapidly degraded by plasma enzymes, giving it a half-life in circulation of about three minutes [70].

Localization	Action
Exocrine	Inhibition of bile secretion, colonic fluid secretion, pancreatic enzymes
Endocrine	Inhibition of growth hormone, thyroid-stimulating hormone, prolactin, insulin, and glucagon
CNS	A neurotransmitter in the lateral septum, cortex, amygdala, and hippocampus

Table VI. The actions of somatostatin based on its location.

Midregional proatrial natriuretic peptide (MR-proANP)

MR-proANP is a precursor of atrial natriuretic peptide [72], which is a hormone secreted from the right atrium, as a response to atrial stretch [72]. ANP increases glomerular filtration rate in the kidney, inhibits sodium and water reabsorption in the nephron, and plays a role in lipid mobilization in white adipose tissue [72]. It also plays a role in vascular remodelling by inhibiting vascular smooth muscle cell proliferation and regulating endothelial cell growth and permeability [73].

Procalcitonin (PCT)

PCT is an inflammatory peptide of which serum levels are very low in normal physiological conditions, but can be increased up to 1000 folds in response to bacterial infection causing release of endotoxins and cytokines [74]. Levels can however also be elevated due to trauma, burns, carcinomas, and cardiogenic chock [74]. Since PCT is an inflammatory marker there has been growing evidence linking it to atherosclerosis and conditions such as AMI [75].

Copeptin

Copeptin is a fragment of pro-arginine vasopressin (AVP), a peptide that is released in response to several inflammatory stimuli [76]. It is synthesised in the hypothalamus and released from the posterior neurohypophysis [76]. It has been described as a quantitative marker of endogenous stress that acts as a nonspecific marker of acute disorder and disease severity. Studies have shown that it has modest diagnostic accuracy in AMI [76].

Secretoglobin family 3A Member 2 (SCGB3A2)

SCGB3A2 is also known as uteroglobin-related protein 1 (UGRP1), it is a small molecular weight protein [77]. It is secreted in airway epithelial cells where it is involved in lung development, inflammatory reactions, and potentially in the development of asthma [77]. It plays an important role in anti-inflammatory activity, acting downstream of inflammation modulators and controlling the inflammatory pathways [77].

Osteoprotegerin (OPG)

OPG is a glycoprotein that is a member of the tumour necrosis factor receptor superfamily [78]. It was originally discovered as an inhibitor of bone resorption but has since then also been found in vascular smooth muscle cells where it acts as a survival factor for endothelial cells [78]. Elevated concentrations of OPG have also been reported in diabetic patients with microvascular complications, indicating a possible involvement in the development of vascular dysfunction in DM [78]. Furthermore, serum OPG levels has been

reported to be significantly associated with both the presence and severity of PAD in diabetic patients [79].

Urokinase-type plasminogen activator surface receptor (uPAR)

The urokinase-type plasminogen activator and its specific receptor (uPAR) are a potent multifunctional system involved in vascular remodelling, they are induced by vascular injury and promote neointima formation in atherosclerosis [80]. They act by controlling vascular smooth muscle cell migration and proliferation, deposition, and by interfering with wound healing [80]. Additionally, uPAR may act as a circulating marker for inflammation, thromobogenesis, immune regulation, and has been associated with PAD, hypertension, DM, CVD, and CKD [81].

Serum macrophage chemokine ligand 16 (CXCL16)

CXCL16 is a transmembrane protein expressed in macrophages and aortic smooth muscle cells, and the expression is enriched in atherosclerotic plaques [82]. It acts as an attractant for cells and scavenger receptors facilitating uptake of oxidized LDL-C [82]. CXCL16 levels have shown a modestly positive association with metabolic dyslipidaemia and inflammatory risk factors [82]. Furthermore, increased levels of CXCL16 has been related to carotid artery intima-media thickness, plaque area, lumen stenosis rate, and increased plaque vulnerability in patients with acute ischemic stroke [83].

Matrix metalloproteinase 9 (MMP-9)

The matrix metalloproteinases (MMPs) proteases act as controllers of extracellular matrix turnover thanks to their ability to degrade extracellular matrix [12, 84]. They are secreted by inflammatory cells and take part of the vascular remodelling during the development and progression of atherosclerosis [12]. Higher levels of MMP-9 have been reported in patients with PAD, of which CLTI patients showed highest levels of MMP-9, thus possibly reflecting higher levels of ischemia with abnormal vascular turnover and inflammation [84].

P-selectin (SELP)

P-selectin (SELP) is a glycoprotein localized in the granules of platelets and Weibel-Palade bodies of endothelial cells [85]. P-selectin mediates interaction of leukocytes and platelets with the endothelium, thus playing a central role in the process of atherosclerosis, arterial injury, thrombosis, and inflammatory responses.
[85]. Furthermore, elevated levels of P-selectin have been reported in patients with PAD [86].

Growth differentiation factor 15 (GDF15)

GDF15 is a member of the transforming growth factor family, the protein modulates macrophage chemotaxis, affects macrophage apoptosis, and inhibits the proliferation of endothelial cells [87]. GDF15 has been proposed as a promising prognostics marker for CV disease [87], and elevated GDF15 levels in PAD patients have been associated with increased mortality and major amputation [87].

Elafin (PI3)

Elafin is an endogenous protein expressed in epithelial tissues such as skin [88], inhibiting destructive and inflammatory neutrophil derived proteases [88]. Preclinical studies have shown that elafin administration limits tissue destruction and preserves organ function in inflammatory vascular injury [88].

Cystatin B (CSTB)

CSTB is expressed in many types of human cells and considered as a biomarker for cancer and CV disease [89]. A study based on participants in the The Malmö Diet and Cancer cohort reported a significant association between levels of CSTB and coronary events during follow-up [89]. Furthermore, CSTB levels are correlated with metabolic syndrome and increased in diabetic patients [89]. Such associations might perhaps also be relevant in PAD.

Trefoil factor 3 (TFF3)

TFF3 is a small-molecule peptide mainly secreted by intestinal goblet cells and is involved in mucosal repair of the gastrointestinal tract [90]. It has been shown to participate in the pathological process of DM, colitis, non-alcoholic fatty liver disease, and cancer [90]. TFF3 can also activate multiple signalling pathways to repair damaged mucosa, regulate glucose and lipid metabolism, and supress inflammation [90]. Furthermore, TFF3 has shown a cardioprotective effect with the ability to reduce infarction size when administered immediately after myocardial ischemia in mice [91].

Fatty acid-binding protein 4 (FABP4)

FABP4 is an intracellular lipid chaperon released into the bloodstream by adipocytes and expressed in activated macrophages [92]. In animal studies, FABP4 deficiency limited to macrophages was related to a reduction in atherosclerotic lesion area and an inhibitor targeting FABP4 in mice was shown to reduce atherosclerotic lesions [92]. In human studies FABP4 has been associated with coronary artery disease and intima-media thickness of the carotid artery [92]. In patients with PAD, higher levels of FABP4 were related to a significantly higher risk of MACE and all-cause mortality [92].

Tumor necrosis factor receptor superfamily member 14	Low-density lipoprotein receptor	Integrin beta-2	Interleukin-17 receptor A	Tumor necrosis factor receptor 2
Matrix metalloproteinase-9	Ephrin type-B receptor 4	Interleukin-2 receptor subunit alpha	Osteoprotegerin	CD166 antigen
Trefoil factor 3	P-selectin	Cystatin-B	Monocyte chemotactic protein 1	Scavenger receptor cysteine-rich type 1 protein M130
Galectin-3	Granulins	Matrix extracellular phosphoglycoprotein	Bleomycin hydrolase	Perlecan
Lymphotoxin-beta receptor	Neurogenic locus notch homolog protein 3	Metalloproteinase inhibitor 4	Contactin-1	Cadherin-5
Trem-like transcript 2 protein	Fatty acid-binding protein, adipocyte	Tissue factor pathway inhibitor	Plasminogen activator inhibitor 1	C-C motif chemokine 24
Transferrin receptor protein 1	Tumor necrosis factor receptor superfamily member 10C	Growth/differentiation factor 15	E-selectin	Azurocidin
Protein delta homolog 1	Spondin-1	Myeloperoxidase	Serum macrophage chemokine ligand 16	Interleukin-6 receptor subunit alpha
Resistin	Insulin-like growth factor-binding protein 1	Chitotriosidase-1	Tartrate-resistant acid phosphatase type 5	C-C motif chemokine 22
Pulmonary surfactant-associated protein D	Elafin	Epithelial cell adhesion molecule	Aminopeptidase N	Tyrosine-protein kinase receptor UFO
Interleukin-1 receptor type 1	Matrix metalloproteinase-2	Tumor necrosis factor receptor superfamily member 6	Myoglobin	Tumor necrosis factor ligand superfamily member 13B
Myeloblastin	Proprotein convertase subtilisin/kexin type 9	Urokinase plasminogen activator surface receptor	Osteopontin	Cathepsin D
Peptidoglycan recognition protein 1	Carboxypeptidase A1	Junctional adhesion molecule A	Galectin-4	Interleukin-1 receptor type 2
Tyrosine-protein phosphatase non-receptor type substrate 1	C-C motif chemokine 15	Caspase-3	Urokinase-type plasminogen activator	Carboxypeptidase B
Chitinase-3-like protein 1	ST2 protein	Tissue-type plasminogen activator	Secretoglobin family 3A member 2	Epidermal growth factor receptor
Insulin-like growth factor-binding protein 7	Complement component C1q receptor	Interleukin-18-binding protein	Collagen alpha-1(I) chain	Paraoxonase
Cathepsin Z	Matrix metalloproteinase-3	Retinoic acid receptor responder protein 2	Intercellular adhesion molecule 2	Kallikrein-6
Platelet-derived growth factor subunit A	Tumor necrosis factor receptor 1	Insulin-like Growth Factor-Binding Protein 2	von Willebrand factor	Platelet endothelial cell adhesion molecule
C-C motif chemokine 16	N-terminal prohormone of brain natriuretic peptide	N-Terminal prosomatostatin	C-terminal endothelin-1	Midregional proatrial natriuretic peptide
Procalcitonin	*Copeptin*			

Table VII. List of all biomarkers analysed in the thesis

Patients and methods

Methodological overview of the studies

	Paper I	Paper II	Paper III	Paper IV
Study design	Retrospective cohort.	Retrospective cohort.	Prospective cohort.	Cross sectional.
Study sample	Patients having undergone elective endovascular infrainguinal surgery for IC registered in Swedvasc (n=1,738).	Patients having undergone elective open infrainguinal surgery for IC registered in Swedvasc (n=1,002).	Individuals participating in baseline and follow-up evaluation of the MPP (n=5,160).	Men participating in AAA screening (n=267).
Enrolment	2010 – 2014.	2010 – 2014.	1974 – 1992, reexamination in 2002 – 2006.	2010 – 2017.
Methods	Identification of patients with and without DM through NDR. Comparison of diabetic and nondiabetic patients regarding CV morbidity and mortality. Median follow-up 5.2 years for patients with DM and 5.4 for patients without.	Identification of patients with and without DM through NDR. Comparison of diabetic and nondiabetic patients regarding CV morbidity and mortality. Median follow-up 5.2 years for patients with and without DM.	Exclusion of individuals with prevalent PAD. Comparison of biomarker levels at baseline in subjects with and without incident PAD. Median follow-up 11.2 years.	Division of men into two categories based on the presence of PAD defined as ankle brachial index <0.9. Comparison of biomarker levels in men with and without PAD.
Data analysis	Propensity score adjusted analyses. Cox regression model. Kaplan–Meier curves with life tables.	Propensity score adjusted analyses. Cox regression model. Kaplan–Meier curves with life tables.	Cox proportional hazards regression model. Kaplan-Meier estimator.	Receiver-operating characteristic. Area under the curve. The Youden Index Method.

Table VIII. Summary of methods used in the papers AAA - abdominal aortic aneurysm, NDR – National Diabetes Register, DM - diabetes mellitus, IC – intermittent claudication, PAD - peripheral arterial disease, CV - cardiovascular.

Patients

Paper I

All 2,858 subjects in Swedvasc having undergone infrainguinal surgery for IC between 2010 and 2014 were identified. For analysis in paper I, the 1,120 subjects having undergone open- or hybrid surgery were excluded. Among the remaining 1,783 subjects meeting the inclusion criteria, we used the NDR for division into two groups based on the presence or absence of DM. We found 626 subjects with DM and 1,112 subjects without DM. Subjects were followed up until 31:st December 2017, enabling a median follow-up of 5.2 years for subjects with DM and 5.4 for those without DM.

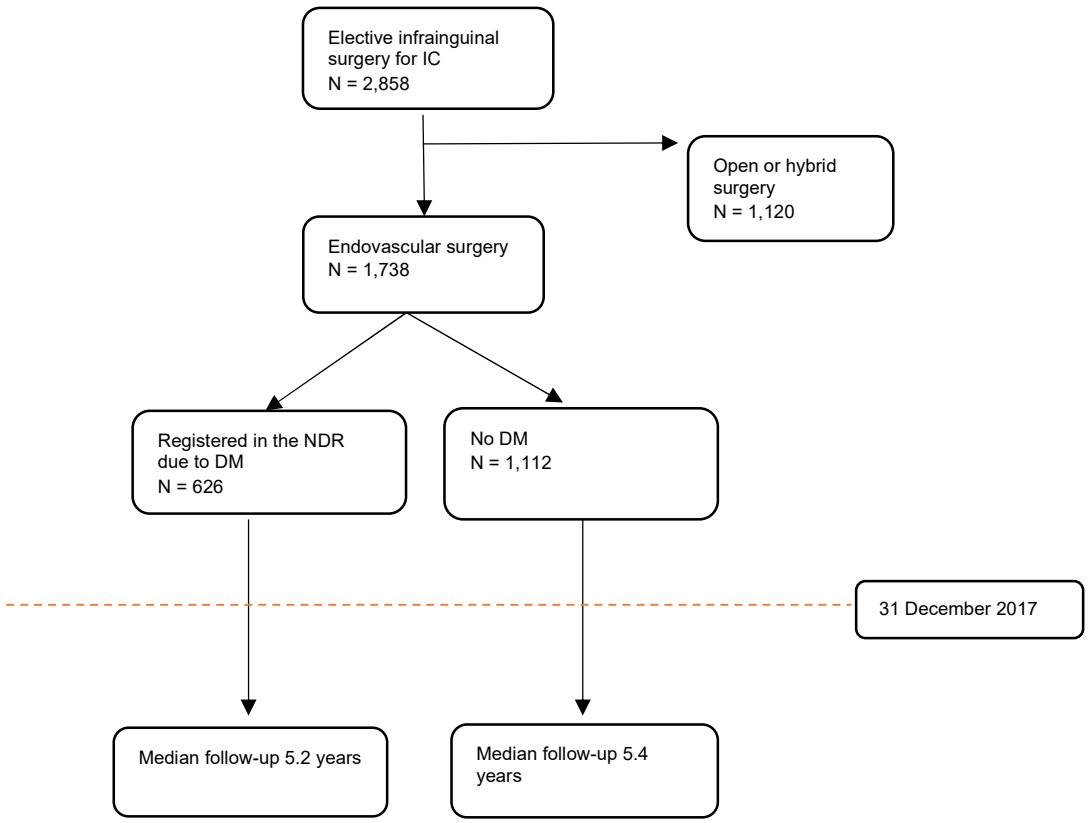

Figure IX. Flowchart of patient selection and follow-up in paper I. IC –Intermittent claudication, DM – Diabetes mellitus.

Paper II

As stated above, all 2,858 subjects in Swedvasc having undergone infrainguinal surgery for IC between 2010 and 2014 were identified. In paper II we excluded the 1,856 subject that had undergone endovascular- or hybrid surgery. The remaining 1,002 subjects meeting the inclusion criteria were divided into two groups based on the presence or absence of DM. We found 323 subjects with DM and 629 subjects without DM. The subjects were followed up until 31st December 2017, enabling a median follow-up of 5.2 years in both groups.

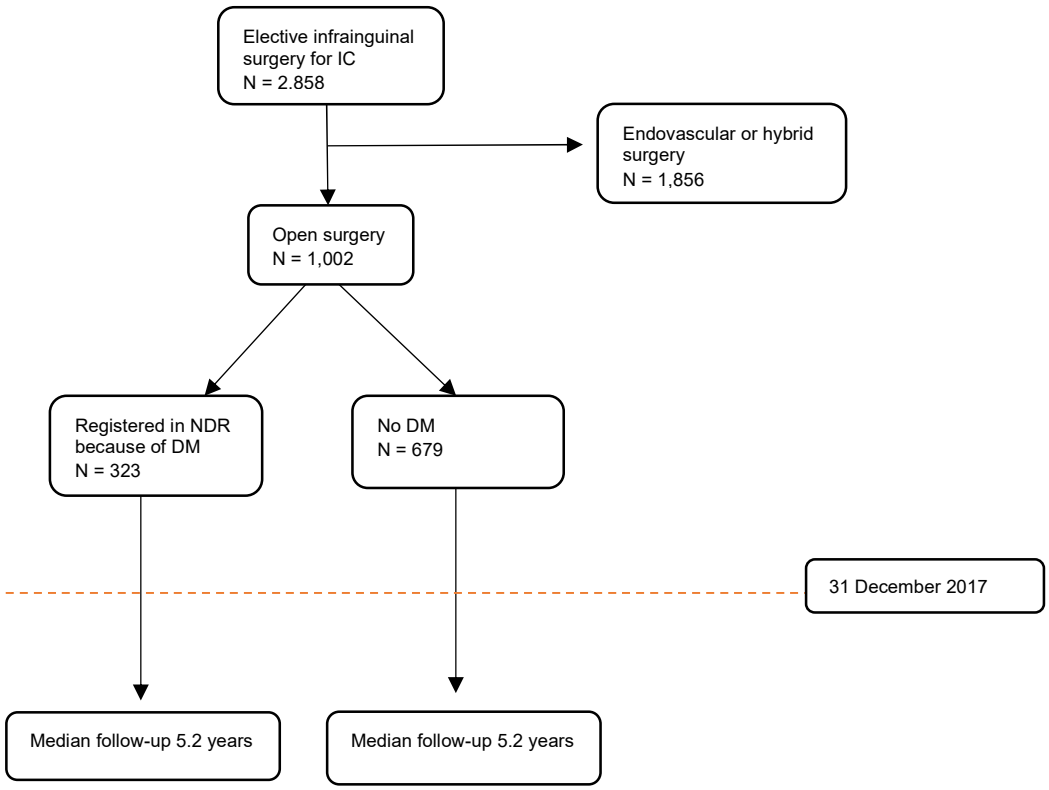

Figure X. Flowchart of patient selection and follow-up in paper II. IC –Intermittent claudication, DM – Diabetes mellitus

Paper III

A total of 33,346 middle aged subjects from the background population were included in the MPP between 1974 and 1992. Among these, 18,240 were re-examined between 2002 and 2006, and analysis of vasoactive biomarkers was performed on 5,160 participants during this re-examination. Subjects were further classified based on PAD diagnosis according to the ICD classification, and those with prevalent PAD at baseline were excluded. Remaining subjects were thereafter followed up until 31:st December 2016. During this median follow-up of 11.2 years, 221 subjects developed incident PAD and 4,939 remained free of PAD.

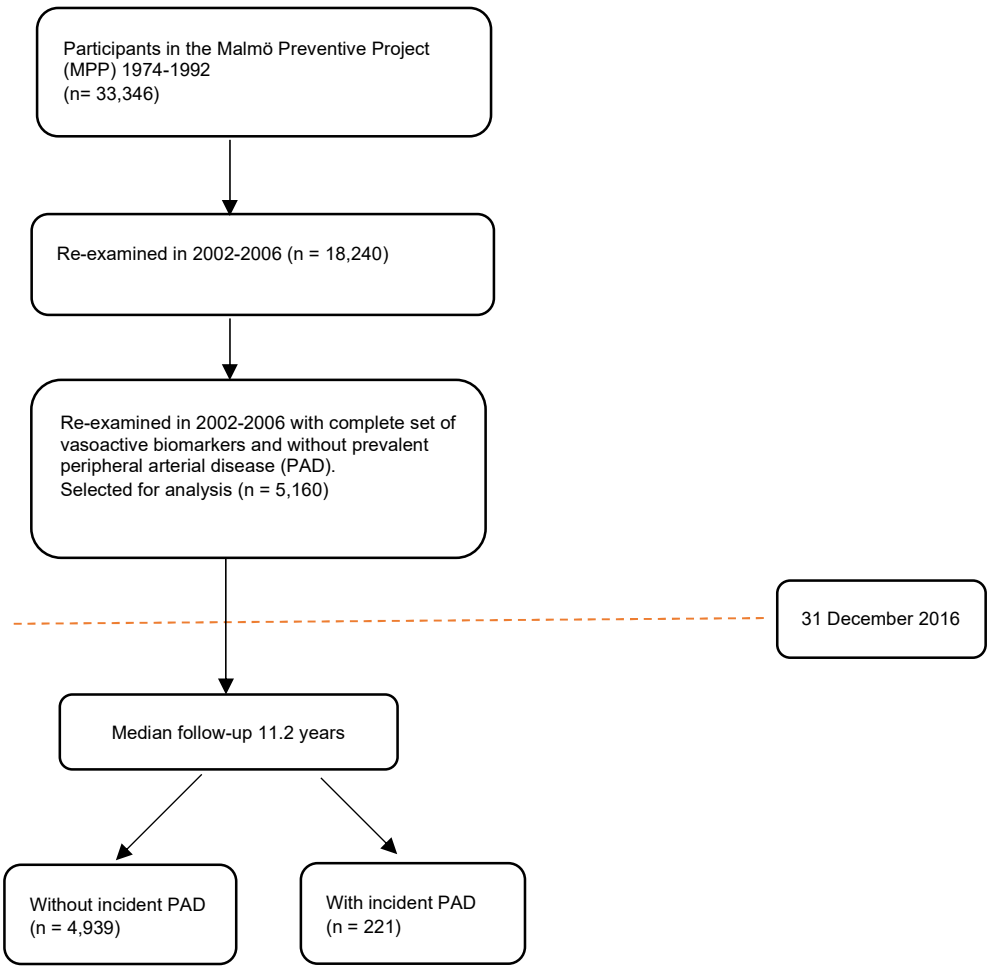

Figure XI. Flowchart of patient selection and follow-up.

Paper IV

During 2010-2017 24,589 men were included in AAA screening at Skåne University Hospital [93], 415 of these men were diagnosed with AAA and 133 of the men with AAA accepted inclusion into the study. Subjects underwent physical examination, measurement of ABI, blood sampling, and assessment of medical history [94]. PAD was defined as ABI <0.9 and 38 individuals were diagnosed with PAD. As a control group, we selected 134 screened men without AAA that were matched for cardiovascular comorbidity and date of examination.

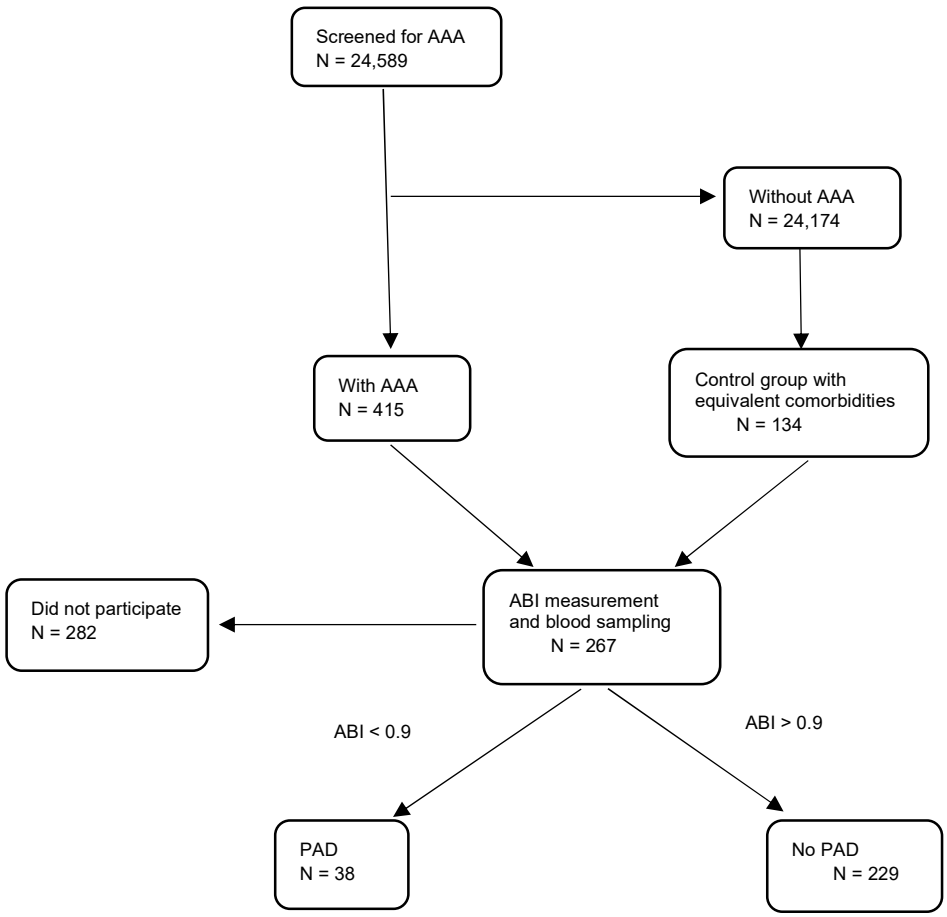

Figure XII. Flowchart of patient selection and follow-up. AAA - Abdominal aortic aneurysm

Data collection

The Swedish Vascular Registry

The Swedish Vascular Registry (Swedvasc) was the world's first population-based registry in vascular surgery when created in 1987, and by 1994 it achieved national coverage [95]. Swedvasc has grown into an integral part of the Swedish healthcare system, providing clinicians, clinics, and researchers with real-world data from an unselected nationwide population [95]. Data is annually analysed, and approximately 10,000–12,000 procedures are yearly registered in Swedvasc [95]. Furthermore, the registry has been validated on several occasions and has shown internal- and external validity of 100% for carotid- and 98.8% for AAA procedures [96, 97]. Access to high quality registry such as Swedvasc enables production of high quality and generalizable nationwide cohorts [95].

For example, in 2021, 5,712 procedures due to PAD were registered, the majority of the operations were due to CLTI and 1,253 of the procedures were due to IC [98]. A standard follow-up in Swedvasc is performed 30 days and one year after surgery [99]. The frequency of complications such as AMI, stroke, reoperation due to bleeding, vascular occlusion, amputation, and mortality are registered at 30 days postoperative follow-up [99].

In papers I and II we used Swedvasc to access information regarding the 2,858 patients that underwent elective infrainguinal intervention for IC during 2010 – 2014.

National Diabetes Register (NDR)

The Swedish NDR was initiated in the beginning of the 1990s, with the first patients reported in 1996 [100]. The purpose of the NDR is to monitor the results of health centres and provide continuous follow-up of national guidelines for diabetes care, treatment, and complications [100]. Annual reports contain characteristics of the patients with T1DM and T2DM such as risk factors, treatments, complications, and procedures such as eye and foot examinations [100]. As of 2022 the NDR has over 470,000 individuals registered [101].

The NDR is validated using automatic controls on newly registered and already existing data. If deviations in the data are discovered the unit responsible for the patient will be contacted [101] . The NDR is estimated to cover 85% of adults with DM, when compared to data on use of antidiabetic medication from the national drug registry. In children with DMT1, however, the NDR is estimated to cover 98% of the patients [101].

The NDR was used to identify patients with DM for papers I and II.

Malmö Preventive Project

Malmö Preventive Project (MPP) is a large-scale screening program for CV risk factors and health promotion that began in 1974 [102]. Initially only men born between 1921 and 1949 were included, but eventually women were also included. By the end of the project in 1992, 22,444 men and 10,902 women had participated [102]. Subjects underwent comprehensive risk factor screening, including physical examination, laboratory testing, and answered a self-administered questionnaire on CVD, hypertension, DM, smoking habits, and alcohol consumption [102]. Laboratory parameters were analysed directly, and serum samples were saved at minus 20 ° C. All surviving participants were invited for re-examination during the years 2002 to 2006; 11,500 men and 6,500 women participated [102]. During this re-examination CV disorders and risk factors were reassessed, a shortened questionnaire and new blood samples were collected [102].

Other registers used in the thesis

The Swedish Cause of Death Register was created in 1952 and comprises data on all deaths of people registered in Sweden [102]. The cause of death registerer was used in papers I and II for identification of causes and time of death.

The Swedish National Inpatient Register (IPR) was created in 1964 and achieved complete coverage in 1978, it contains more than 99% of the somatic and psychiatric discharges, and validation has shown that 85 – 95% of the diagnoses were valid [103]. The IPR was used in Papers I and II for data regarding primary and secondary discharge diagnoses and lengths of hospitalization.

The Prescribed Drug Register (PDR) was established in 2005 and contains information about all prescribed drugs dispensed at pharmacies[104]. The PDR was used for information regarding prescribed drug usage in papers I and II.

The National Cancer Register was founded in 1958 and contains data on the patient, medical- and follow-up data [105]. The National Cancer Register was used in papers I and II for information about comorbidities. The longitudinal integration for health insurance and job market studies (LISA; Statistics Sweden) was used in papers I and II to obtain socioeconomic characteristics, such as marital status and educational level.

Ethical approval

Studies I and II were approved by The Regional Board for Research Ethics in Lund, Sweden with registration numbers 2016/232 and 2016/544.

Study III was approved by The Regional Board for Research Ethics in Lund, Sweden with registration number 2014/643.

Study IV was approved by the Regional Board for Research Ethics in Lund with registration numbers 2010/239 and 2014/643.

In studies I-II all patients had given written informed consent to registration in NDR and Swedvasc. In studies III-IV all subjects gave written consent to participation.

Definitions used in papers I-IV

Acute myocardial infarction was defined by international classification of diseases, tenth revision (ICD-10) code I21.

Body mass index was calculated using the following equation where kg represents a person's weight in kilograms and m^2 their height in meters squared $BMI = \frac{kg}{m^2}$

Chronic limb-threatening ischemia was defined by the presence of ischaemic rest pain, with or without tissue loss for more than two weeks.

Diabetes mellitus was defined as being registered in the NDR in papers I and II, as fasting plasma glucose ≥7.0 mmol/L in paper III, and patient reported and verified in patient files in paper IV.

Duration of DM was in papers I and II defined as the years since DM diagnosis.

Drug treatment, including lipid lowering drugs, acetylsalicylic acid, metformin and other glucose-lowering medications, and anticoagulant therapy was defined according to the Prescribed Drug Register in papers I and II.

Hypertension was in papers I and II defined as collecting a minimum of one prescription of antihypertensive drugs one year prior to index operation and one prescription is equivalent of three months of medicine.

Major adverse cardiovascular events (MACE) in papers I and II included angina pectoris, acute myocardial infarction, papillary muscle rupture, hemopericardium, ventricular septal rupture, chronic ischemic heart disease (ICD-10 codes I20-I25) ; cerebral infarction, intracerebral haemorrhage, and subdural haemorrhage (ICD-10 codes I61-I64).

Major amputation was defined as above ankle-level amputation.

Peripheral arterial disease was in paper IV defined as an ABI <0.9.

Psychiatric disorders in papers I and II excluded dementia.

Smoking was in paper I and II defined as currently smoking at baseline according to data collected from Swedvasc or NDR. In paper III, smoking status was based on a self-administered questionnaire, and "ever smoker" was defined as a participant who has been smoking on a daily basis for ≥6 months. In paper IV, smoking habits was categorized into never-, previous- or current smoker.

Plasma biomarkers

Circulating levels of CT-proET-1 were assessed using Thermo Scientific B·R·A·H·M·S CT-proET-1 KRYPTOR [106].

Circulating levels of NT-proSST were assessed using chemiluminescence/coated tube format (B·R·A·H·M·S GmbH) with a detection limit of 4 pmol/L [107].

Circulating levels of MR-proANP were assessed Thermo Scientific B·R·A·H·M·S MR-proANP KRYPTOR (B·R·A·H·M·S, Hennigsdorf, Germany) [106].

Circulating levels of copeptin were assessed using Thermo Scientific B·R·A·H·M·S CT-proAVP LIA [106].

Serum PCT concentrations were measured by by an ultrasensitive assay (ProCa-S; BRAHMS GmbH, Hennigsdorf, Germany) [108].

Circulating levels of NT-proSST were assessed using chemiluminescence/coated tube format (B·R·A·H·M·S GmbH) with a detection limit of 4 pmol/L [107].

Circulating levels of SCGB3A2, OPG, U-PAR, CXCL16, MMP-9, SELP, GDF-15, PI3, CSTB, TFF3, and FABP4 were assessed with with Proseek Multiplex CVD III96x96 (Olink Biosciences) panel. This high-throughput, multiplex immunoassay allows simultaneous measurement of 91 CVD-related proteins by proximity extension assay and was performed in accordance with the Proseek Multiplex CVD III96x96 user manual at SciLifeLab at the Clinical Biomarker Facility, Uppsala University, Sweden [109, 110].

Statistical analyses

Descriptive statistics were presented using median, interquartile range (IQR), mean, standard deviation (SD), counts, and percentages with 95% confidence intervals (CIs). P-values <0.05 were considered as significant.

In papers I and II all analyses were propensity score adjusted, using Cox regression analysis adjusted for several factors. We estimated propensity scores using a generalized boosted multinomial regression model with an interaction depth of 3, a maximum of 10,000 trees, and a shrinkage of 0.01. Standardized mean difference was used to describe similarity between infrainguinal IC patients with and without DM. Incidence rates were estimated as the number of events per 1,000 person-years with exact 95%

Poisson CIs. Kaplan-Meier curves were used to describe cumulative total and CV mortality, MACE, AMI, stroke, major amputation, and a composite of death or major amputation. The analyses compared infrainguinal IC patients with and without DM using both unadjusted and inverse probability of treatment weighting (IPTW) adjusted Cox regression presented as hazard ratio (HR). Analyses were performed using R 3.4.3.

In paper III quantitative normal and skewed distributed variables were presented as mean with standard deviation (SD) and median with interquartile range (IQR). Differences between groups were assessed with t test for continuous parametric variable, Chi-Square test for nominal variables, and the Mann-Whitney U test for continuous nonparametric variables. Log transformed values of CT-proET-1, NT-proSST, MR-proANP, PCT, and copeptin were used in the Cox proportional hazards regression models to determine HRs. Risk factors for PAD and plasma biomarkers were included in a multivariable-adjusted model. Kaplan-Meier estimator was used to make the survival analysis. Analyses were performed using SPSS version 25.0.

In paper IV Mann–Whitney U test was used for quantitative variables and the two-sided Fisher's exact test for nominal variables, and the Bonferroni method for multiple-comparison correction. Univariate and multivariate logistic regression models were used to examine the association between biomarkers and clinical variables with PAD. Diagnostic potential of the different protein biomarkers were evaluated by analysing receiver-operating characteristic (ROC) curves and accuracy was measured as the area under the curve (AUC). The Youden Index Method was used to establish the optimal cut-off point to discriminate those with and without PAD. Statistical analyses were done in SPSS version 20.

Propensity score adjusted analysis

Propensity score-adjusted analysis is an approach that was developed in the 1980s and is defined as the probability that an individual would have been allocated to a particular treatment group as a function of observed baseline characteristics [111]. These conditional probabilities can be estimated using multivariable logistic regression where the treatment group is the dependent variable and the baseline characteristics and risk factors are the independent variables. Propensity scores ranging from 0 to 1 for each patient are produced as predicted probabilities of a given treatment by the regression model featuring identified risk factors. It is commonly used in observational studies to reduce selection bias, mitigate confounding, and improve internal validity [111].

In papers I and II we used inverse probability of treatment weighting (IPTW), which is a method that entails allocating a weight to each patient in the cohort. This method allowed us to generate a synthetic sample with virtually balanced covariates to reduce confounding between those with and without DM. [111].

Aims

Papers I and II: The primary aim of these studies was to compare propensity score-adjusted short- (30-day) and long-term total and CV mortality, AMI, MACE, stroke, and major amputation after elective surgery for infrainguinal IC in patients with and without DM. Paper I evaluated patients having undergone elective endovascular surgery and paper II evaluated patients after open vascular surgery. The secondary aim of these studies was to evaluate potential relationships between glycaemic control and outcomes in diabetic patients.

Paper III: The primary aim was to evaluate if biomarkers associated with other CVD can predict mortality and incident PAD during long-term follow-up.

Paper IV: The aim was to evaluate 91 proteins associated with inflammation, angiogenesis, platelet activation, coagulation, wound healing, chemotaxis, cell adhesion, metabolism, and with AAA development for a potential role as new diagnostic biomarkers for PAD.

Results

Paper I

Main findings

- During short-term follow-up, the IPTW-adjusted Cox regression showed no differences between patients with and without DM in total- and CV mortality, MACE, AMI, stroke, and major amputation.
- During long-term follow-up, the IPTW-adjusted Cox regression showed higher rates of MACE (HR 1.26, CI 1.07-1.48; p< 0.01), AMI (HR 1.48, CI 1.09-2.00; p=0. 01), and major amputation (HR 2.31, CI 1.24-4.32; p< 0.01) in patients with DM (Table IX).
- Higher HbA1c in diabetic patients was associated with higher risk of total mortality (HR 1.01, CI 1.00-1.03; p<0.05), whereas neither duration of diabetes nor HbA1c level was related to CV mortality, MACE, AMI, stroke, or major amputation during follow-up.

	HR	p-value	95% CI
Short-term			
Mortality	0.86	0.8470	0.19 – 3.89
CV mortality	0.77	0.7795	0.13 – 4.66
MACE	1.06	0.7703	0.73 – 1.52
AMI	1.68	0.3364	0.58 – 4.86
Stroke	0.38	0.3844	0.04 – 3.39
Major amputation	1.80	0.5613	0.25 – 13.23
Major amputation or death	0.91	0.8619	0.32 – 2.60
Long-term			
Mortality	1.12	0.2901	0.91 – 1.40
CV mortality	1.14	0.3225	0.88 – 1.49
MACE	1.26	0.0051	1.07 - 1.48
AMI	1.48	0.0113	1.09 – 2.00
Stroke	1.25	0.2515	0.86 - 1.81
Major amputation	2.31	0.0087	1.24 – 4.32
Major amputation or death	1.18	0.1336	0.95 – 1.45

Table IX Propensity score adjusted of short- and long-term complications after elective infrainguinal endovascular surgery for intermittent claudication in patients with and without DM. Hazard ratio (HR) for total and cardiovascular (CV) mortality, major adverse CV events (MACE), acute myocardial infarction (AMI), stroke, major amputation, and the composite of major amputation and death. P-values and 95% confidence interval (CI).

Paper II

Main findings

- During short-term follow-up, the IPTW-adjusted Cox regression showed no differences between patients with and without DM in total- and CV mortality, MACE, AMI, stroke, and major amputation (table X).
- During long-term follow-up, the IPTW-adjusted Cox regression showed higher rates of MACE (HR 1.33, CI 1.08–1.62; p<0.01), and AMI (HR 2.21, CI 1.46–3.35; p<0.01) in patients with DM (table X).
- Higher HbA1c in diabetic patients was associated with higher risk of MACE (HR 1.02, CI 1.00–1.03; p= 0.02), stroke (HR 1.05, CI 1.00–1.11; p=0.04), and total mortality (HR 1.03, CI 1.01–1.06; p<0.01). Duration of diabetes was associated with higher risk of major amputation (HR 1.08, CI 1.02–1.15; p < 0.01).

	HR	p-value	95% CI
Short-term			
MACE	1.18	0.5330	0.70 – 2.02
AMI	1.24	0.7713	0.29 – 5.21
Stroke	0.49	0.5315	0.05 – 4.67
Major amputation*			
Major amputation or death	1.79	0.5300	0.29 – 1.04
Total mortality	0.69	0.7511	0.07 – 6.68
CV mortality	0.69	0.7511	0.07 – 6.68
Long-term			
MACE	1.33	0.0062	1.08 – 1.62
AMI	2.21	0.0002	1.46 – 3.35
Stroke	1.10	0.7381	0.63 – 1.91
Major amputation	1.17	0.6988	0.52 – 2.65
Major amputation or death	1.25	0.0877	0.97 – 1.62
Total mortality	1.22	0.1450	0.93 – 1.59
CV mortality	1.38	0.0514	1.00 – 1.92

Table X. Propensity score adjusted of short- and long-term complications after elective infrainguinal open surgery for intermittent claudication in patients with and without DM. Hazard ratio (HR) for total and cardiovascular (CV) mortality, major adverse CV events (MACE), acute myocardial infarction (AMI), stroke, major amputation, and the composite of major amputation and death. P-values and 95% confidence interval (CI). *Not calculated as only one patient underwent amputation.

Papers I and II

Differences between non-diabetic and diabetic patients after endovascular- and open surgery during long-term follow up are shown below, enabling comparison of results after intervention with different surgical techniques (table XI).

	Endovascular surgery	Open surgery
Mortality	0	0
CV mortality	0	0
MACE	+	+
AMI	+	+
Stroke	0	0
Major amputation	+	0
Major amputation or death	0	0

Table XI. Summary of long-term outcomes endovascular- and open surgery for IC. Results are based on propensity score adjusted Cox regression analysis. Increased risk in diabetic patients is represented as + and no increased risk in diabetic patients is represented as 0. Major adverse cardiovascular events (MACE). Acute myocardial infarction (AMI).

The importance of diabetes duration and HbA1c in diabetic patients during long-term follow up after endovascular- and open surgery during long-term follow up are shown below, enabling comparison of results after intervention with different surgical techniques (table XII).

	Diabetes duration		HbA1c	
	Endovascular surgery	Open surgery	Endovascular surgery	Open surgery
Mortality	0	0	+	+
CV mortality	0	0	0	0
MACE	0	0	0	+
AMI	0	0	0	0
Stroke	0	0	0	+
Major amputation	+	0	0	0
Major amputation or death	0	0	0	0

Table XII Summary of effects of diabetes duration and HbA1c in diabetic patients undergoing revascularization for IC. Results are based on propensity score adjusted Cox regression analysis. Increased risk in diabetic patients is represented as + and no increased risk in diabetic patients is represented as 0. Major adverse cardiovascular events (MACE). Acute myocardial infarction (AMI).

Paper III

Main findings

- Adjusted Cox proportional hazards regression model showed that higher levels of CT-proET-1 (HR 1.8; 95% CI 1.4-2.3; p< 0.001), NT-proSST (HR 1.5; 95% CI 1.2-2.0; p=0.002), and MR-proANP (HR 1.7; 95% CI 1.3-2.3; p=0.001) were independently associated with incident PAD during 11.2 years of follow-up (table XIII).
- Adjusted Cox proportional hazards regression model showed that higher levels of CT-proET-1 (HR 1.3; 95% CI 1.2-1.5; p<0.001), NT-proSST (HR 1.2; 95% CI 1.1-1.3; p<0.001), MR-proANP (HR 1.4; 95% CI 1.3-1.6; p< 0.001), PCT (HR 1.1; 95% CI 1.0-1.2; p=0.011), and copeptin (HR 1.2; 95% CI 1.1-1.4; p<0.001) were independently associated with mortality during 11.2 years of follow-up.

Variables	β	HR (95% CI) for incident PAD	p
CT-proET-1	0.6	1.8 (1.4-2.3)	< 0.001
NT-proSST	0.4	1.5 (1.2-2.0)	0.002
MR-proANP	0.5	1.7 (1.3-2.3)	0.001
PCT	0.1	1.1 (0.9-1.4)	0.380
Copeptin	0.2	1.2 (0.9-1.7)	0.207

Table XIII. Cox regression analysis of plasma biomarkers at baseline and the risk of incident PAD. HR; Hazard ratio, SD; standard deviation (SD) PAD; peripheral arterial disease, BMI; Body Mass Index; CI; Confidence interval, CT-proET-1; C-terminal endothelin-1, NT-proSST; N-Terminal prosomatostatin, MR-proANP; midregional proatrial natriuretic peptide, PCT; Procalcitonin.

Paper IV

Main findings

- Both uni- and multivariate analysis showed that men with PAD had significantly higher levels of SCGB3A2, OPG, U-PAR, CXCL16, MMP-9, SELP, GDF-15, elafin, CSTB, TFF3, and FABP4.
- SCGB3A2, OPG, U-PAR, CXCL16, MMP-9, SELP, GDF-15, and elafin were significantly associated with higher risk of PAD in men with AAA.
- CXCL16, MMP-9, SELP, GDF-15, elafin, CSTB, and FABP4 were significantly associated with PAD in men without AAA.

Biomarker	Univariate			Multivariate		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Secretoglobin Family 3A Member 2 (SCGB3A2)	1.7	1.2-2.3	0.001	1.7	1.2-2.4	0.001
Osteoprotegerin (OPG)	1.8	1.3-2.5	0.001	1.8	1.3-2.5	0.001
Urokinase-type plasminogen activator surface receptor (U-PAR)	1.9	1.4-2.7	<0.0001	1.9	1.3-2.7	0.001
Serum macrophage chemokine ligand 16 (CXCL16)	2.5	1.6-3.8	<0.0001	2.4	1.5-3.9	<0.0001
Matrix metalloproteinase 9 (MMP-9)	2.0	1.4-2.9	<0.0001	1.9	1.3-2.8	0.001
P-selectin (SELP)	2.0	1.4-3.0	<0.0001	1.9	1.9-2.8	0.001
Growth differentiation factor 15 (GDF-15)	1.9	1.4-2.6	<0.0001	1.7	1.2-2.4	0.005
Elafin (PI3)	1.9	1.4-2.7	<0.0001	1.9	1.3-2.6	0.001
Cystatin B (CSTB)	1.7	1.3-2.4	0.001	1.7	1.2-2.3	0.001
Trefoil factor 3 (TFF3)	1.7	1.2-2.4	0.001	1.6	1.1-2.3	0.009
Fatty acid-binding protein 4 (FABP4)	1.8	1.3-2.5	0.001	1.7	1.2-2.4	0.002

Table XIV. Biomarkers and their association with risk for peripheral arterial disease Odds Ratio (OR), Confidence interval (CI).

Discussion

Outcome of interventional revascularization

Results of papers I and II showed no difference in short-term complications between patients with and without diabetes. As events were few and confidence intervals wide, caution must be applied when drawing the conclusion that both open- and endovascular procedures can be safely performed in diabetic patients with IC caused by infrainguinal atherosclerotic lesions. Patients with IC that have not responded favorably to BMT and supervised exercise training or have debilitating symptoms that hinders their daily life activities should still in corroboration with current European guidelines [26] be offered appropriate intervention regardless of the presence or absence of DM.

Results from our study differ from previously published articles in regard to short-term complication rates. A retrospective study of 174 diabetic and non-diabetic patients with infrainguinal IC and CLTI undergoing endovascular- and open surgery reported 21% (n=37) readmission rate due to post-operative complication of which the majority of complications were due to infection at the surgical site or the index limb [112]. Our narrower patient selection, however, makes it difficult to compare with these results, as CLTI patients suffer greater short- and long-term complication rates than those with IC due to their more advanced risk factor profile already at initial presentation [112]. A large study of 1,647 patients with IC and 2,802 patients with CLTI undergoing infrainguinal endovascular intervention reported that 6.5% (n=107) of IC patients had an unplanned hospital readmission within 30 days after surgery [113]. They identified smoking, DM, preoperative dialysis, and procedure time exceeding 120 minutes as risk factors for unplanned readmissions [113]. Furthermore, a cohort of 6,112 diabetic and non-diabetic patients undergoing lower extremity bypass due to CLTI and IC reported a 9.4% (n=11,180) 30-day hospital readmission rate of which majority of readmission reasons were related to the patients index operation and >50% of readmissions were attributable to wound complications [114].

The abovementioned findings should however to interpreted with caution as it is important to note that the Swedish NDR includes 400 000 adult patients with DM [101] and 22,803 infrainguinal operations were registered in the years of 2010 – 2014 [99](4,331 in 2010, 4448 in 2011, 4938 in 2012, 4251 in 2013, and 4835 in 2014 [99]). We did, however, only include 1,738 patients in paper I and 1,002 patients in paper II, thus we must take into consideration that the few short-term complications registered may be too few to provide sufficient statistical power in the analysis. Therefore, we cannot exclude that the short-term were due to a type II statistical error.

In contrast, long-term follow up in paper I showed that diabetic patients undergoing endovascular revascularization due to IC caused by infrainguinal atherosclerotic lesions had higher risk of MACE, AMI, and major amputation. Whereas paper II showed that diabetic patients undergoing open revascularization due to IC caused by infrainguinal atherosclerotic lesions had higher rates MACE and AMI, but similar risk of major amputation.

The higher risk of major amputation in diabetic patients undergoing endovascular surgery compared to open surgery may be attributed to the greater patency and durability of surgical interventions [42]. CLTI and IC are clearly different stages of PAD, but patients in both disease stages undergoing endovascular surgery are reported to be older, more functionally dependent, have more severe comorbidities such as renal insufficiency and dialysis compared to those undergoing open surgery [115]. Furthermore, a retrospective long-term study [116] suggested that open methods might be preferable in CLTI patients with DM who have developed heel ulcers [116]. A large retrospective cohort study [117] of 75,189 subjects undergoing surgery due to symptomatic PAD selected 14,339 patients undergoing open- and 14,399 patients undergoing endovascular surgery 1:1 matched using propensity score model. The study group consisted of diabetic and non-diabetic patients undergoing supra- and infrainguinal surgery due to CLTI and IC. The study reported patients undergoing open surgery had lower rates of major adverse limb events (MALE) and mortality compared to the group undergoing endovascular surgery [117].

The increased rates of MACE and AMI in diabetic patients during long-term follow-up in both paper I and II may be a result of potential micro- and macrovascular complications of DM. Both micro- and macrovascular complications of DM have, however, decreased in recent years, resulting in a reduction of AMI and stroke rates in diabetic patients [47]. Furthermore, an American study has shown lower incidence rates of CVD in patients

diagnosed with DM in the years 2012-2014 compared to those diagnosed 2003-2005 [47].

Somewhat similar results have been reported by another Swedvasc based observational study analysing mortality and amputation rates in a large group of patients (n=16,889) undergoing either open- or endovascular surgery due to supra- or infrainguinal IC or CLTI [118] which showed initially low but linearly increasing amputation and mortality rates [118]. The patients in this study were of higher age (mean 74 years) compared to our study (mean 70 years), whereas comorbidities were relatively similar in both studies and 26% of their included patients had DM [118].

Role of HbA1c

Long-term follow up in paper I showed that increased HbA1c in diabetic patients after endovascular surgery was associated with higher risk of total mortality, and elevated HbA1c levels in diabetic patients after open surgery were related to higher risk of MACE, stroke, and total mortality during long term follow-up in paper II.

These results highlight the importance of good diabetic care aiming to reach HbA1c targets and corroborate established knowledge regarding the importance of intensive glycemic control to reduce potential diabetic complications [119].

Role of diabetes duration

Paper I showed no association between diabetes duration and short- or long-term complications, whereas long-term follow up in paper II showed that longer duration conferred a higher risk of major amputation in the diabetic group. It is, however, estimated that a patient with DM has a 23 times higher risk of undergoing lower limb amputation compared to a non-diabetic individual [120]. Thus, the increased risk of major amputation may be a reflection of diabetic complications, despite the fact that only paper II showed higher risk of major amputation.

Plasma biomarkers in relation to PAD and mortality

The plasma biomarkers studied in this thesis can be classified as endothelial, cardiac, inflammatory, and hormonal biomarkers (Table XV).

Classification	Biomarker
Endothelial	C-terminal endothelin-1 (CT-proET-1)
	Osteoprotegerin (OPG)
	P-selectin (SELP)
Cardiac	Midregional proatrial natriuretic peptide (MR-proANP)
	Copeptin
Inflammatory	Growth differentiation factor 15 (GDF-15)
	Urokinase-type plasminogen activator surface receptor (uPAR)
	Fatty acid-binding protein 4 (FABP4)
	Trefoil factor 3 (TFF3)
	Elafin (PI3)
	Cystatin B (CSTB)
	Secretoglobin Family 3A Member 2 (SCGB3A2)
	Matrix metalloproteinase 9 (MMP-9)
	Serum macrophage chemokine ligand 16 (CXCL16)
	Procalcitonin (PCT)
Hormonal	N-Terminal prosomatostatin (NT-proSST)

Table XV Biomarker type

Endothelial biomarkers

Among the evaluated endothelial biomarkers, CT-proET-1 could be independently used for prediction of incident PAD in paper III. Due to the cross-sectional design of paper IV, we cannot determine whether or not OPG and SELP also have predictive properties in addition to their ability to identify prevalent PAD.

The relationship between these biomarkers and PAD may be explained by their various effects on the endothelium; CT-proET-1 has shown atherogenic and pro-inflammatory effects [69], OPG has been linked to development of vascular dysfunction and arterial calcification [78], and SELP has been shown to mediate interaction of leukocytes and platelets of the endothelium [85].

A case-control study consisting of 238 men with IC and 245 men in the control group matched for age and prevalence of DM reported significantly higher levels of CT-proET-1 in the IC group [121]. A comparably smaller study consisting of 165 patients with T2DM reported significant association between serum OPG levels and both presence and severity of PAD [79]. OPG has also been reported as an independent predictor of PAD in patients with peritoneal dialysis [122]. Elevated OPG levels have also been reported in PAD patients requiring endovascular revascularization compared to age- and sex-matched individuals without CVD, thus OPG may be used as a biomarker of PAD progression and prognosis after endovascular surgery [123]. P-selectin is a glycoprotein encoded by SELP, and a prospective population-based cohort of 6,814 individuals reported elevated levels of this marker to be significantly associated with lower ABI and prevalent PAD, suggesting a potential predictive ability [124].

Furthermore, a study of 40 individuals undergoing endovascular surgery due to PAD showed that elevated levels of both ET-1 and SELP prior to surgery were associated with higher risk of post-operative restenosis [125].

Cardiac biomarkers

Paper III showed that elevated levels of MR-proANP may be used for both prediction and identification of incident PAD. The increased levels of MR-proANP in PAD patients may be a reflection of a physiological response to inflammatory and hemodynamic changes which might lead to atherosclerosis and CVD. The previously mentioned case-control study [121] also showed that the IC group had higher levels of MR-proANP compared to the control group. Furthermore, copeptin was shown to be independently associated with mortality which may be due to atherosclerotic changes resulting in tissue hypoperfusion, consequent osmotic alteration, and endogenous stress increasing the risk of fatal CV events [126].

Inflammatory biomarkers

The inflammatory markers GDF-15, uPAR, FABP4, TFF3, Elafin, CSTB, SCGB3A2, MMP-9, and CXCL16 were shown to be indicative of PAD in paper IV. Atherosclerosis is considered to be a disease of chronic inflammation altering the functions of the cells in the arterial cell wall and unleashing inflammatory pathways leading to activation and involvement of the immune system [6]. Paper III showed that elevated levels of PCT were associated with reduced long-term survival, although infectious diseases are the most common reasons for elevated PCT levels, noninfectious causes such as neuroendocrine malignancies and cardiac arrest have also reported [127] .

Furthermore, an inability to reduce serum PCT in patients with severe sepsis has shown to increase the risk of mortality [128].
A cross sectional study of 121 patients reported higher levels of MMP-9 in patients with PAD, with higher MMP-9 levels in patients with CLTI than those with IC [84].

Jönelid et al. [129] also used the Proseek 96 Multiplex CVD III 96^{x}96 proximity extension assay for plasma profiling of 92 CVD related proteins [129] in 390 patients included three to five days after AMI and followed up after 3, 12, and 24 months. Blood sampling was performed three to five days after AMI and at the three months follow-up visit, and ABI was measured two to three weeks after the index event [129]. In patients with PAD, levels of GDF-15, uPAR, FABP-4, and CSTB levels were elevated compared to in those with normal ABI. Furthermore, levels of GDF-15 were found to predict PAD [129]. These results enable an interesting comparison with our paper IV, as both articles used the Proseek Multiplex analysis and the median age of 67 years in the AMI patients is comparable to the age of 65 years in our study group. Our findings regarding the above-mentioned biomarkers partly corroborate the results of Jönelid, but it is unclear why our findings regarding other biomarkers differ. There are differences between the two studies, however, the AMI patients were of both sexes and had suffered an acute ischaemic event prior to sampling which might have affected biomarker levels.

To the best of our knowledge, there are no previous studies showing associations between elafin, SCGB3A2, TTF3, and PAD. CXCL16 levels have been associated with carotid artery intima thickness in patients with acute ischemic stroke [83], whereas there are no prior findings linking it to lower extremity PAD.

Hormonal biomarkers

Paper III showed that elevated levels of NT-proSST may be used for both prediction and identification of incident PAD. This association may be as a result of the somatostatin induced inhibition of key hormones of metabolism leading to development of hypertension and hyperglycemia [130]. Furthermore, at the time of writing this thesis there are no articles showing associations between NT-proSST and PAD.

Methodological considerations and limitations

Selection bias

Papers I and II were based on materials derived from the national registers Swedvasc and NDR. Swedvasc has an external validity of 100% for carotid and 98.8% for AAA procedures [96], however, no validation of PAD procedures is available.

The participation rate in MPP was 71% [102], which can be considered relatively high, strengthening the generalizability of paper III. However, the subjects represent a homogenous ethnic group from Malmö with 83% of the participants born in Sweden. Furthermore, studies have shown that subjects complying with the invitation can be expected to have better overall health status than those not choosing to participate [131].

Paper IV is based on individuals participating in AAA screening, to which only men aged ≥65 years are invited [131]. Thus, only men have been included in paper IV, creating a selection bias as women are innately excluded.

Information bias

Papers I and II rely on data derived from mandatory health data or population registries such as the IPR and cause of death registry (as explained in page 47) and therefore have virtually no missing values. Swedvasc has an external validity of 93% for infrainguinal bypass operations [97]. There have, however, been reports of misclassification of CLTI as IC in Swedvasc and thus the reported association between DM and the increased risk of major amputation should be interpreted with caution [132]. Furthermore, an observational cohort study based on data from Swedvasc showed that 59% of subjects undergoing surgery due to IC did not have smoking status confirmed [118]. In our studies I and II data on smoking status was retrieved from Swedvasc and NDR and was missing for 15% of patients.

Incident PAD was not an endpoint of the original MPP study and therefore ABI and biomarkers were not analysed at the baseline. Thus, identification of PAD in paper III relied on ICD codes which have been recorded by different systems over the years. Furthermore, we were unable to assert PAD incident and mortality on participants that emigrated during follow-up.

In paper IV data was collected when subjects were included in the study, minimizing the risk of informational bias.

Confounding

Papers I and II were nationwide retrospective observational cohort studies and paper III was a prospective cohort study. To minimize the confounding resulting from the retrospective nature of the studies we adjusted for potential confounders in the statistical models used. As papers I, II, and III were observational cohort studies, causality between exposure and outcome cannot be determined. Paper IV was a cross sectional study and thus it cannot establish any causal relationship between exposure and outcome.

In papers I – III the statistical analyses were adjusted for several factors including age, gender, smoking status, CVD, and malignant disease. In paper IV we did, however, not adjust for gender and age since only 65-year-old men were included in the study. Therefore, we cannot identify gender- or age specific changes related to biomarker levels.

Papers I and II utilized a propensity score model adjusted for 34 potential confounders, which allowed us to observe differences between patients with and without DM, and thus reliably isolate the effect of DM upon our outcomes. There is always a risk of residual confounding as we only can adjust for variables entered in the model.

Other limitations

Papers I and II lacked detailed data on location and extent of the treated atherosclerotic lesions, this information would have been desirable as it is well established that the effect of interventional revascularization, especially endovascular revascularization diminishes with more complex atherosclerotic lesions [8, 26, 27, 42]. We also lacked crucial information regarding the use of insulin and types of antidiabetic medications since novel antidiabetic medications have been introduced during the five-year follow-up and have shown great reduction in mortality and CV morbidity (discussed in greater details on pages 31-32 and page 70.

Asymptomatic PAD patients are estimated to represent approximately 25 – 50% of individuals with PAD [16], and without baseline ABI measurement in paper III we were unable to identify these patients with asymptomatic PAD. Furthermore, decreased smoking rates in Sweden [13] and increased use of lipid-lowering drugs and antithrombotic medications since the start of the MPP may have impacted the CV related outcomes over the 11 year long follow-up time [133].

Strengths

Papers I and II are large nationwide studies with over 5 years of follow-up, narrow and specific inclusion criteria, data retrieved from Swedvasc and NDR and propensity score adjustment. Furthermore, use of the IPR, national cancer register, and the PDR guarantees near perfect data coverage.
Strengths of paper III arise from its large size with 5,160 subjects included and the long 11-year follow-up.

The main strength of paper IV is the reliable measurement of ABI, enabling identification of both asymptomatic and symptomatic PAD patients. Furthermore, the study used data derived from screening materials which can be seen as a double-edged sword as it confers both its strength and its weakness. All 65-year-old men in Sweden are invited to AAA and thus the individuals participating in the screening examinations can be seen as a reflection of the general population, however, previous studies have reported that subjects participating in screening are of higher socioeconomic status and more health aware [131].

Reproducibility

The use of ICD-10 codes in papers I – III ensures reproducibility of our studies. Patient selection and choice of interventional treatment for IC varies markedly [134], however, between countries and thus our results may not be generalized to other populations. Countries with highly developed national registries such as the Danish adult diabetes registry [135] may use similar methods to evaluate the risk of postoperative complications in diabetic and non-diabetic complications in their respective countries.

Ethical considerations

As mentioned earlier, subjects in paper I and II gave written consent to registration in Swedvasc and NDR, individuals studied in paper III gave written consent to participation in the MPP, and subjects in paper IV gave written consent to participation in the study. Since papers I-II utilized data derived from patient registers, subjects included in the study have not been made aware of their participation in the studies, nor the outcome of these studies. One may therefore argue that involuntary participation in the studies may be unethical. This is also applicable to paper III in which subjects were not informed about their levels of plasma biomarkers.

On the other hand, to inform an individual of a possibly dangerous, but unknown and not yet treatable condition may bring more psychological harm than benefit; as demonstrated in individuals participating in the AAA screening [136]. Subjects participating in the studies have not received any direct benefits of study participation, but neither has their study participation caused them any harm, risk, or unpleasant experiences.

Future considerations

The current diabetic management recommendations in Sweden from 2017 (updated 2019) [55] recommend initial treatment with metformin in all patients without contraindications to this drug. If optimal glycemic control cannot be achieved with metformin alone, addition of SGLT2 inhibitors is recommended for patients with concomitant CVD and GLP1-RA or SGLT2 inhibitors is recommended for those with obesity [55]. Recent studies have shown decreased risk of new acute coronary syndrome events, heart failure and renal impairment in subjects treated with SGLT2 inhibitors or GLP1-RA, independent of baseline HbA1c levels [137]. With increasing implementation of novel antidiabetic medications giving us better tools to manage DMT2, we may assume further reduction of CV mortality and morbidity in diabetic patients with PAD in the future. We also hope to see earlier implementation of the novel antidiabetic medications in accordance with the latest European guidelines published in 2020 recommending initiation of diabetic treatment with SGLT2 inhibitors or GLP1-RA in patients with DMT2 and CVD, or at very high or high CV risk. Guideline definitions of very high-risk individuals are those with DM and target organ damage or three or more major risk factors; high risk individuals are DM patients with duration ≥10 years without target organ damage but with any other additional risk factor [56].

Novel non-interventional revascularization methods such as gene- and cell therapy have been developed. Gene therapy aims to stimulate angiogenesis by targeting different genes involved in angiogenesis, and systematic reviews and meta-analysis of RCTs have been made, however, they have shown no significant benefit in symptomatic PAD patients.[12] Cell therapy on the other hand, uses autologous cells from the PAD patients or allogenic cells from donors; meta-analysis of placebo-controlled trials have however shown no significant advantages of stem cell therapy [12]. These novel treatment methods are currently considered as experimental treatments and only used in scientific trials, but they have accumulated great scientific popularity and may one day become the mainstay of symptomatic PAD treatment.

Results from the VOYAGER study showed lower rates of acute limb ischemia, major amputation, AMI, ischemic stroke, and death from cardiovascular causes with a combination of low dose rivaroxaban and ASA compared to ASA alone, 2,629 of the 6,564 patients followed for 28 months after infrainguinal surgery for PAD had DM. Swedish guidelines for prevention of atherosclerosis [138] were published in 2014 and have not yet

been updated to include low-dose rivaroxaban (2.5 mg twice daily) in addition to aspirin in patients with high atherosclerotic burden. Our findings, however, support the notion that it might therefore be reasonable to routinely add rivaroxaban after infrainguinal revascularization for IC to further decrease the risk of CV events during the postoperative period without increasing the risk of bleeding.

Nether diagnostic nor predictive biomarkers for PAD are currently used clinically [68]. We do, however, believe that with further focus on researching and developing the biomarkers included in this thesis we may one day implement predictive biomarkers into clinical practice. This would prove beneficial as early identification and treatment of PAD has proven major benefits for both the patient and the healthcare systems [26, 27].

In the recent years there has been a rapid development of artificial intelligence (AI) and the improvement in its ability to rapidly process vast amounts of data, recognize patterns and solve various problems in various different field. Current biomarker research relies on various statistical analysis performed on programs such as SPSS and R, whereas future biomarker analysis may be performed using newly developed AI models and programs. Furthermore, various novel biomarkers analysis sampling methods such as Proseek 96 Multiplex CVD III $96^{x}96$ proximity extension assay analyzing 92 biomarkers may benefit of the AI processing power; results and data may be instantaneously processed and scores of biomarkers in relation to subject comorbidities may provide more knowledge than an individual biomarker in itself.

Conclusions

- Diabetic patients had increased rates of MACE, AMI, and major amputation during 5 years of follow-up after endovascular surgery for IC. HbA1c was associated with total mortality among diabetic patients.

- Diabetic patients had increased rates of AMI and MACE during 5 years of follow-up after open surgery for IC. Among diabetic patients elevated HbA1c levels were associated with stroke, MACE, and total mortality, whereas diabetes duration was associated with major amputation.

- Elevated levels of CT-proET-1, NT-proSST, and MR-proANP independently predicted the risk of incident PAD during 11 years of follow up of middle-aged individuals, whereas CT-proET-1, NT-proSST, MR-proANP, PCT, and copeptin were independently associated with mortality during 11 years of follow-up.

- Elevated plasma levels of SCGB3A2, OPG, U-PAR, CXCL16, MMP-9, SELP, GDF-15, PI3, CSTB, TFF3, and FABP4 can potentially be used to identify PAD in men, regardless of the presence of concomitant AAA or not.

Populärvetenskaplig sammanfattning

Benartärsjukdom (BAS) innebär att man har förträningar på pulsådrorna (artärerna) i benen, vilka kan leda till minskat blodflöde till benen som i sin tur kan orsaka claudicatio intermittens (tidigare kallad fönstertittarsjuka). Alla med benartärsjukdom har dock inte symptom, trots att de har ökad risk för förträningar på andra kärl (åderförkalkning) som leder till ökad risk för bland annat hjärtinfarkt och stroke (hjärninfarkt). Förträning av benartärerna gör att musklerna inte kan få tillräckligt mycket blod vid ansträngning ledande till kraftiga vad och bensmärtor som gör att en individ måste stanna upp så att musklerna hinner försörjas med blod. I slutstadiet för BAS kan dessa smärtor även uppträda i vila, tex när man ligger och sover. Detta är då ett kritiskt tillstånd som kan leda till amputation om man inte agerar snabbt.

BAS har flertalet riskfaktorer som till exempel rökning, höga blodfetter, övervikt, högt blodtryck (hypertension) och diabetes och drabbar oftast äldre människor. BAS upptäckts oftast i primärvården genom att man mäter blodtrycket i benen och jämför det med blodtrycket i armarna, ett så kallat ankel-brakialindex (ABI). Vid påvisad BAS är totalt rökstopp den viktigaste delen av behandlingen, fysisk aktivitet och behandling av andra riskfaktorer som tex övervikt och hypertoni är också viktiga delar i behandlingen. Patienter med claudicatio brukar rekommenderas blodförtunnande och kolesterolsänkande mediciner för att minska risken för att drabbas av hjärtinfarkt och stroke.

De patienter som upplever allt för kraftiga bensmärtor efter endast kort gångsträcka och har kraftiga begränsningar i sin vardag trots att de får rätt mediciner och tränar aktivt kan vara aktuella för operation. Det finns olika metoder för att operera kärlen, ett alternativt är att göra en ballongvidgning (endovaskulär åtgärd) av en förträning (på liknande sätt som man gör i hjärtat). Detta görs genom att man för in en plastslang (kateter) i en artär via ljumsken, kirurgen använder sig då utav en röntgenmaskin för att lokalisera förträningen och då blåsa upp en ”ballong” med ”fjädrar” som återställer blodflödet genom förträningen. Fördelarna med denna metod är att det inte är ett lika komplicerat ingrepp som den klassiska kirurgin och oftast behöver man bara övernatta en natt på sjukhuset efter operationen. Den klassiska öppna kirurgin kan man göra genom att sy in ett nytt kärl och förbi leda blodet från det förträngda kärlet (bypass), fördelarna med denna metod är att den kan ha bättre resultat på långt skikt.

Diabetes är en vanlig folksjukdom i vårt samhälle, där diabetes typ 2 är den vanligaste och står för cirka 90% av alla diabetesfallen. Vid diabetes har man för mycket socker i blodet, vid diabetes typ 1 orsakas detta av otillräcklig

produktion av insulin (ett hormon som gör så att sockret går in i cellerna) och vid typ 2 orsakas det av resistens mot insulin, dvs att insulinet har en sämre verkan. Diabetes kan på sikt öka risken för andra sjukdomar så som åderförkalkning, hjärtinfarkt, stroke, skador på ögats näthinna, njurskador och skador på nerver. Diabetes diagnostiseras relativt enkelt med hjälp av ett blodprov som visar kroppens blodsockernivåer. Det finns flera olika typer av behandling mot diabetes, principerna bakom dessa behandlingar är att man försöker sänka blodsockret genom att tex få kroppen att ta upp mindre socker från maten, att skapa mindre socker och att utsöndra socker i urinen.

En biomarkör är ofta ett blodprov på ett ämne som kan tala för ökad risk att utveckla en sjukdom eller indikera en sjukdom. Biomarkörer används redan i sjukvården och bland de vanligaste är snabbsänkan (CRP) som vanligtvis tas i primärvården för att särskilja mellan tex lunginflammation och förkylning. Det finns vetenskapliga studier som tyder på att biomarkörer även kan användas för att undersöka om en individ har en ökad risk till att utveckla BAS. Tidig upptäckt av en individ med BAS eller vid hög risk till att utveckla BAS leder till att man redan vid ett tidigt skede kan inleda medicinsk behandling och minska risken för att individen ska drabbas av hjärtinfarkt eller stroke.

Målen med den här avhandlingen är att undersöka om individer med diabetes har ökad risk till att drabbas av komplikationer efter endovaskulär och öppen kirurgi för claudicatio. Vi har även som mål att finna nya biomarkörer som ska kunna användas för tidigt upptäckt av BAS.

Långtidsuppföljning visade att individer med diabetes ökad risk för hjärtinfarkt, stroke och benamputation efter att endovaskulär kirurgi. Långtidsuppföljning visade även att individer med diabetes hade ökad risk för att drabbas av hjärtinfarkt och stroke efter öppen kirurgi.

Vi upptäckte att förhöjda blodvärden av olika biomarkörer (C-terminal endothelin-1, N-Terminal prosomatostatin, midregional proatrial natriuretic peptide) kunde både påvisa BAS och upptäcka individer med ökad risk för att utveckla BAS. Förhöjda nivåer av C-terminal endothelin-1, N-Terminal prosomatostatin, midregional proatrial natriuretic peptide, procalcitonin och copeptin talade även för ökad dödlighet under 11 års uppföljning. Vi fann att individer med BAS hade högre nivåer av 11 olika biomarkörer (secretoglobin family 3A member 2, osteoprotegerin, urokinase-type plasminogen activator

surface receptor, serum macrophage chemokine ligand 16, matrix metalloproteinase 9, p-selectin, growth differentiation factor 15, elafin, cystatin B, trefoil factor 3 och fatty acid-binding protein 4) jämfört med individer utan BAS.

Sammanfattningsvis visar denna avhandling att individer med diabetes och claudicatio bör erbjudas operation i samma utsträckning som de utan diabetes. Långtidsuppföljningen visade att patienter med diabetes drabbades av mer hjärt- och kärlsjukdomar än de utan diabetes, vilket stärker vikten av god diabetesbehandling för att minska dessa risker. Vi har även identifierat flertalet biomarkörer som eventuellt kan användas som redskap för att utvärdera vilka som kommer att utveckla framtida BAS.

Acknowledgments

This thesis would not have been possible without the collaboration, support, guidance, and contribution of numerous people through both my clinical and academic journey. The people I would like to give special thanks to are:

Professor **Anders Gottsäter,** my principal supervisor and role model when it comes to research. I sincerely want to thank you for introducing me to research and your continuous support, and guidance throughout all the years I have been on my research journey. I am thankful for all the hours you have put into teaching me how to research effectively, how to improve my English and how to write better.

Professor **Stefan Acosta**, my co-supervisor for being so kind and supportive. I will always be amazed by how knowledgeable and humble you are, and I aspire to be like you in that regard.

Associate Professor **Moncef Zarrouk,** not only have you been of great help in my research endeavours, but you have also continuously guided and supported me in my clinical work, even at times I have not worked at the vascular centre. In you I see an inspiration and a friend.

Thank you, all my **co-authors**, for all the work they have put into each paper, all the valuable input has been greatly appreciated.

I want to thank my wife **Sedra** for all your love and support, helping me get through the countless hours spent working on my thesis. You have never failed to cheer me up when I have been feeling down and you never have or will seize to amaze me.

I want to thank my family. My mother **Areej** for always believing in my and being there when I need her, you are the reason to why I started researching in the first place. My father **Mutaz** for always guiding me into taking the right decisions in life and reminding me that every obstacle can be overcome. My brother **Nofel** for all the fond memories from our childhood and all your support and encouragement. Thank you, **Naisa**, **Carmen**, and **Lucas**, for being the best additions to the family that I can wish for. My **grandparents** that despite living in Australia I have always felt connected to and always had their support. Thank you to my **in-laws** for your support, it has been a

pleasure getting to know you. To all my **friends** that I have gotten to know over the years, you have helped shape me into the person I am today.

References

1. Criqui MH and Aboyans V. Epidemiology of peripheral artery disease. *Circ Res* 2015; 116: 1509-1526. 2015/04/25. DOI: 10.1161/CIRCRESAHA.116.303849.
2. Lin J, Chen Y, Jiang N, et al. Burden of Peripheral Artery Disease and Its Attributable Risk Factors in 204 Countries and Territories From 1990 to 2019. *Front Cardiovasc Med* 2022; 9: 868370. 2022/05/03. DOI: 10.3389/fcvm.2022.868370.
3. Song P, Rudan D, Zhu Y, et al. Global, regional, and national prevalence and risk factors for peripheral artery disease in 2015: an updated systematic review and analysis. *Lancet Glob Health* 2019; 7: e1020-e1030. 2019/07/16. DOI: 10.1016/S2214-109X(19)30255-4.
4. Fowkes FG, Rudan D, Rudan I, 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: 1329-1340. 2013/08/07. DOI: 10.1016/S0140-6736(13)61249-0.
5. Sigvant B, Wiberg-Hedman K, Bergqvist D, et al. A population-based study of peripheral arterial disease prevalence with special focus on critical limb ischemia and sex differences. *J Vasc Surg* 2007; 45: 1185-1191. 2007/06/05. DOI: 10.1016/j.jvs.2007.02.004.
6. Libby P, Buring JE, Badimon L, et al. Atherosclerosis. *Nat Rev Dis Primers* 2019; 5: 56. 2019/08/20. DOI: 10.1038/s41572-019-0106-z.
7. Weber C and Noels H. Atherosclerosis: current pathogenesis and therapeutic options. *Nat Med* 2011; 17: 1410-1422. 2011/11/09. DOI: 10.1038/nm.2538.
8. Norgren L, Hiatt WR, Dormandy JA, et al. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). *J Vasc Surg* 2007; 45 Suppl S: S5-67. 2007/01/16. DOI: 10.1016/j.jvs.2006.12.037.
9. Wang W, Zhao T, Geng K, et al. Smoking and the Pathophysiology of Peripheral Artery Disease. *Front Cardiovasc Med* 2021; 8: 704106. 2021/09/14. DOI: 10.3389/fcvm.2021.704106.
10. Siasos G, Tsigkou V, Kokkou E, et al. Smoking and atherosclerosis: mechanisms of disease and new therapeutic approaches. *Curr Med Chem* 2014; 21: 3936-3948. 2014/09/02. DOI: 10.2174/092986732134141015161539.

11. United States Public Health Service Office of the Surgeon General; National Center for Chronic Disease Prevention and Health Promotion (US) Office on Smoking and Health. *Chapter 4, The Health Benefits of Smoking Cessation*. https://www.ncbi.nlm.nih.gov/books/NBK555590/: Washington (DC): US Department of Health and Human Services, 2020.
12. Nordanstig J, Behrendt CA, Bradbury AW, et al. Peripheral arterial disease (PAD) - A challenging manifestation of atherosclerosis. *Prev Med* 2023; 171: 107489. 2023/04/10. DOI: 10.1016/j.ypmed.2023.107489.
13. folkhalsomyndigheten. https://www.folkhalsomyndigheten.se/livsvillkor-levnadsvanor/andts/utveckling-inom-andts-anvandning-och-ohalsa/anvandning/anvandning-av-tobaks-och-nikotinprodukter/vuxnas-bruk-av-tobaks--och-nikotinprodukter/.
14. Van't Hof JR, Wang W, Matsushita K, et al. Association of Smokeless Tobacco Use With Incident Peripheral Artery Disease: Results From the Atherosclerotic Risk in Communities Study. *Am J Prev Med* 2023; 64: 728-733. 2023/01/23. DOI: 10.1016/j.amepre.2023.01.001.
15. Mancia Chairperson G, Kreutz Co-Chair R, Brunstrom M, 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 Endorsed by the European Renal Association (ERA) and the International Society of Hypertension (ISH). *J Hypertens* 2023 2023/06/22. DOI: 10.1097/HJH.0000000000003480.
16. McDermott MM, Guralnik JM, Ferrucci L, et al. Asymptomatic peripheral arterial disease is associated with more adverse lower extremity characteristics than intermittent claudication. *Circulation* 2008; 117: 2484-2491. 2008/05/07. DOI: 10.1161/CIRCULATIONAHA.107.736108.
17. Zemaitis MR, Boll JM and Dreyer MA. Peripheral Arterial Disease. *StatPearls*. Treasure Island (FL), 2022.
18. Hamburg NM and Balady GJ. Exercise rehabilitation in peripheral artery disease: functional impact and mechanisms of benefits. *Circulation* 2011; 123: 87-97. 2011/01/05. DOI: 10.1161/CIRCULATIONAHA.109.881888.
19. McDermott MM. Lower extremity manifestations of peripheral artery disease: the pathophysiologic and functional implications of leg ischemia. *Circ Res* 2015; 116: 1540-1550. 2015/04/25. DOI: 10.1161/CIRCRESAHA.114.303517.
20. Conte MS, Bradbury AW, Kolh P, et al. Global Vascular Guidelines on the Management of Chronic Limb-Threatening Ischemia. *Eur J Vasc Endovasc Surg* 2019; 58: S1-S109 e133. 2019/06/12. DOI: 10.1016/j.ejvs.2019.05.006.
21. Abu Dabrh AM, Steffen MW, Undavalli C, et al. The natural history of untreated severe or critical limb ischemia. *J Vasc Surg* 2015; 62: 1642-1651 e1643. 2015/09/24. DOI: 10.1016/j.jvs.2015.07.065.

22. Xu D, Zou L, Xing Y, et al. Diagnostic value of ankle-brachial index in peripheral arterial disease: a meta-analysis. *Can J Cardiol* 2013; 29: 492-498. 2012/08/29. DOI: 10.1016/j.cjca.2012.06.014.
23. Wilkes S, Stansby G, Sims A, et al. Peripheral arterial disease: diagnostic challenges and how photoplethysmography may help. *Br J Gen Pract* 2015; 65: 323-324. 2015/05/27. DOI: 10.3399/bjgp15X685489.
24. Aboyans V, Criqui MH, Abraham P, et al. Measurement and interpretation of the ankle-brachial index: a scientific statement from the American Heart Association. *Circulation* 2012; 126: 2890-2909. 2012/11/20. DOI: 10.1161/CIR.0b013e318276fbcb.
25. Ankle Brachial Index C, Fowkes FG, Murray GD, et al. Ankle brachial index combined with Framingham Risk Score to predict cardiovascular events and mortality: a meta-analysis. *JAMA* 2008; 300: 197-208. 2008/07/10. DOI: 10.1001/jama.300.2.197.
26. Aboyans V, Ricco JB, Bartelink MEL, 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 arteriesEndorsed 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* 2018; 39: 763-816. 2017/09/10. DOI: 10.1093/eurheartj/ehx095.
27. Society for Vascular Surgery Lower Extremity Guidelines Writing G, Conte MS, Pomposelli FB, et al. Society for Vascular Surgery practice guidelines for atherosclerotic occlusive disease of the lower extremities: management of asymptomatic disease and claudication. *J Vasc Surg* 2015; 61: 2S-41S. 2015/02/02. DOI: 10.1016/j.jvs.2014.12.009.
28. Jansen-Chaparro S, Lopez-Carmona MD, Cobos-Palacios L, et al. Statins and Peripheral Arterial Disease: A Narrative Review. *Front Cardiovasc Med* 2021; 8: 777016. 2021/12/10. DOI: 10.3389/fcvm.2021.777016.
29. Sigvant B, Lundin F and Wahlberg E. The Risk of Disease Progression in Peripheral Arterial Disease is Higher than Expected: A Meta-Analysis of Mortality and Disease Progression in Peripheral Arterial Disease. *Eur J Vasc Endovasc Surg* 2016; 51: 395-403. 2016/01/19. DOI: 10.1016/j.ejvs.2015.10.022.
30. Kumbhani DJ, Steg PG, Cannon CP, et al. Statin therapy and long-term adverse limb outcomes in patients with peripheral artery disease: insights from the REACH registry. *Eur Heart J* 2014; 35: 2864-2872. 2014/03/04. DOI: 10.1093/eurheartj/ehu080.
31. Oyama K, Giugliano RP, Tang M, et al. Effect of evolocumab on acute arterial events across all vascular territories : results from the FOURIER trial. *Eur Heart J* 2021; 42: 4821-4829. 2021/09/20. DOI: 10.1093/eurheartj/ehab604.

32. Williams B, Mancia G, Spiering W, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. *Eur Heart J* 2018; 39: 3021-3104. 2018/08/31. DOI: 10.1093/eurheartj/ehy339.
33. Ostergren J, Sleight P, Dagenais G, et al. Impact of ramipril in patients with evidence of clinical or subclinical peripheral arterial disease. *Eur Heart J* 2004; 25: 17-24. 2003/12/20. DOI: 10.1016/j.ehj.2003.10.033.
34. Fowkes FG, Price JF, Stewart MC, 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: 841-848. 2010/03/04. DOI: 10.1001/jama.2010.221.
35. Belch J, MacCuish A, Campbell I, 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. 2008/10/18. DOI: 10.1136/bmj.a1840.
36. Twine CP, Kakkos SK, Aboyans V, et al. European Society for Vascular Surgery (ESVS) 2023 Clinical Practice Guidelines on Antithrombotic Therapy for Vascular Diseases. *Eur J Vasc Endovasc Surg* 2023 2023/04/06. DOI: 10.1016/j.ejvs.2023.03.042.
37. Committee CS. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). CAPRIE Steering Committee. *Lancet* 1996; 348: 1329-1339. 1996/11/16. DOI: 10.1016/s0140-6736(96)09457-3.
38. Eikelboom JW, Connolly SJ, Bosch J, et al. Rivaroxaban with or without Aspirin in Stable Cardiovascular Disease. *N Engl J Med* 2017; 377: 1319-1330. 2017/08/29. DOI: 10.1056/NEJMoa1709118.
39. Bonaca MP, Bauersachs RM, Anand SS, et al. Rivaroxaban in Peripheral Artery Disease after Revascularization. *N Engl J Med* 2020; 382: 1994-2004. 2020/03/30. DOI: 10.1056/NEJMoa2000052.
40. Khuri SF, Henderson WG, DePalma RG, et al. Determinants of long-term survival after major surgery and the adverse effect of postoperative complications. *Ann Surg* 2005; 242: 326-341; discussion 341-323. 2005/09/02. DOI: 10.1097/01.sla.0000179621.33268.83.
41. Vartanian SM and Conte MS. Surgical intervention for peripheral arterial disease. *Circ Res* 2015; 116: 1614-1628. 2015/04/25. DOI: 10.1161/CIRCRESAHA.116.303504.
42. Gerhard-Herman MD, Gornik HL, Barrett C, et al. 2016 AHA/ACC Guideline on the Management of Patients With Lower Extremity Peripheral Artery Disease: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation* 2017; 135: e686-e725. 2016/11/15. DOI: 10.1161/CIR.0000000000000470.

43. Sitlinger A and Zafar SY. Health-Related Quality of Life: The Impact on Morbidity and Mortality. *Surg Oncol Clin N Am* 2018; 27: 675-684. 2018/09/15. DOI: 10.1016/j.soc.2018.05.008.

44. Gunnarsson T, Bergman S, Parsson H, et al. Long Term Results of a Randomised Trial of Stenting of the Superficial Femoral Artery for Intermittent Claudication. *Eur J Vasc Endovasc Surg* 2023; 65: 513-519. 2023/01/16. DOI: 10.1016/j.ejvs.2023.01.004.

45. Lindgren H, Qvarfordt P, Akesson M, et al. Primary Stenting of the Superficial Femoral Artery in Intermittent Claudication Improves Health Related Quality of Life, ABI and Walking Distance: 12 Month Results of a Controlled Randomised Multicentre Trial. *Eur J Vasc Endovasc Surg* 2017; 53: 686-694. 2017/04/05. DOI: 10.1016/j.ejvs.2017.01.026.

46. Lindgren HIV, Qvarfordt P, Bergman S, et al. Primary Stenting of the Superficial Femoral Artery in Patients with Intermittent Claudication Has Durable Effects on Health-Related Quality of Life at 24 Months: Results of a Randomized Controlled Trial. *Cardiovasc Intervent Radiol* 2018; 41: 872-881. 2018/03/10. DOI: 10.1007/s00270-018-1925-0.

47. An J, Nichols GA, Qian L, et al. Prevalence and incidence of microvascular and macrovascular complications over 15 years among patients with incident type 2 diabetes. *BMJ Open Diabetes Res Care* 2021; 9 2021/01/06. DOI: 10.1136/bmjdrc-2020-001847.

48. Collins BH. Transplantation, Bioengineering, and Regeneration of the Endocrine Pancreas Eds: Giuseppe Orlando, Lorenzo Piemonti, Camillo Ricordi, Robert J. Stratta Rainer W.G. Gruessner Cambridge, MA: Academic Press, 2020; Vol. 1, 865 pages, Vol. 2, 546 pages. *Am J Transplant* 2020 2020/09/25. DOI: 10.1111/ajt.16317.

49. Huang D, Refaat M, Mohammedi K, et al. Macrovascular Complications in Patients with Diabetes and Prediabetes. *Biomed Res Int* 2017; 2017: 7839101. 2017/12/15. DOI: 10.1155/2017/7839101.

50. Vinik AI, Erbas T, Park TS, et al. Platelet dysfunction in type 2 diabetes. *Diabetes Care* 2001; 24: 1476-1485. 2001/07/27. DOI: 10.2337/diacare.24.8.1476.

51. Viigimaa M, Sachinidis A, Toumpourleka M, et al. Macrovascular Complications of Type 2 Diabetes Mellitus. *Curr Vasc Pharmacol* 2020; 18: 110-116. 2019/04/10. DOI: 10.2174/1570161117666190405165151.

52. Vithian K and Hurel S. Microvascular complications: pathophysiology and management. *Clin Med (Lond)* 2010; 10: 505-509. 2010/12/02. DOI: 10.7861/clinmedicine.10-5-505.

53. Hageman D, Gommans LN, Scheltinga MR, et al. Effect of diabetes mellitus on walking distance parameters after supervised exercise therapy for intermittent claudication: A systematic review. *Vasc Med* 2017; 22: 21-27. 2016/12/03. DOI: 10.1177/1358863X16674071.

54. Nauck MA, Wefers J and Meier JJ. Treatment of type 2 diabetes: challenges, hopes, and anticipated successes. *Lancet Diabetes Endocrinol* 2021; 9: 525-544. 2021/06/29. DOI: 10.1016/S2213-8587(21)00113-3.

55. Läkemedelsverket If. tt förebygga aterosklerotisk hjärt-kärlsjukdom med läkemedel – behandlingsrekommendation, https://www.lakemedelsverket.se/sv/behandling-och-forskrivning/behandlingsrekommendationer/sok-behandlingsrekommendationer/lakemedel-for-glukoskontroll-vid-typ-2-diabetes---behandlingsrekommendation#hmainbody1 (2017).

56. Cosentino F, Grant PJ, Aboyans V, et al. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. *Eur Heart J* 2020; 41: 255-323. 2019/09/10. DOI: 10.1093/eurheartj/ehz486.

57. Sanchez-Rangel E and Inzucchi SE. Metformin: clinical use in type 2 diabetes. *Diabetologia* 2017; 60: 1586-1593. 2017/08/05. DOI: 10.1007/s00125-017-4336-x.

58. Padda IS, Mahtani AU and Parmar M. Sodium-Glucose Transport Protein 2 (SGLT2) Inhibitors. *StatPearls*. Treasure Island (FL), 2022.

59. Marchiori E, Rodionov RN, Peters F, et al. SGLT2 Inhibitors and Peripheral Vascular Events: A Review of the Literature. *Heart Fail Clin* 2022; 18: 609-623. 2022/10/11. DOI: 10.1016/j.hfc.2022.03.001.

60. Liarakos AL, Tentolouris A, Kokkinos A, et al. 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. 2023/01/08. DOI: 10.1016/j.jdiacomp.2022.108390.

61. Yoshiji S, Minamino H, Tanaka D, et al. Effects of glucagon-like peptide-1 receptor agonists on cardiovascular and renal outcomes: A meta-analysis and meta-regression analysis. *Diabetes Obes Metab* 2022; 24: 1029-1037. 2022/02/10. DOI: 10.1111/dom.14666.

62. Golledge J. Abdominal aortic aneurysm: update on pathogenesis and medical treatments. *Nat Rev Cardiol* 2019; 16: 225-242. 2018/11/18. DOI: 10.1038/s41569-018-0114-9.

63. Wanhainen A, Verzini F, Van Herzeele I, 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: 8-93. 2018/12/12. DOI: 10.1016/j.ejvs.2018.09.020.

64. Ying AJ and Affan ET. Abdominal Aortic Aneurysm Screening: A Systematic Review and Meta-analysis of Efficacy and Cost. *Ann Vasc Surg* 2019; 54: 298-303 e293. 2018/08/07. DOI: 10.1016/j.avsg.2018.05.044.

65. Wanhainen A, Hultgren R, Linne A, et al. Outcome of the Swedish Nationwide Abdominal Aortic Aneurysm Screening Program. *Circulation* 2016; 134: 1141-1148. 2016/10/19. DOI: 10.1161/CIRCULATIONAHA.116.022305.

66. Benson RA, Poole R, Murray S, et al. Screening results from a large United Kingdom abdominal aortic aneurysm screening center in the context of optimizing United Kingdom National Abdominal Aortic Aneurysm Screening Programme protocols. *J Vasc Surg* 2016; 63: 301-304. 2015/10/21. DOI: 10.1016/j.jvs.2015.08.091.
67. Califf RM. Biomarker definitions and their applications. *Exp Biol Med (Maywood)* 2018; 243: 213-221. 2018/02/07. DOI: 10.1177/1535370217750088.
68. Ziegler L, Hedin U and Gottsater A. Circulating Biomarkers in Lower Extremity Artery Disease. *Eur Cardiol* 2022; 17: e09. 2022/04/12. DOI: 10.15420/ecr.2021.58.
69. Barton M and Yanagisawa M. Endothelin: 30 Years From Discovery to Therapy. *Hypertension* 2019; 74: 1232-1265. 2019/11/05. DOI: 10.1161/HYPERTENSIONAHA.119.12105.
70. Martinez V. *Handbook of Biologically Active Peptides*. Second Edition ed.: Academic Press, 2013.
71. O'Toole TJ SS. *Somatostatin*. StatPearls, 2022.
72. Sandefur CC J. *Atrial Natriuretic Peptide*. StatPearls 2022
73. Song W, Wang H and Wu Q. Atrial natriuretic peptide in cardiovascular biology and disease (NPPA). *Gene* 2015; 569: 1-6. 2015/06/16. DOI: 10.1016/j.gene.2015.06.029.
74. Cleland DA EA. *Procalcitonin*. StatPearls Publishing, 2022
75. Schuetz P, Daniels LB, Kulkarni P, et al. Procalcitonin: A new biomarker for the cardiologist. *Int J Cardiol* 2016; 223: 390-397. 2016/08/21. DOI: 10.1016/j.ijcard.2016.08.204.
76. Mueller C, Mockel M, Giannitsis E, et al. Use of copeptin for rapid rule-out of acute myocardial infarction. *Eur Heart J Acute Cardiovasc Care* 2018; 7: 570-576. 2017/06/09. DOI: 10.1177/2048872617710791.
77. Kim SK, Seok H, Park HJ, et al. Association Between Secretoglobin Family 3A Member 2 (SCGB3A2) Gene Polymorphisms and Asthma in a Korean Population. *Med Sci Monit* 2017; 23: 1880-1885. 2017/04/20. DOI: 10.12659/msm.903983.
78. Knudsen ST, Foss CH, Poulsen PL, et al. Increased plasma concentrations of osteoprotegerin in type 2 diabetic patients with microvascular complications. *Eur J Endocrinol* 2003; 149: 39-42. 2003/06/26. DOI: 10.1530/eje.0.1490039.
79. Demkova K, Kozarova M, Malachovska Z, et al. Osteoprotegerin concentration is associated with the presence and severity of peripheral arterial disease in type 2 diabetes mellitus. *Vasa* 2018; 47: 131-135. 2018/01/10. DOI: 10.1024/0301-1526/a000682.

80. Kiyan Y, Limbourg A, Kiyan R, et al. Urokinase receptor associates with myocardin to control vascular smooth muscle cells phenotype in vascular disease. *Arterioscler Thromb Vasc Biol* 2012; 32: 110-122. 2011/11/15. DOI: 10.1161/ATVBAHA.111.234369.

81. Samman Tahhan A, Hayek SS, Sandesara P, et al. Circulating soluble urokinase plasminogen activator receptor levels and peripheral arterial disease outcomes. *Atherosclerosis* 2017; 264: 108-114. 2017/07/22. DOI: 10.1016/j.atherosclerosis.2017.06.019.

82. Lehrke M, Millington SC, Lefterova M, et al. CXCL16 is a marker of inflammation, atherosclerosis, and acute coronary syndromes in humans. *J Am Coll Cardiol* 2007; 49: 442-449. 2007/01/30. DOI: 10.1016/j.jacc.2006.09.034.

83. Jin G. The relationship between serum CXCL16 level and carotid vulnerable plaque in patients with ischemic stroke. *Eur Rev Med Pharmacol Sci* 2017; 21: 3911-3915. 2017/10/05.

84. Tayebjee MH, Tan KT, MacFadyen RJ, et al. Abnormal circulating levels of metalloprotease 9 and its tissue inhibitor 1 in angiographically proven peripheral arterial disease: relationship to disease severity. *J Intern Med* 2005; 257: 110-116. 2004/12/21. DOI: 10.1111/j.1365-2796.2004.01431.x.

85. Merten M and Thiagarajan P. P-selectin in arterial thrombosis. *Z Kardiol* 2004; 93: 855-863. 2004/11/30. DOI: 10.1007/s00392-004-0146-5.

86. Signorelli SS, Anzaldi M, Libra M, et al. Plasma Levels of Inflammatory Biomarkers in Peripheral Arterial Disease: Results of a Cohort Study. *Angiology* 2016; 67: 870-874. 2016/02/19. DOI: 10.1177/0003319716633339.

87. De Haan JJ, Haitjema S, den Ruijter HM, et al. Growth Differentiation Factor 15 Is Associated With Major Amputation and Mortality in Patients With Peripheral Artery Disease. *J Am Heart Assoc* 2017; 6 2017/09/01. DOI: 10.1161/JAHA.117.006225.

88. Alam SR, Newby DE and Henriksen PA. Role of the endogenous elastase inhibitor, elafin, in cardiovascular injury: from epithelium to endothelium. *Biochem Pharmacol* 2012; 83: 695-704. 2011/11/22. DOI: 10.1016/j.bcp.2011.11.003.

89. Goncalves I, Hultman K, Duner P, et al. High levels of cathepsin D and cystatin B are associated with increased risk of coronary events. *Open Heart* 2016; 3: e000353. 2016/02/06. DOI: 10.1136/openhrt-2015-000353.

90. Yang Y, Lin Z, Lin Q, et al. Pathological and therapeutic roles of bioactive peptide trefoil factor 3 in diverse diseases: recent progress and perspective. *Cell Death Dis* 2022; 13: 62. 2022/01/19. DOI: 10.1038/s41419-022-04504-6.

91. Liu SQ, Tefft BJ, Roberts DT, et al. Cardioprotective proteins upregulated in the liver in response to experimental myocardial ischemia. *Am J Physiol Heart Circ Physiol* 2012; 303: H1446-1458. 2012/10/16. DOI: 10.1152/ajpheart.00362.2012.
92. Hobaus C, Herz CT, Pesau G, et al. FABP4 and Cardiovascular Events in Peripheral Arterial Disease. *Angiology* 2018; 69: 424-430. 2017/08/30. DOI: 10.1177/0003319717728226.
93. Vats S, Sundquist K, Wang X, 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: 137-142. 2020/07/01. DOI: 10.1016/j.ijcard.2020.06.022.
94. Lindberg S, Zarrouk M, Holst J, et al. Inflammatory markers associated with abdominal aortic aneurysm. *Eur Cytokine Netw* 2016; 27: 75-80. 2016/12/03. DOI: 10.1684/ecn.2016.0381.
95. Sigvant B, Mani, K. & Björck, M. The Swedish vascular registry Swedvasc 1987–2018. *Gefässchirurgie* 2019. DOI: https://doi.org/10.1007/s00772-018-0473-x.
96. Venermo M and Lees T. International Vascunet Validation of the Swedvasc Registry. *Eur J Vasc Endovasc Surg* 2015; 50: 802-808. 2015/09/05. DOI: 10.1016/j.ejvs.2015.07.021.
97. Troeng T, Malmstedt J and Bjorck M. External validation of the Swedvasc registry: a first-time individual cross-matching with the unique personal identity number. *Eur J Vasc Endovasc Surg* 2008; 36: 705-712. 2008/10/15. DOI: 10.1016/j.ejvs.2008.08.017.
98. Nationella, kvalitetsregistret and kärlkirurgi f. Årsrapport för 2021, https://www.ucr.uu.se/swedvasc/arsrapporter/swedvasc-2022/viewdocument/416 (2022).
99. Swedvasc. Årsrapporter, https://www.ucr.uu.se/swedvasc/arsrapporter.
100. Eliasson B and Gudbjornsdottir S. Diabetes care--improvement through measurement. *Diabetes Res Clin Pract* 2014; 106 Suppl 2: S291-294. 2015/01/01. DOI: 10.1016/S0168-8227(14)70732-6.
101. Diabetesregistret N. Årsrapport 2022, https://www.ndr.nu/#/arsrapport (2022).
102. Berglund G, Nilsson P, Eriksson KF, et al. Long-term outcome of the Malmo preventive project: mortality and cardiovascular morbidity. *J Intern Med* 2000; 247: 19-29. 2000/02/15. DOI: 10.1046/j.1365-2796.2000.00568.x.
103. Ludvigsson JF, Andersson E, Ekbom A, et al. External review and validation of the Swedish national inpatient register. *BMC Public Health* 2011; 11: 450. 2011/06/11. DOI: 10.1186/1471-2458-11-450.
104. Socialstyrelsen. National Prescribed Drug Register, https://www.socialstyrelsen.se/en/statistics-and-data/registers/national-prescribed-drug-register/ (2022).

105. socialstyrelsen. National Cancer Register, https://www.socialstyrelsen.se/en/statistics-and-data/registers/national-cancer-register/ (2019).
106. Johansson M, Ricci F, Di Martino G, et al. Cardiovascular biomarkers predict fragility fractures in older adults. *Heart* 2019; 105: 449-454. 2018/10/17. DOI: 10.1136/heartjnl-2018-313741.
107. Holm H, Nagga K, Nilsson ED, et al. N-Terminal Prosomatostatin and Risk of Vascular Dementia. *Cerebrovasc Dis* 2017; 44: 259-265. 2017/08/31. DOI: 10.1159/000479940.
108. Cotoi OS, Manjer J, Hedblad B, et al. Plasma procalcitonin is associated with all-cause and cancer mortality in apparently healthy men: a prospective population-based study. *BMC Med* 2013; 11: 180. 2013/08/14. DOI: 10.1186/1741-7015-11-180.
109. Assarsson E, Lundberg M, Holmquist G, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLoS One* 2014; 9: e95192. 2014/04/24. DOI: 10.1371/journal.pone.0095192.
110. Lundberg M, Eriksson A, Tran B, et al. Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of low-abundant proteins in human blood. *Nucleic Acids Res* 2011; 39: e102. 2011/06/08. DOI: 10.1093/nar/gkr424.
111. Lalani N, Jimenez RB and Yeap B. Understanding Propensity Score Analyses. *Int J Radiat Oncol Biol Phys* 2020; 107: 404-407. 2020/06/13. DOI: 10.1016/j.ijrobp.2020.02.638.
112. Han SM, Wu B, Eichler CM, et al. Risk Factors for 30-Day Hospital Readmission in Patients Undergoing Treatment for Peripheral Artery Disease. *Vasc Endovascular Surg* 2015; 49: 69-74. 2015/07/07. DOI: 10.1177/1538574415593498.
113. Bodewes TC, Soden PA, Ultee KH, et al. Risk factors for 30-day unplanned readmission following infrainguinal endovascular interventions. *J Vasc Surg* 2017; 65: 484-494 e483. 2017/01/28. DOI: 10.1016/j.jvs.2016.08.093.
114. Jones CE, Richman JS, Chu DI, et al. Readmission rates after lower extremity bypass vary significantly by surgical indication. *J Vasc Surg* 2016; 64: 458-464. 2016/10/21. DOI: 10.1016/j.jvs.2016.03.422.
115. Bodewes TCF, Darling JD, Deery SE, et al. Patient selection and perioperative outcomes of bypass and endovascular intervention as first revascularization strategy for infrainguinal arterial disease. *J Vasc Surg* 2018; 67: 206-216 e202. 2017/08/29. DOI: 10.1016/j.jvs.2017.05.132.
116. Butt T, Lilja E, Orneholm H, et al. Amputation-Free Survival in Patients With Diabetes Mellitus and Peripheral Arterial Disease With Heel Ulcer: Open Versus Endovascular Surgery. *Vasc Endovascular Surg* 2019; 53: 118-125. 2018/11/24. DOI: 10.1177/1538574418813746.

117. Parvar SL, Ngo L, Dawson J, et al. Long-term outcomes following endovascular and surgical revascularization for peripheral artery disease: a propensity score-matched analysis. *Eur Heart J* 2021; 43: 32-40. 2021/02/25. DOI: 10.1093/eurheartj/ehab116.
118. Baubeta Fridh E, Andersson M, Thuresson M, et al. Amputation Rates, Mortality, and Pre-operative Comorbidities in Patients Revascularised for Intermittent Claudication or Critical Limb Ischaemia: A Population Based Study. *Eur J Vasc Endovasc Surg* 2017; 54: 480-486. 2017/08/12. DOI: 10.1016/j.ejvs.2017.07.005.
119. Boye KS, Thieu VT, Lage MJ, et al. The Association Between Sustained HbA1c Control and Long-Term Complications Among Individuals with Type 2 Diabetes: A Retrospective Study. *Adv Ther* 2022; 39: 2208-2221. 2022/03/23. DOI: 10.1007/s12325-022-02106-4.
120. Butt T, Lilja E, Elgzyri T, et al. Amputation-free survival in patients with diabetic foot ulcer and peripheral arterial disease: Endovascular versus open surgery in a propensity score adjusted analysis. *J Diabetes Complications* 2020; 34: 107551. 2020/02/18. DOI: 10.1016/j.jdiacomp.2020.107551.
121. Kollerits B, Sturm G, Lamina C, et al. Comparison and evaluation of cardiac biomarkers in patients with intermittent claudication: results from the CAVASIC study. *Clin Chem* 2013; 59: 692-702. 2013/01/15. DOI: 10.1373/clinchem.2012.193656.
122. Lin WC, Tsai JP, Lai YH, et al. Serum osteoprotegerin level is positively associated with peripheral artery disease in patients with peritoneal dialysis. *Ren Fail* 2020; 42: 131-136. 2020/01/18. DOI: 10.1080/0886022X.2020.1714654.
123. Kadoglou NPE, Kapetanios D, Korakas E, et al. Association of serum levels of osteopontin and osteoprotegerin with adverse outcomes after endovascular revascularisation in peripheral artery disease. *Cardiovasc Diabetol* 2022; 21: 171. 2022/09/02. DOI: 10.1186/s12933-022-01605-6.
124. Wassel CL, Berardi C, Pankow JS, et al. Soluble P-selectin predicts lower extremity peripheral artery disease incidence and change in the ankle brachial index: the Multi-Ethnic Study of Atherosclerosis (MESA). *Atherosclerosis* 2015; 239: 405-411. 2015/02/16. DOI: 10.1016/j.atherosclerosis.2015.01.022.
125. Chen N, Chen L, Jiang S, et al. Predictive value of P-selectin and endothelin-1 for vascular restenosis after interventional procedures for peripheral artery disease. *Exp Ther Med* 2019; 17: 3907-3912. 2019/04/17. DOI: 10.3892/etm.2019.7407.
126. Vargas KG, Kassem M, Mueller C, et al. Copeptin for the early rule-out of non-ST-elevation myocardial infarction. *Int J Cardiol* 2016; 223: 797-804. 2016/08/31. DOI: 10.1016/j.ijcard.2016.08.304.
127. Alexandra Binnie JL, Claudia C. Dos Santos,. *Evidence-Based Practice of Critical Care*. third ed.: Elsevier, 2020.

128. Schuetz P, Birkhahn R, Sherwin R, et al. Serial Procalcitonin Predicts Mortality in Severe Sepsis Patients: Results From the Multicenter Procalcitonin MOnitoring SEpsis (MOSES) Study. *Crit Care Med* 2017; 45: 781-789. 2017/03/04. DOI: 10.1097/CCM.0000000000002321.
129. Jonelid 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: 353-360. 2021/08/05. DOI: 10.1080/00365513.2021.1921839.
130. Hedback T, Almgren P, Nilsson PM, et al. N-Terminal Prosomatostatin as a Risk Marker for Cardiovascular Disease and Diabetes in a General Population. *J Clin Endocrinol Metab* 2016; 101: 3437-3444. 2016/07/12. DOI: 10.1210/jc.2016-1736.
131. Zarrouk M, Holst J, Malina 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: 50-55. 2013/04/02. DOI: 10.1016/j.jvs.2012.12.080.
132. Djerf H, Hellman J, Baubeta Fridh E, et al. Low Risk of Procedure Related Major Amputation Following Revascularisation for Intermittent Claudication: A Population Based Study. *Eur J Vasc Endovasc Surg* 2020; 59: 817-822. 2019/12/24. DOI: 10.1016/j.ejvs.2019.11.023.
133. Bijlsma MJ, Janssen F, Bos J, et al. Brief Report: Association Between Statin Use and Cardiovascular Mortality at the Population Level: An Ecologic Study. *Epidemiology* 2015; 26: 802-805. 2015/08/27. DOI: 10.1097/EDE.0000000000000370.
134. Behrendt CA, Sigvant B, Kuchenbecker J, et al. Editor's Choice - International Variations and Sex Disparities in the Treatment of Peripheral Arterial Occlusive Disease: A Report from VASCUNET and the International Consortium of Vascular Registries. *Eur J Vasc Endovasc Surg* 2020; 60: 873-880. 2020/10/03. DOI: 10.1016/j.ejvs.2020.08.027.
135. Jorgensen ME, Kristensen JK, Reventlov Husted G, et al. The Danish Adult Diabetes Registry. *Clin Epidemiol* 2016; 8: 429-434. 2016/11/16. DOI: 10.2147/CLEP.S99518.
136. Ericsson A, Holst J, Gottsater A, et al. Psychosocial consequences in men taking part in a national screening program for abdominal aortic aneurysm. *J Vasc Nurs* 2017; 35: 211-220. 2017/11/21. DOI: 10.1016/j.jvn.2017.06.001.
137. Byrne RA, Rossello X, Coughlan JJ, et al. 2023 ESC Guidelines for the management of acute coronary syndromes. *Eur Heart J* 2023 2023/08/25. DOI: 10.1093/eurheartj/ehad191.
138. Läkemedelsverket If. Att förebygga aterosklerotisk hjärt-kärlsjukdom med läkemedel – behandlingsrekommendation. 2014. DOI: 014;25(5):20–33.

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Printed by Media-Tryck, Lund 2023 NORDIC SWAN ECOLABEL 3041 0903

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Department of Clinical Sciences, Malmö

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2023:136
ISBN 978-91-8021-478-0
ISSN 1652-8220