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HYBRID PET/CTA IMAGING FOR CORONARY ARTERY DISEASE

Studies on the effect of sex and genetic factors on diagnosis and outcome

Iida Kujala

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ABSTRACT

Coronary computed tomography angiography (CTA) enables non-invasive detection of coronary atherosclerosis and obstructive coronary artery disease (CAD). In turn, positron emission tomography angiography (PET) myocardial perfusion imaging (MPI) enables detection of hemodynamically significant coronary artery stenoses and myocardial ischemia. In hybrid imaging, data from coronary CTA and PET MPI are combined providing complementary information about coronary anatomy and physiology.

We have comprised an observational retrospective registry of patients who have been imaged in Turku PET Centre with a hybrid imaging protocol due suspected obstructive CAD.

We evaluated sex differences in disease profile and outcomes after hybrid imaging. We found that women have lower prevalence of ischemic CAD and lower rate of adverse events (unstable angina pectoris, myocardial infarction, death). Importantly, hybrid imaging predicted outcomes equally in women and men. We also investigated the incremental value of PET imaging over CTA in predicting short- and long-term outcomes. PET MPI improved the prediction of adverse events beyond CTA imaging for the first 4 years of follow-up. After this period MPI did not add prognostic power over CTA. This illustrates the complementary nature of anatomic and functional imaging in the prediction of outcome in patients with suspected CAD.

Above this, we collected blood samples from the registry patients and assessed the predictive power of the 3 most promising polygenic risk scores (PRS) in prediction of coronary atherosclerosis and obstructive CAD. We found that the addition of PRS to classical clinical risk factors did not clinically significantly improve the predictive accuracy for either coronary atherosclerosis or obstructive CAD, indicating that current PRSs are not justified for clinical routine for CAD.

KEYWORDS: coronary artery disease, hybrid imaging, polygenic risk score, prognosis

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TIIVISTELMÄ

Sepelvaltimoiden tietokonetomografia tutkimus (CTA) on kajoamaton ja tarkka kuvantamismenetelmä, jonka avulla voidaan luotettavasti poissulkea ahtauttava sepelvaltimotauti. CTA kuvantamisessa todettu ahtauma ei kuitenkaan aina ole hemodynaamisesti merkittävä. Positroniemissiontomografia (PET) mahdollistaa hemodynaamisesti merkittävien ahtaumien ja sydänlihasiskemian arvioimisen. Hybridikuvantamisessa yhdistämme nämä kaksi kuvantamismenetelmää saadaksemme tietoa sekä potilaan sepelvaltimoiden anatomiasta että tautimuutosten merkityksestä.

Olemme koostaneet havainnoivan retrospektiivisen rekisterin Turun PET-keskuksessa tutkituista potilaista, joilla on epäilty oireiden aiheuttajana ahtauttavaa sepelvaltimotautia.

Me tutkimme eroavaisuuksia hybridikuvantamisen ennustearvossa miesten ja naisten välillä, sekä eroavaisuuksia sepelvaltimotaudin luonteessa. Havaitsimme, että naisilla esiintyy miehiä harvemmin sydänlihaksen iskemiaa aiheuttavaa sepelvaltimotautia. Naisilla havaitsimme esiintyvän myös vähemmän päätetapahtumia (epästabiili angiina, sydäninfarkti, kuolema). Hybridi kuvantamisen tulokset ennustivat päätetapahtumia naisilla ja miehillä yhtäläisesti. Tutkimme myös PET kuvauksen tuomaa lisähyötyä ennustearvossa CTA kuvantamisen jälkeen. PET kuvantaminen toi lisäarvoa päätetapahtumien ennustamisessa ensimmäisen 4 vuoden seuranta-ajan aikana. Tämän jälkeen ennustearvossa ei havaittu eroa.

Tämän lisäksi keräsimme osalta potilaista verinäytteitä ja selvitimme geneettisten riskipisteytyksien tarkkuutta sepelvaltimotaudin todennäköisyyden arvioinnissa oireisilla potilailla. Totesimme, että geneettiset riskipisteytykset eivät tuoneet kliinisesti merkittävää lisäarvoa kliinisiin riskitekijöihin lisättynä. Tämän perusteella geneettisten riskipisteytysten käyttö ei ole perusteltua kliinisessä työssä sepelvaltimotaudin todennäköisyyttä arvioitaessa.

AVAINSANAT: sepelvaltimotauti, hybrikuvantaminen, geneettinen riskipisteytys, ennustearvo

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Abbreviations

AUC	area under curve
CABG	coronary artery bypass graft
CAD	coronary artery disease
CFR	coronary flow reserve
CMD	coronary microvascular dysfunction
CMR	Cardiovascular magnetic resonance
CTA	computed tomography angiogr q<zweaphy
ECG	electrocardiogram
FFR	fractional flow reserve
FH	familial hypercholesterolemia
GWAS	genome-wide association study
ICA	invasive coronary angiography
MBF	myocardial blood flow
NYHA	New York Heart Association
PCI	percutaneous coronary intervention
PET	positron emission tomography
PRS	polygenic risk score
SNP	single nucleotide polymorphism
SPECT	single-photon emission computerized tomography
ROC	receiver operating characteristic
OR	odds ratio

List of Original Publications

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

I Kujala I, Nammas W, Maaniitty T, Stenström I, Klén R, Bax J, Knuuti J, Saraste A. Prognostic value of combined coronary CT angiography and myocardial perfusion imaging in women and men. *European Heart Journal Cardiovascular Imaging.* 2023 Aug 23;24(9):1201-1209

II Lehtonen E*, Kujala I*, Tamminen J, Maaniitty T, Saraste A, Teuho J, Knuuti J, Klén R. Incremental prognostic value of downstream positron emission tomography perfusion imaging after coronary computed tomography angiography: a study using machine learning. *European Heart Journal Cardiovascular Imaging.* 2024 Jan 29;25(2):285-292
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III Kujala I, Vangipurapu J, Maaniitty T, Saraste A, Kere J, Knuuti J. Polygenic risk scores in predicting coronary artery disease in symptomatic patients. A validation study. *Journal of Atherosclerosis and thrombosis.* 2024 Feb 23. Doi:10.5551/jat.64623

1 Introduction

Coronary computed tomography angiography (CTA) is an accurate non-invasive imaging method to diagnose or exclude coronary atherosclerosis and obstructive coronary artery disease (CAD) (Budof et al. 2008). However, the degree of stenosis seen on coronary CTA doesn´t always match with the haemodynamic significance of the lesion (Mejboom et al., 2008). Positron emission tomography angiography (PET) perfusion imaging (MPI) enables detection of hemodynamically significant coronary artery stenoses and myocardial ischemia. In hybrid imaging, coronary CTA and PET MPI are combined to accurately assess anatomical changes as well as ischaemia. (Danad et al. 2017)

Coronary artery disease (CAD) is a leading cause of mortality and morbidity around globe (Virani et al. 2023). Accurate and early detection as well as appropriate risk evaluation is needed to guide treatment strategies and tailor therapy. Chronic CAD with stable symptoms can evolve to an acute coronary syndrome at any time. Thus, identification of high-risk patients is crucial.

This thesis is comprised of clinical retrospective studies, investigating the use of hybrid imaging in prognostic evaluation of CAD. As previously known, there are differences in prognostic performance between imaging modalities in men and women (Pagidipati et al, 2016; Mangion et al., 2020; Lubbers et al., 2017). We investigated the differences in prognostic value of hybrid imaging between both sexes. Above that, we assessed the incremental prognostic value of PET MPI after coronary CTA.

Strategies to identify patients with a higher likelihood of CAD are needed to target diagnostic testing appropriately. Currently, clinical risk scores incorporating clinical risk factors and presenting symptoms are used widely in clinical practice. In addition to these clinical factors, genetic risk scores have been shown to independently predict the development of CAD (Khera et al., 2018; Agbaedeng et al., 2021; Elliot et al., 2020; Mars et al., 2020; Inouye et al., 2018). In this thesis we evaluated the clinical benefit of risk stratifying patients with suspected obstructive CAD by their genetic risk.

2 Review of Literature

2.1 Coronary artery disease

2.1.1 Pathophysiology of CAD

Atherosclerosis is a progressive disease of medium- and large sized arteries. It is initiated by activation of the endothelium (inner cell layer of the artery), followed by cascade of events which leads to narrowing of the vessel lumen and activation of inflammatory pathways (Jebari-Benslaiman et al. 2022). Formation of atherosclerotic plaque along with these processes results in chronic ischemic cardiovascular diseases as well as acute complications. Atherosclerosis is the main cause of cardiovascular diseases. CAD is most common manifestation of atherosclerosis along with ischemic stroke and peripheral vascular disease in lower extremities (Bentzon et al., 2014). The clinical presentations of CAD include chronic and acute coronary syndromes (myocardial infarction (MI), unstable angina pectoris (UAP)) and sudden death.

Progression of the disease takes years to decades along with various stages of severity and symptoms (Insull 2009). The progression is mainly driven by cardiovascular risk factors, which include age, male sex, smoking history, hypertension, diabetes, hyperlipidemia, and family history of premature CAD (Wilson et al 1998.). Together these risk factors explain >90% of the occurrence of myocardial infarctions globally (Yusuf et al. 2004)

The vascular wall consists of three layers. The inner layer (intima) is formed by endothelial cells, collagen and elastic fibers and acts as a first barrier for molecules circulating in blood stream. The middle layer (tunica media) consists of smooth muscle cells with elastic and collagenous tissue. Finally, the outer layer (tunica adventitia) is mainly consisted of dense connective tissue. (Jebari-Benslaiman et al., 2022, Rhodin et al., 1968).

Atherosclerosis initiates when endothelial dysfunction leads to low-density lipoprotein (LDL) infiltration and retention in intima. High plasma levels of LDL are thought to be the major determinant of this process (Tabas et al. 2007). LDL undergoes various modifications in intima and the modified LDL stimulates pro-inflammatory cytokine production, which is responsible for capturing leukocytes

from bloodstream (Gimbrone et al. 2016). Once monocytes are captured and they invade intima they become either pro-inflammatory M1-like macrophages or M2-like anti-inflammatory macrophages (Leitinger et al. 2013). Once in the intima, macrophages act as deposit of lipids and become foam cells by mechanisms incompletely understood (Steinberg et al. 2009). When the foam cell formation has been going on long enough, finally we can see fatty streaks along the vessel walls (xanthomas). Xanthomas are still fully reversible and are a physiologic response to blood flow. Xanthomas are found as early as in neonates (Nakashima et al. 2002). However, they can develop into progressive atherosclerotic lesions if the cause of their formation does not dissipate (Bentzon et al. 2014). Growing lipid pools in the intima can lead to adaptive intimal thickening or can further grow into lipid-rich necrotic cores due apoptosis of the cells. This process is irreversible. The connective tissue around the plaques also changes gradually in the process. The former loose fibro cellular tissue is replaced and expanded by collagen-rich fibrotic tissue and becomes the dominant component of plaques (Kragel et al. 1989). Calcification of progressive plaques is common and increases with age. Calcification of the plaque can still expand the size of the plaque (Otsuka et al. 2014).

During the formation of atherosclerotic plaques, simultaneously vessel remodeling and autoregulation occurs to retain intra luminal cavity and blood flow. Once the atherosclerosis progresses and remodeling fails to address the growing plaques, stenosis occurs. Stenosis becomes flow-limiting usually when the luminal diameter is reduced by 50% or more (Duncker et al., 2015). Vasodilation works as a compensatory mechanism in flow-limiting stenosis. However, it becomes exhausted if the oxygen demand rises enough during exercise. When the luminal stenosis reaches 90% it becomes exhausted even at rest (Duncker et al., 2015). When the vessels fail to respond to the need of sufficient flow and oxygen demand of the myocardium, ischaemia occurs.

As the cascade of the events continue to go further, growing plaques may finally get vulnerable and hypoxia-induced neovascularization boosts this even further. The weak and destabilized fibrotic cap on the vulnerable plaque can lead to plaque rupture or erosion. Exposure of inner content of ruptured plaque to blood initiates platelet activation and aggregation resulting in occlusive thrombosis and its clinical complications, such as MI or UAP (Badimon et al. 2009, Fuster et al., 1988).

MI is defined pathologically as cell death in the myocardium due prolonged ischaemia and deprivation of oxygen. Clinically it is detected by circulating biomarkers of cardiomyocyte death in the setting of evidence of acute ischaemia (Thygesen 2018; Ojha 2022, Alpert et al., 2000). Patients may suffer from chest pain, discomfort in the neck, back or upper limbs. Similar symptoms can occur in unstable angina pectoris (UAP) which can be distinguished from stable angina pectoris by worsening symptoms or symptoms that doesn't completely resolve with rest or

nitrogen (Goyal et al. 2022). However, in UAP we do not see similar elevation of cardiac biomarkers as in MI. Thrombus in UAP is not occlusive but can develop into it and thus result in MI (Hombach et al. 1988). Similarly, to plaque rupture and thrombus formation, also vasospastic disease can manifest as MI and is an etiological differential diagnosis (Beijk et al. 2019).

2.1.2 Sex differences in disease profile and pathogenesis

Table 1. Sex differences in disease profile and pathogenesis.

Later onset of disease in women	Van rosendael et al., 2023; Crea et al. 2015
Atypical symptoms in women	Arslanian-Engoren et al 2006, Ilner et al 2005
Lower prevalence of obstructive CAD in women	Crea et al. 2015, Waheed et al 2020
Differences in plaque morphology	Burke et al.,1998; Reynolds et al.,2011; Lansky et al., 2012
Higher prevalence of CMD in women	Wong et al., 2002
Abnormal coronary reactivity in women	von Mering et al., 2004
Lower CFR in women	Kobayashi et al. 2015
Higher rest flow in women	Kobayashi et al. 2015
Hormonal differences	Iorga et al., 2017
More often non-atherosclerotic causes of acute coronary syndromes in women; vasospasm, dissection, stress-induced cardiomyopathy	Ruiz-Garcia et al., 2012

Cardiovascular diseases are the major cause of death in both sexes (Maas et al. 2011). Over past decades, there is growing evidence on differences in prevalence, characteristics, and outcomes of CAD between men and women.

Women present acute or chronic CAD typically at an older age than men (Van rosendael et al., 2023; Crea et al. 2015). In middle aged women and men, it was found in swedish SCAPIS study that women typically have better cardiovascular health than men (Higueras-Fresnillo et al., 2023). Women are also more likely to experience atypical symptoms such as fatigue, dyspnea, and indigestion (Arslanian-Engoren et al 2006, Ilner et al 2005). In invasive and non-invasive imaging, it is seen that CAD characteristics differ between sexes. Women with chronic CAD have less frequently obstructive and extensive CAD than men (Crea et al. 2015, Waheed et al 2020). Symptomatic women are more likely to have normal coronary angiography than men (Sullivan et al 1994), which is also seen after confirmed MI (Hvelplund et al 2010). The absence of obstructive CAD in angiography is seen in 10-25% of women during acute coronary syndrome while the occurrence in men is only 6-10% (Bugiardini et al., 2005; Hochman et al., 1999; Hochman et al., 1997; Andersson et

al., 2007). Yet, women with ischaemic disease have similar (Benjamin et al. 2018, Taqueti 2017) or even worse outcome than men (Van Rosendael et al., 2023; Baldassarre et al. 2016, Izadnegahdar et al 2016, Parvand et al 2018). Some studies have also reported that plaque morphology differs between sexes and acute coronary syndromes are present among women more often than men via plaque erosion/distal microembolization rather than rupture as causative to female-specific CAD pathophysiology (Burke et al.,1998; Reynolds et al.,2011; Lansky et al., 2012). However, studies are inconclusive as some are showing no significant difference (Chia et al. 2007; Bharadwaj et al., 2016).

The inconsistency between unobstructed arteries and poor prognosis in women have been explained with differences in the pathophysiology of women´s CAD. Those include the higher prevalence of coronary microvascular dysfunction (CMD) (Wong et al., 2002), abnormal coronary reactivity (von Mering et al., 2004) and lower coronary flow reserve (CFR) (Kobayashi et al. 2015). The coronary microvasculature alters the luminal cavity and flow by vasoconstriction and -dilatation (Shaw et al. 2016). Dysregulation of this system is referred to as CMD, which refers to whole subset of disorders independent from epicardial CAD. Classical risk factors such as smoking, age and hypertension may be associated with CMD (Mygind et al. 2016) for which women are more sensitive to, because of lower microvascular arterial compliance (Coutinho et al. 2018). It has been noted that CMD can induce ischaemia and even non-ST-elevation infarction independent of obstructive CAD (Shinha et al., 2020). Women are reported having more often impaired myocardial perfusion due CMD than men (Reis et al. 2001, Murthy et al., 2014). Also, non-atherosclerotic causes such as coronary vasospasm, coronary dissection and stress-induced cardiomyopathy are more common among women experiencing clinical symptoms of acute coronary syndromes than men (Ruiz-Garcia et al., 2012).

Low coronary flow reserve (CFR) is a result of effects of epicardial CAD, but also diffuse atherosclerosis and CMD. Low CFR affects heart's ability to adapt to stress and maintain myocardial perfusion and has been shown to be an independent predictor of clinical events (Lee et al. 2018). CFR is defined as the heart´s capability to increase the coronary blood flow at maximal hyperemia compared to baseline flow at rest (Gould et al., 1974). Worse prognosis of severely impaired CFR may be related to coronary vasomotor dysfunction arising from a mix of CAD phenotypes (Taqueti 2018). Women are also suggested to have naturally lower CFR due higher resting flow than men, but similar stress flow (Kobayashi et al. 2015). This may be one factor of poorer prognosis related to non-obstructive CAD in women than in men. Whereas outcomes in men are closely associated with obstructive findings, in women only those with impaired CFR had increased risk of events (Taqueti 2018). Low CFR is associated with similar increase of events in both sexes, but it is more

prevalent in women and in a population with severely impaired CFR women have even greater risk of events than men (Taqueti 2017).

In addition to lower CFR and higher prevalence of CMD, smaller epicardial coronary arteries along with higher rest flow results in differences in shear stress and inflammatory mediators over the life span of women. It is hypothesized that this may also modify the development of CAD, resulting in more diffuse disease with vasomotor dysfunction in women than men (Taqueti 2018).

Apart from this, the prevalence of cardiovascular disease in women peaks after menopause. It is believed that estrogen has a cardioprotective effect and at least partly explains why women present CAD 10 years later than men (Iorga et al., 2017)

2.1.3 Diagnosis of chronic CAD

The lifetime risk of developing CAD in men and women after 40 years of age is 49% and 32%, respectively (Sanchis-Gomar et al 2016). According to 2019 ESC guidelines for the diagnosis and management of chronic coronary syndromes, evaluation of a patient with suspected chronic CAD is a six-step process. The first step is to evaluate a patient's symptoms and risk factors and determine whether the underlying cause for symptoms is chronic coronary syndrome. On the second step, physician evaluates patient's overall physical health and quality of life, which could affect treatment options. Step 3 includes basic tests, such as laboratory tests and ECG, and in selected patient's chest x-ray and echocardiography. Thereafter in step 4, the clinical likelihood of CAD is estimated before applying diagnostic tests. In 2019 ESC Guidelines, clinical likelihood is determined according to age, sex, and type of symptoms, but modified based on the presence of risk factors and results from previous tests such as ECG, exercise test and coronary calcium score when available (Knuuti et al., 2020; Diamond et al., 1979).

Depending on the clinical likelihood, diagnostic tests are performed (Step 5). Clinical likelihood, local routines, available testing methods aa well as patient´s characteristics and preferences affect which noninvasive or invasive test modality is chosen. Fragile patients with a lot of comorbidities and contraindication for invasive treatment options can be diagnosed clinically without further testing, and medical therapy initiated.

Anatomical evaluation of epicardial coronary arteries can be conducted with either coronary computed tomography angiography (CTA) or invasive coronary angiography (ICA). In patients with lower likelihood or uncertain etiology of symptoms, non-invasive imaging is preferred. Patients with high likelihood of having CAD, difficult symptoms, or high risk of events can be admitted directly to ICA, which enables combining diagnostic evaluation with therapeutic interventions. (Knuuti et al., 2020)

Functional and anatomical testing are both suitable in the evaluation of coronary artery disease if physician understands the nature and limitations of each modality. Functional non-invasive tests include exercise-ECG (currently not preferred as diagnostic test), stress echocardiography, single-photon emission CT (SPECT), positron emission tomography (PET) and contrast-enhanced *cardiovascular* magnetic resonance imaging (CMR). In these methods, myocardial ischaemia or perfusion abnormalities is provoked either by physical exercise, pharmacological stressors, or vasodilators. Functional imaging has high accuracy in detecting flow-limiting coronary stenosis. However, lower grade atherosclerosis without ischaemia remains undetected. (Knuuti et al. 2020). Differences in non-invasive testing modalities are further discussed in chapter 2.2.

As the final step, once CAD is diagnosed, patient´s event risk is evaluated as it has major impact on selection of treatment.

2.1.4 Prognosis and follow-up of chronic CAD

Non-modifiable risk factors including age, male sex, ethnicity, and family history, explain 63% to 80% of the prognosis while modifiable have smaller contribution (Pencina et al. 2019). Modifiable risk factors include hypertension, hyperlipidemia, diabetes mellitus, obesity, smoking, unhealthy diet, and low physical activity. In addition to these conventional risk factors, fatty liver disease, chronic kidney disease, chronic systemic inflammatory diseases, thyroid disease, and low socioeconomic status have been associated with elevated risk of CAD (Brown et al. 2023). Modifiable and non-modifiable risk factors together explain >90% of the occurrence of myocardial infarctions globally (Yusuf et al. 2004).

In 2020, American Heart Association reported CAD age-adjusted death rates per 100 000 to be 128.5 for White males, 153.6 for Black males, and 102.2 for Hispanic males. For White females, the rate was 63.8, for Black females, it was 85.9, and for Hispanic females, it was 54.2. (Tsao et al., 2023)

CAD mortality was highest in Africa and the Middle East, Eastern Europe and Central Asia. It has been shown that the increase in CAD severity is associated with higher likelihood of death and MI independently of ischeamia severity or other clinical predictors (Reynoldst al. 2021). The increase in mortality reflects the relationship between the burden of atherosclerosis and the likelihood of having unstable plaques causing adverse outcomes (Reynolds et al., 2021). Moreover, patients are considered as high risk if they have a three-vessel disease with proximal stenoses, left main disease or proximal anterior descending disease in anatomical imaging modalities (Emond et al., 1994; Leipsic et al., 2013; Carrigan et al., 2009). It has also been shown that moderate or severe abnormalities on functional testing

among patients with suspected CAD were associated with increased risk of cardiovascular death or MI (Hoffmann et al., 2017)

Patients with chronic CAD require a life-long treatment to prevent progression of atherosclerosis and adverse events (Gibbons et al., 2017; Virani et al., 2023). The most suitable treatment strategy should be tailored for each patient, combining life-style intervention, medical therapy (antithrombotic drugs, lipid lowering drugs, blood pressure drugs, anti-ischemic drugs and in case of incident diabetes mellitus also blood glucose lowering drugs) and revascularization by percutaneous coronary intervention (PCI) or coronary artery bypass graft surgery (CABG) (Virani et al., 2023).

Clinical outcome in patients with CAD is generally good. It has been shown that with optimal medical therapy there would be a substantial reduction of CAD events (Mozaffarian et al. 2015). However, patients can develop a variety of cardiovascular complications related directly to CAD or in the interaction with CAD. These include, MI, UAP, sudden death and heart failure. CAD is a dynamic process with a spectrum of disease types and patients can develop acute coronary syndromes at any point of this progression. Adverse outcomes can occur with both symptomatic patients and asymptomatic patients and are typically due to an acute atherothrombotic event caused by either plaque rupture or erosion. Risk scores including clinical parameters and biomarkers have been shown to predict outcomes in chronic CAD patients (Daly et al 2005, Lindholm et al. 2017) as well as the results of anatomical and functional evaluation of coronary arteries (Knuuti et al.,2020, Hoffmann 2017, Reynolds et al., 2021)

Patients with non-obstructive CAD require attention as well. It has been shown that non-obstructive CAD is associated with increased risk of adverse events over patients without coronary atherosclerosis (Jespersen et al 2012). The outcome of atherosclerosis can be unfavorable even without epicardial disease. The higher risk may be explained by microvascular disease, non-obstructive plaques that become unstable and cause thrombotic events as well as dynamic stenoses caused by spasm or intramyocardial bridges (Knuuti et al 2019). Particularly, patients with diabetes without epicardial disease, but with abnormal CFR, have similarly poor prognosis than those with obstructive epicardial disease (Murthy et al. 2012). In women it is considered that microvascular dysfunction precedes the development of epicardial CAD more commonly than in men and it was shown that women with low CFR without epicardial CAD have increased risk for major adverse outcomes (Pepine et al. 2010).

Secondary prevention after an acute coronary syndrome or revascularization is important due to increased risk of recurrent events in these patients (Daly et al 2006). Also, patients with any comorbidities, signs of heart failure or patients with worsening symptoms or shorter duration of symptoms were at a greater risk of

having adverse outcome (Daly et al 2006). In a recent large study, it was found that the risk of recurrent event during 5-year follow up after first MI was high, 33.4% for second non-fatal MI or cardiac death (Steen et al. 2022). The risk for experiencing second event was found to be 6-fold higher within first 12 months of the primary event compared to period after the first year (Steen et al. 2022). Rates of second adverse event were higher in the patient population without revascularisation than those who were revascularised. (Steen et al. 2022).

However, it has been shown that the residual risk for event after revascularisation is still high. This is seen in patients treated for acute coronary syndrome as well as for stable angina (Madhavan et al. 2020). ISCHEMIA trial found that there was no mortality benefit from invasive treatments as compared to medical therapy alone in patients within chronic CAD and extensive ischaemia based on non-invasive testing (Maron et al., 2020). In fact, optimal medical therapy appeared to be as effective as revascularisation in terms of overall events (Maron et al. 2020). However, revascularisation reduced anginal symptoms more effectively than medical therapy alone (Spertus et al., 2020). In another publication of the ISCHEMIA trial, it was found that the rate of cardiovascular death and MI was lower among patients with most severe anatomic CAD (those with 3-vessel CAD with ≥70% stenosis or 2-vessel CAD with ≥70% stenosis, including in the proximal LAD) who were admitted to invasive treatment than to medical therapy (Reynolds et al. 2021). Invasive treatment might not reduce the risk of overall death rate in this patient population.

2.2 Non-invasive imaging of CAD

2.2.1 Coronary computed tomography angiography

Coronary computed tomography angiography (CTA) has an important role in the non-invasive assessment of CAD. It enables precise three-dimensional detection of coronary plaques, plaque features and their impact on coronary lumen. Coronary CTA allows the detection of both non-obstructive and obstructive atherosclerosis, which cannot be assessed with other noninvasive methods (Hoffman et al. 2006). Coronary CTA has high sensitivity of 97% (93% to 99%) to rule out anatomically significant CAD (Knuuti et al. 2018). However, specificity is lower, 78% (67% to 86%) for the detection of anatomical stenosis and 53% (37 to 68%) for the detection of functionally significant stenosis defined by abnormal fractional flow reserve (FFR) (Knuuti et al., 2018). Coronary CTA is increasingly used as a first-line test for diagnosis as well as to guide treatment strategies in patients with suspected obstructive CAD (Stenström et al. 2019)

Contraindications for coronary CTA are severe allergy for iodine contrast agent, pregnancy, or severe renal dysfunction. Furthermore, extensive coronary

calcification, irregular heart rate, significant obesity, or inability to co-operate with breath-hold commands can result in non-diagnostic image quality (Gueret et al., 2013). Major calcification and motion artifacts hamper the proper evaluation of epicardial vessels, which might lead to overestimation of disease severity. This partly explains the relatively low specificity of this imaging method. However, the negative predictive value of coronary CTA is close to 100% (Meijboom. et al 2008).

Coronary CTA is based on the use of multi-detector CT scanners. This reduces examination time and radiation dose as well as improves image quality. Each x-ray detector rows records data independently as the gantry rotates (Webb 2006). According to SCCT Guideline, minimum requirements for coronary CTA are a 64-row CT with maximum collimation of each detector element ≤0.75mm and rotation speed ≤ 500ms. A typical result consists of 200-300 trans axial slices. Intravenous iodine contrast agent is used to achieve vascular enhancement for the evaluation of coronary lumen. Prospective ECG triggering reduces the radiation dose and allows the reconstruction of images at selected times of the cardiac cycle, but retrospective triggering is needed for functional assessment of ventricles and valves. Apart from evaluation of suspected CAD, coronary CTA can be used for other purposes as well. Those include preoperative evaluation of coronary arteries or aorta, etiological evaluation of heart failure and assessment of CABG grafts or stents. (Narula et al., 2021)

Anatomic evaluation of coronary artery stenosis does not directly correlate with the hemodynamic significance of a coronary lesion. Therefore, functional evaluation is recommended before the decision on revascularization (Neumann et al. 2019). This can be assessed with fractional flow reserve (FFR) measurements based on invasive intracoronary pressure measurements or using non-invasive functional imaging. Also, recently a CTA based method on computational fluid dynamics or machine learning (Taylor et al., 2013) has been introduced. FFR from CTA have been validated against invasive FFR, measured during ICA, with improved diagnostical accuracy to identify lesion specific ischemia (Taylor et al. 2013). CT-based FFR was found to be non-inferior to invasive FFR in decision making and in revascularization target identification (Collet et al., 2018). However, one in four CTA scans are unevaluable for non-invasive FFR (Driessen et al. 2019).

2.2.2 Non-invasive functional imaging

The aim of cardiac functional imaging is to detect myocardial ischaemia. This is usually caused by flow-limiting stenosis but can be a result of CMD or vasospastic disease. Previously, the myocardial ischaemia during stress was mostly evaluated with exercise ECG. Currently, more accurate noninvasive functional imaging tests have been suggested as a preferred diagnostic test instead of exercise ECG. The most

common techniques for functional assessment of the myocardial perfusion include SPECT MPI as well as stress echocardiography (Neglia et al., 2023). Moreover, PET MPI and CMR are used to an increasing extent, but due to high cost and lower availability they are not utilized as widely as SPECT or stress echocardiography. Functional imaging is recommended as a primary test, especially when clinical likelihood of CAD is high to intermediate or as a secondary test when coronary CTA shows suspicion of obstructive stenosis or coronary CTA is non-diagnostic (Knuuti et al. 2020).

2.2.2.1 Stress echocardiography

Stress echocardiography is a combination of echocardiography with physical or pharmacological stress. It is used for assessment of obstructive CAD as well as other conditions, such as cardiomyopathies or valvular disease. It enables the assessment of ischaemic wall motion abnormalities with semi-quantitative visual measurements. Simultaneously blood pressure, ECG changes and symptoms can be assessed (Sicari et al., 2009). The sensitivity and specificity for the detection of obstructive CAD are 85% and 82%, respectively (Knuuti et al., 2018).

Dobutamine is preferred as a pharmacological stressor. No differences in diagnostic accuracy between pharmacological and physical stressors have been observed (Heijenbrok-Kal et al., 2018). Contrast agents can be used to improve diagnostic accuracy by improving endocardial visualization (Senior et al., 2017; Senior et a., 2009). Sensitivity and specificity have been found to be 68-77% and 72-88% for perfusion stress echocardiography (Senior et al., 2009)

Advantages of this method are good diagnostic accuracy, low cost, lack of radiation exposure and good availability. However, a major limitation is its dependence of the physician´s skills.

2.2.2.2 Stress cardiovascular magnetic resonance imaging

Stress cardiovascular magnetic resonance imaging (CMR) can assess myocardial ischaemia, presence of myocardial scar as well as cardiac function globally and regionally without exposure to radiation and with an excellent safety profile. It can accurately detect hemodynamically significant CAD through evaluation of myocardial perfusion and wall motion in response to stress. Sensitivity has been reported 90% (83-94) and specificity 80% (69-88) to detect anatomically significant CAD (Knuuti et al. 2018).

In stress CMR, pharmacological stress by vasodilator or dobutamine is used, and gadolinium contrast agent may be injected to assess perfusion and scar. During the first pass of contrast agent, reduced myocardial signal is indicative of a flow-limiting

stenosis. The result is based typically on visual assessment of low-signal areas, but semi-quantitative analytical methods (van Dijk et al., 2017) and fully quantitative (Engblom et al., 2017; Engblom et al., 2024) methods have also been under development. One of the strengths of CMR is that it can detect scars of silent myocardial infarction (Kramer et al., 2020) by persistent gadolinium enhancement in delayed-enhancement CMR.

Various logistical issues, limited availability and need of special expertise limit the widespread use of CMR in the detection of ischaemia.

2.2.2.3 Single-photon emission computerized tomography

Functional evaluation of myocardium with single-photon emission computerized tomography (SPECT) MPI is the most widespread non-invasive functional imaging method for assessment of suspected obstructive CAD globally. Patients are given radiotracer on a peripheral vein and its myocardial distribution is visualized with a SPECT camera during rest and either exercise or pharmacological stress. Stress-only imaging can also be used for lower radiation dose (Bhalodkar et al., 2010). The radiotracers are actively internalized by the viable myocardial cells in proportion to regional myocardial blood flow. The tracers emit high-energy photons that are detected three-dimensionally by SPECT camera. Technetium-99m labeled tracers are the most used radiotracers in SPECT imaging.

A typical SPECT camera is formed with two or multiple camera heads rotating with the gantry around patient. It generates multiple myocardial projections, at least 64, to form a three-dimensional image that show radiotracer´s distribution in the myocardium (Hyafil et al., 2019). Visual analysis of perfusion is often complemented with semiquantitative analysis of segmental perfusion. A per-patient or per vessel summed scores can be further calculated from the segments. It has been previously reported that the sensitivity and specificity of SPECT for diagnosis of CAD are 73-87% and 70-83%, respectively (Knuuti et al. 2018).

2.2.2.4 Positron emission tomography

Cardiac positron emission tomography (PET) is a powerful quantitative method for assessing myocardial perfusion. PET enables even greater accuracy for detecting ischaemia than SPECT (Boyden & Murthy 2014). Advantages over SPECT are higher spatial resolution, shorter living radioisotopes, better kinetics of flow tracers and the ability to provide quantitative measurements of physiologic parameters. PET enables quantification of global and regional MBF in milliliters per gram per minute (ml/g/min) during hyperemic stress and at rest. From rest and stress flows, myocardial flow reserve can be calculated. This gives important diagnostic and

prognostic information. (Bateman et al., 2016; Schindler et al., 2010; Patel et al., 2020)

PET has been used for decades to noninvasively investigate cardiovascular disease. Due to limited availability and higher costs, it has been used mainly for research purposes for a long time. The increasing number of PET scanners, methodologic advances and improved radiotracer availability have contributed to its more widespread use. For PET MPI, a generator or cyclotron produced nuclear tracers is needed.

PET imaging defines accurate distribution of the tracer in an organ by producing three-dimensional images. It enables measuring tracer kinetics and uptake during pharmacological stress. Thus, the presence or absence, location, and extent of myocardial ischaemia can be obtained (Bengel etl al. 2009). The emission of the two photons results in higher detection efficiency and thus higher imaging resolution than the radionuclide emitting single gamma-ray photons detected by gamma camera in SPECT. (Bengel et al. 2009).

For PET MPI, three tracers are mostly used. These are 15O-labeled water (H2 15O), 13N-labeled ammonia (13NH3), and 82Rubidium (82Rb) (Saraste et al., 2012). 15O-water is freely diffusible and an ideal flow tracer for quantitation. 15O-water has a half-life only of 112 seconds, which enables short study time. However, the tracer does not accumulate in myocardium and therefore visual PET image analysis without image processing cannot be performed. 13NH3 has half-life of about 10 minutes and the second imaging can be performed after 30 minutes of tracer injections to allow the tracer to decay (Knuuti et al., 2009). 13NH3 has relatively high myocardial retention fraction and thus, it is possible to perform flow quantitation, visual image analyses and measure ejection fraction as well. A generator product Rubidium-82 does not need an on-site cyclotron and is currently the most widely used tracer for PET MPI. It has a short half-life of 76 seconds. Quantification of MBF is more difficult than with other PET MPI tracers due to lower tracer extraction fraction and image quality is poorer than with 13NH3.

In previous studies, sensitivity of PET for diagnosis of obstructive CAD varied between 84-95% and specificity 81-92% respectively (Knuuti et al., 2018; Jaarsma et al., 2012; Mc Ardle et al., 2012; Kajander et al., 2011; Machac et al. 2005; Carli et al., 2007). Chosen nuclear tracer, applied cut-off values and quantification methodology might explain the differences in diagnostic performance between these studies. Significantly higher sensitivity and specificity (95% and 91%) was detected when 15O-water PET MPI was analyzed with absolute quantification of stress MBF (Kajander et al., 2011). Quantitative measurement of MBF seems to be the most accurate as relative differences in regional myocardial radiotracer uptake may underestimate the severity of CAD (Kajander et al., 2011). In relative assessment, the area with highest tracer retention is used as a reference and assumed to be normal.

Therefore, in the case of extensive multi-vessel disease or microvascular disease with global reduction of perfusion, CAD may be completely missed in relative image analysis. It has been found that stress MBF <2.3 mL/g/min is the best predictor of ischaemia defined as reduced invasive FFR with 15O-water (Danad et al.,2014) in patients with suspected CAD. The optimal cut-off values of MBF for the detection of obstructive CAD may vary depending on the used tracer and imaging technique and due to myocardial damage e.g. in patients with heart failure (Knuuti et al., 2009).

2.2.3 Hybrid Imaging

Hybrid imaging enables the anatomical and functional evaluation of coronary artery disease. It combines data from two separate imaging modalities and fuses it to create a more comprehensive evaluation of underlying disease. In hybrid imaging both imaging methods equally contribute to the formation of the final image together. When imaging CAD, hybrid imaging can be performed e.g. by combining CTA with either SPECT or PET (Gaemperli et al., 2012). Combination of CTA with CMR is also possible.

As the nature of functional and anatomical evaluation is different, the imaging methods complement each other (Kajander et al., 2010). The studies have shown that only 30-50% of >50% luminal stenosis is associated with a reversible perfusion defect on MPI. On the other hand, many patients with normal MBF may have extensive subclinical CAD (Hacker et al., 2007; Gaemperli et al.,2007; Gaemperli et al., 2008; Schuijf et al., 2006). Hybrid imaging appears to offer superior diagnostic accuracy than stand-alone imaging. This has been found with both coronary CTA and SPECT (Gaemperli et al., 2011, Schaap et el., 2014) as well as with coronary CTA and PET (Danad et al.,2013; Kajander et al., 2010). Dan-NICAD is also studying whether patients can avoid unnecessary ICAs by performing MPI with CMR or PET in patients where obstructive CAD cannot be ruled out with CTA only. Although hybrid imaging seems ideal, there are several factors that need to be considered including logistics, radiation dose and cost issues. Optimal patient selection for hybrid imaging also needs further clarification.

To save patients from inappropriate excessive radiation and to use resources cost-effectively, a selective hybrid imaging approach has also been introduced. In a selective imaging, patients will undergo complementary imaging only if the first modality shows abnormal or equivocal results (Maaniitty et al., 2017; Kauffmann & Buechel, 2016; Saraste et al., 2012). It has been suggested that due to the high negative predictive value, patients could be evaluated with coronary CTA before MPI, especially when clinical likelihood is low. If, however, CTA shows suspicion of obstructive CAD the functional assessment of myocardial perfusion reveals the hemodynamic significance of coronary artery lesions. This protocol has been

suggested to be appropriate with patients with low to moderate clinical likelihood of CAD.

On the other hand, coronary CTA alone cannot identify ischemia accurately or CMD. Therefore, MPI first approach might be beneficial in patients with higher clinical likelihood of ischaemia. In the case of SPECT imaging, the order of MPI first could, however, be problematic, as SPECT is semi-quantitative by nature and can miss patients with three-vessel disease or CMD in some cases. This can be, however, overcome mostly by utilizing coronary calcium score derived from standard attenuation CT.

Noninvasive hybrid imaging provides important information on the location, severity, and extent of atherosclerosis and ischaemia. This can be used to guide the decision to further admit patients into revascularization or medical therapy. To further use resources effectively and save patients from unnecessary radiation, selective PET after CTA protocol has been applied. In the selective referral, only patients with obstructive CAD in CTA are admitted to PET MPI. Selective assessment of MBF by PET after CTA has been shown to guide referral to ICA and potentially reduce the number of unnecessary ICAs and revascularizations (Stenström et al. 2019)

2.2.4 Prognostic value of non-invasive imaging

Non-invasive cardiac imaging, such as CTA, CMR, PET, SPECT and stress echocardiography provide important information also for risk stratification of patients with suspected or CAD (Martijn et al., 2017). In addition to making a diagnosis, evaluation of patient's risk for adverse events is clinically one of the most important aspects of cardiac imaging (Budof et al. 2006). Identification of patients at high risk for future events enables optimization of medical treatment and recognition of those who might benefit from revascularization (Fihn et al.2019, Knuuti et al., 2020.) In contrast, negative test results should assure physicians and patients that no further downstream testing or therapy is needed (Smulders et al., 2017). In this paragraph we are focusing on the prognostic value of non-invasive imaging modalities, a greater attention is given to CTA and PET as they are in the center of interest of this thesis.

Recently, a large meta-analysis has been conducted comparing prognostic value of non-invasive imaging methods (Smulders et al., 2017). It was found that a negative test result of any of these methods (stress echocardiography, CMR, CTA, PET, SPECT) was associated with low risk for future cardiac death or MI. Negative CTA was associated with the lowest pooled event risk and differed significantly from stress echocardiography and SPECT, although prognostic value of CMR or PET did not significantly differ from CTA. However, after adjustment of population true

event risk based on pre-test probabilities, CTA showed similar prognostic value as other modalities after negative test result (Smulders et al.,2017). However, the meta-analysis detected multiple confounding factors related to patient selection between modalities. In conclusion, differences in outcome after a negative test result between modalities can partly be explained by variations in clinical likelihood and proportion of pre-existing CAD among patients admitted to testing.

It has been found that there is a correlation between the extent of atherosclerotic findings on coronary CTA and adverse events (Bamberg et al., 2011; Bittencourt et al., 2014; Cho et al., 2018). Apart from the extent of atherosclerosis, high-risk plaque features have also been shown to predict unfavorable outcomes (Williams et al., 2020; Stone et al., 2011; Erlinge et al., 2021). However, coronary artery disease is also driven by inflammation and is not fully reflected by severity of stenoses or plaque features, leaving us with unexplained and undetectable residual risk after coronary CTA. New cardiac CT techniques aim to detect inflammation by imaging perivascular fat (Channon et al.,2022). Currently, it is not clear how this enables us to assess the residual risk for coronary artery disease (Channon et al., 2022). Also, patients with CMD might get unrealistically good prognosis because CTA alone cannot rule out CMD. It has been shown that CMD alone can induce ischaemia and even non-ST-elevation infarction independently without obstructive CAD (Knuuti et al. 2020).

Normal PET scan has been shown to indicate low risk for future CAD events (<1% annual event rate), while an abnormal scan indicates worse prognosis (Dorbala et al., 2013). Adverse events risk gradually increases from normal to severely abnormal MPI. Stress MPI has been shown to provide incremental prognostic value over rest imaging (Lertspurapa et al., 2008; Dorbala et al., 2009) and the assessment of PET derived CFR even further adds the prognostic value of PET (Murthy et al.,2011; Ziadi et al.,2011). Patients with reduced CFR in PET MPI have been shown to experience higher cardiac mortality independent of clinical and other imaging-based risk markers. (Murthy et al.,2011)

There is only limited information about the benefit of assessing CAD with hybrid imaging and its incremental value in prognosis. Hybrid imaging may be useful for identification of patients who are at high risk of developing acute coronary syndrome and can add value to CTA imaging alone. In patients with intermediate coronary plaques, occurrence of ischaemia at an anatomically appropriate location is associated with an increased risk of death or cardiac events after hybrid imaging (Pazhenkottil et al., 2011, Pazhenkottil et al.,2018). In contrast, patients with obstructive lesions but normal MBF, have favorable prognosis (Maaniitty et al., 2017). Although the complementary nature of anatomical and functional imaging is well established, there are only limited data on how each method can predict events in the short and the long term.

2.2.5 Sex differences in performance of non-invasive testing

Table 2. Sex differences in performance of non-invasive testing.

Lower peak exercise capacity in women	Fairbairn et al., 2020
Lower incidence of obstructive CAD in women	Fairbairn et al., 2020
Smaller body size in women	Fairbairn et al., 2020
Smaller left ventricle size in women	Fairbairn et al., 2020
Smaller epicardial arteries in women	Fairbairn et al., 2020
Breast attenuation	Fairbairn et al., 2020

There are differences in the diagnostic accuracy of non-invasive tests to assess obstructive CAD between women and men (Dolor et al., 2019; Mieres et al., 2014; Haase et al.,2019). Multiple confounding factors exist, which affect the assessment of CAD in women as compared to men. Lower peak exercise capacity, smaller body and left ventricle size, smaller epicardial arteries, breast attenuation and lower incidence of obstructive CAD are all confounding factors that limit the accuracy of non-invasive testing in women (Fairbairn et al., 2020). In addition, current strategy for diagnosing CAD focuses on the detection of obstructive plaques, which are less frequently present in women (Shaw et al., 2009). Therefore, sex specific strategies and test interpretation are recommended (Mieres et al., 2014) and even sex specific cut-off values are being suggested (Georgiopoulos et al., 2022).

Exercise ECG has been shown to have similar negative predictive value in men and women (81% and 78% respectively) and provides important prognostic information in both sexes (Mieres et al., 2014; Gibbons et al., 2002; Gulati et al., 2003). However, a large meta-analysis showed that the positive predictive value is significantly lower in women (Dolor et al., 2012). Other non-invasive modalities than exercise ECG have been shown to provide comparable diagnostic and prognostic information in women and men (Chamsi-Pasha, 2017). Multiple studies have found improved accuracy of stress echocardiography over exercise ECG in women with sensitivity and specificity of 79% and 83%, respectively, which is similar than in men (Dolor et al., 2012). In comparison, the sensitivity and specificity of exercise ECG in women was found to be 62% and 68% (Dolor et al., 2012). Even further, MPI has been shown to have better accuracy than exercise ECG (Mieres et al., 2003). Comparative studies have shown similar diagnostic and prognostic value of SPECT (Dolor et al., 2012; Mieres et al., 2014; Gebhard et al., 2018; Kay et al., 2013) and stress echocardiogram in women and men (Pellikka et al., 2007). PET MPI has shown significantly better specificity for diagnosis of CAD in women than SPECT (Bateman et al., 2006). CMR has been shown to have similar performance

in women versus men (specificity 83.5% versus 82.8%) and (sensitivity (88.7% versus 85.6%) (Greenwood et al., 2014).

CTA has important prognostic and diagnostic implications particularly in women who have greater heterogeneity of CAD. Recent comparative studies indicate that in women, anatomic evaluation by coronary CTA is associated with lower rate of CAD diagnosis than noninvasive functional tests as compared to men (Pagidipati et al., 2016; Mangion et al., 2020; Lubbers et al.,2017). No difference of diagnostic accuracy in CTA between men and women have been observed (Dolor et al., 2012; Mieres et al., 2014)

There is only limited data on differences in prognostic value of noninvasive diagnostic tests for CAD according to sex (Dolor et al., 2012). It is suggested that women may gain similar or even more prognostic information from coronary CTA than stress testing for ischaemia, whereas men benefit equally from both testing modalities (Pagidipati et al., 2016; Mangion et al., 2020; Lubbers et al.,2017). More recently, retrospective studies have reported equal predictive value of per vessel extent of CAD by coronary CTA in women and men (Schulman-Marcuse et al.,2016). In the Scottish Computed Tomography of the Heart (SCOT-HEART) trial, addition of coronary CTA to standard evaluation of patients with chest pain showed similar benefits in women and men (Mangion et al 2020). In the Prospective Multicenter Imaging Study for Evaluation of Chest Pain (PROMISE) trial, women appeared to derive even more prognostic information from CTA than men who tend to derive similar prognostic value from both anatomical and functional tests (Pagidipati et al., 2019). Previous evidence supports the prognostic value of SPECT perfusion imaging in women as well as in men (Dolor et al., 2012; Mieres et al., 2014; Gebhard et al.,2018; Kay et al.,2013). Studies have also reported similar prognostic value of myocardial perfusion abnormalities detected by ^{82}Rb or ^{13}N-ammonia PET perfusion imaging in women and men (Gebhard et al., 2018; Kay et al., 2013). Currently, the prognostic value of combined anatomical and functional imaging in men versus women remains unknown.

2.3 Polygenic risk scores (PRS) in prediction of coronary artery disease

2.3.1 Genetical background of CAD

Genetic factors have an important contribution on the disease development in CAD (McPherson & Tybjaerg-Hansen, 2016). Heritability has been estimated to explain 40-60% of the development of CAD (Vinkhuyzen et al., 2013, McPherson & Lyman, 2019; Pen et al., 2014) with the strongest interplay in early onset CAD (Zdravkovic et al.,2002) and early-onset CAD events (Schunkert et al., 2011). For common

multifactorial diseases, including CAD, polygenic inheritance plays a greater role than rare monogenic mutations. Genome-wide association studies (GWAS) have previously shown that the genetic load for CAD is due to common genetic variants with small effect sizes, in addition to rare variants with stronger effects (McPherson & Lyman, 2019; Aragam & Natarajan 2020, McPherson & Tybjaerg-Hansen, 2016). Common low risk to rare high risk genetic variants most likely act cumulatively to drive the overall risk of an individual (McPherson & Lyman, 2019; Katsanis, 2016)

Rare coding variants, mainly in genes regulating low-density lipoprotein metabolism such as LDLR (low density lipoprotein receptor), PCSK9 (*Proprotein convertase subtilisin/kexin type 9*) and APOB (Apolipoprotein b-100), have a significant contribution to the early-onset CAD via syndrome called familial hypercholesterolemia (FH) (McPherson & Lyman, 2019). The importance of this association is well known and established. Individuals with FH have been shown to have almost 4-fold risk for future CAD (Khera et al., 2016). However, FH is rare and most individuals with premature CAD do not carry these mutations (McPherson & Lyman, 2019). Recent meta-analysis suggested that only 5.9% of patients with premature CAD carries a FH-mutation (Hu et al., 2020)

The knowledge behind genetic basics of CAD has been evolving during the past half-century. Candidate genes coding for proteins of known biological significance seemed to be logical first steps in understanding CAD genetics. However, except for rare monogenic disorders of lipid metabolism, the causality of single-gene disorders to coronary atherosclerosis or plaque rupture was found to be modest. McPherson & Tybjaerg-Hansen, 2016).

Recent developments in understanding polygenic diseases have been driven by technological advances. High-throughput DNA microarray technology, which uses chips containing up to a million markers consisting of single-nucleotide polymorphisms (SNPs), are used to tag common variations across the human genome. Comparison of the frequency of the alleles in each SNPs in cases and controls provides an approach for genetic research, which doesn´t involve preliminary assumptions on candidate genes or traits. This approach is called genome-wide association studies (GWAS). Unlike monogenic mutations, these SNPs points causative locus, but rarely act themselves as functional variants. GWAS makes use of linkage disequilibrium, which is nonrandom coinheritance of genetic variants across the human genome and allows to identify genetic markers that tag the actual causal variants. Allele frequency and effect sizes with genome-wide significance are derived from GWAS studies. (McPherson & Tybjaerg-Hansen, 2016). To this day, over 320 loci with association to CAD, with local or long-distance effects on the expression of genes via number of complex mechanisms, have been found with GWAS (Chen & Schunkert, 2021)

However, it is noted that heritability of complex traits lies mainly in SNPs that do not reach genome-wide significance. Recently it was found that only 22% of CAD heritability (and 55% of narrow sense heritability) is explained by common autosomal SNPs (Nikpay et al., 2017). To interpret this, narrow sense heritability is defined as the fraction of phenotypic variance that can be explained by the variation of additive effects of these genes (Otto, 2001). The missing heritability of CAD is under interest of geneticists and more advanced genotyping panels and customized sequencing methods are under development. (Nikpay et al., 2017)

2.3.2 Construction of PRS

GWAS has identified millions of genetic variants associated to CAD. Solely these variants carry only a small effect on disease genetic burden, but when aggregated to polygenic risk scores (PRS), they could provide significant advances in risk prediction and understanding disease etiology. PRS are defined as a single value of an individual´s common genetic liability to a phenotype (Choi et al., 2020).

Derived from GWAS, multiple different PRS have been previously created. PRS can be calculated by summing risk alleles which are preferably weighted by their effect size estimates derived from summary statistics (Agbaedeng et al., 2021). The use of summary statistics for the effect size estimate distinguishes PRS from phenotypic prediction approaches that only uses individual level data (Choi et al., 2020). Heterogeneity in cohorts, error in the effect size estimates, selection of included SNPs and chosen weighting modalities affects and limits the accuracy of PRS. There are also multiple quality requirements that should be considered when designing PRS, such as removal of duplicate SNP, reassuring there is no sample overlap and appropriate handling of sex chromosomes.

Even from the same trait, PRS can be constructed in a range of different ways and thus, multiple different PRS scores can be made (Newman et al., 2022). Proper construction of the PRS is important and have been shown to influence in predictive power. PRS that sums only risk mutations together without any weighting modalities are called unweighted-PRS. Previous meta-analysis shows that unweighted-PRS were only associated with 8% increase in CAD risk, while weighted were associated with 26-49% risk, respectively (Agbaedeng et al., 2021). In addition, it seems that more complex weighting modalities such as “metaGRS” (meta-analysis approach with CAD 1,745,180 variants) and “LDpred” (matrix and summary statistics based on Bayesian method) derived PRS are associated with the strongest effect on CAD development (Agbaedeng et al., 2021). Among with different weighting modalities, estimation of effect sizes and tailoring PRS to target certain population also dealing with linkage disequilibrium is an important factor in the development of PRS. Controlling linkage disequilibrium is extremely challenging as there is complicated

and strong correlation structure among SNP across the genome. (Choi et al., 2020, Loh et al., 2018). As calculating techniques and sample sizes increase, it is targeted to design even more advanced and accurate PRS scores. (Choi et al., 2020).

2.3.3 PRS as predictors of atherosclerosis and obstructive CAD and their clinical utility

Strategies to identify patients with a higher likelihood of CAD are needed to target diagnostic testing appropriately and to tailor preventive therapy. Current risk scores incorporating known clinical risk factors – smoking, hypertension, diabetes, dyslipidaemia, age, sex, and family history – are used widely in clinical practice. However, there has been widespread interest in stratifying patients by their genetic risk scores as well as parental history of premature CAD that has been shown to independently predict future CAD in offspring (Vinkhuyzen et al., 2013, McPherson &Lyman, 2019; Pen et al., 2013). For this purpose, PRS has been suggested to be included in clinical care.

Previous studies have shown that current PRS can independently predict development of CAD (Khera et al 2018; Agbaedeng et al., 2021; Elliot et al., 2020, Mars et al., 2020; Inouye et al. 2018) and are an incremental predictor of incident CAD along with clinical cardiovascular risk factors (Agbaedeng et al., 2021). Thus, it has been suggested that PRS could be implemented into clinical care for better risk stratification of underlying CAD (Khera et al., 2018; Inouye et al., 2018). However, the clinical usability has not been previously evaluated at individual level, and a validation of various PRS in independent population is not yet available (Agbaedeng et al. 2021)

In very recent PROMISE trial analysis, it was hypothesized that PRS would be independently associated with high-risk CAD phenotypes in patients with previously known CAD. However, no association between PRS and high-risk CAD features was observed (Newman et al. 2022). In another study, it was suggested that polygenic risk increases risk for incident CAD through an increased burden of coronary atherosclerosis rather than promoting adverse plaque features or stenosis severity (Christiansen et al., 2020). So far it seems that unfavourable genetics plays an independent role in CAD development, but not in disease severity (Christiansen et al., 2020).

One issue is that different PRS scores can give quite varying risk estimates for healthy individuals for given disease. This indicates that an individual could receive vastly different medical advice depending on which PRS was used. (Clifton et al., 2022; Ding et al., 2022). This hampers the usability of PRS in clinical real-world setting. There are only limited investigations on how different PRS diverge from each other in risk prediction of CAD. In addition, there is only limited information

on how different PRS can be implemented into different populations and ancestries. The uncertainty of PRS and the effect of ancestry in the development of PRS needs to be further explored before implementing PRS in clinical care (Ding et al., 2022). Also, age and sex related analyses are needed. It is suggested that younger patients might derive more prognostic information than older patients from PRS calculation among patients without known CAD (Marston et al., 2023) and with known CAD (Manikpurage et al., 2021), but research on predictive accuracy according to sex is controversial (Manikpurage et al., 2021; Elliot et al., 2020)

3 Aims

The purpose of this study was to evaluate the value of hybrid PET/CTA imaging for diagnosis and outcomes of CAD among patients with suspect obstructive CAD; focusing on effects of sex and genetic factors. The detailed objectives in this thesis are as follows:

1. Evaluate sex differences in disease profile and outcomes after combined CTA and PET perfusion imaging in patients with suspected obstructive CAD. (Study I).
2. To assess the incremental value of PET perfusion imaging over coronary CTA in prediction of short- and long-term cardiac events using machine learning approaches (Study II).
3. To assess the predictive power of the previously published most promising three PRS in the prediction of CAD in symptomatic patients with suspected CAD in which the coronary phenotype has been carefully characterized using both anatomic and functional imaging. (Study III).

4 Materials and Methods

4.1 Study design and patient population

The purpose of Turku Cardiac CTA Registry is to preserve imaging and clinical data of all patients who have undergone coronary CTA and PET imaging in Turku PET Centre since January 2006. It is observational by nature. Most patients were referred to coronary CTA due clinically suspected obstructive CAD with at least intermediate pre-test likelihood (>15%). Pre-test probability is calculated by considering patients' sex, age and symptoms (ESC 2019). Other indications for referral included e.g. preoperative scans, arrhythmias, and evaluation of the aetiology of heart failure or cardiomyopathies. Patients with other indications than suspected CAD, however, were excluded from current analyses (Study I-III). Patients with previous coronary revascularisations (PCI or CABG) or known previous CAD were also excluded from Studies I and II.

In Studies I-III patients were referred to hybrid PET/CTA imaging due clinically suspected obstructive CAD and imaging protocols followed our local clinical practice. In our routine clinical practice, patients with suspected CAD undergo first coronary CTA, and immediately after that, PET perfusion imaging if coronary CTA alone cannot rule out obstructive CAD. All patients in whom CTA shows a suspicion of an obstructive coronary plaque (e.g., stenosis diameter ≥50%), the hemodynamic significance of the plaque is evaluated using ^{15}O-water PET perfusion imaging during adenosine stress. If obstructive CAD can be ruled out using only CTA, no further testing is performed.

In study I, we retrospectively identified all consecutive patients referred for hybrid PET/CTA in the Turku PET Centre in the period of 2008-2016. In cases of repeated tests during the study period, the earliest successful test was included in the analysis. We excluded trial patients, as they did not follow our selective imaging protocol. Out of the total 2212 patients, we yet excluded 122 patients with non-diagnostic CTA, 28 patients with non-diagnostic PET, 100 patients in whom PET was not performed despite obstructive CAD on CTA (61 of these referred directly for invasive coronary angiography), and 14 patients with PET performed although CTA was normal. Consequently, the final analysis included 1948 patients.

For Study II we retrospectively collected data from patients who have been admitted to hybrid PET/CTA between 2008-2016. Patients with prior known CAD were not included. Our cohort consists of a total of 2411 patients. After removal of incomplete data entries, data from 2284 patients was retained for the final analysis.

Study III cohort consisted of 998 patients who have undergone coronary PET/CTA during 2006-2019 and given voluntary consent for collection of a blood sample for genetic analyses. Blood samples were collected either on the day of imaging or afterwards on a separate visit. After excluding patients who did not complete the imaging protocol, 943 patients were included in the final analysis.

As the thesis consists of three studies from observational, retrospectively collected, cardiac registry patients, cohorts in Studies I-III are partly overlapping.

Studies comply with the Declaration of Helsinki. The Ethics Committee of the Hospital District of Southwest Finland approved the study protocol and waived the need for written informed consent for retrospective evaluation of clinically collected data. Permission from the National Institute for Health and Welfare (Finland) was gained for the purpose of collecting patient information. For study III written informed consent was obtained from patients and they received oral and/or written information of the study protocol.

4.2 Data acquisition and analysis

4.2.1 Imaging data acquisition

4.2.1.1 Coronary CTA imaging

Imaging acquisitions were standard for each Study I-III. Coronary CTA scans were performed using a 64-row hybrid PET-CT scanner (GE Discovery VCT or GE D690, General Electric Medical Systems, Waukesha, Wisconsin). Collimation was set at 64 × 0.625 mm, gantry rotation time was 350 ms, tube current 500 to 750 mA, and voltage 100 to 120 kV, depending on patient size. Prior to coronary CTA, beta-blocker (metoprolol, 0 to 30 mg) was given intravenously to achieve an ideal heart rate of <60 beats/min. For coronary dilatation, isosorbide dinitrate aerosol (1.25 mg) was administered. Coronary CTA was performed using intravenously injected low-osmolal iodine contrast agent (Depending on patient's weight amount varied between 320-400 mg iodine/ml; with injection velocity 4-5 ml/s) followed by saline flush. Prospective ECG-triggering for CTA was applied when feasible, to reduce the radiation dose. The presence, extent, and severity of coronary atherosclerosis were evaluated by experienced physicians according to the 17-vessel system by using the GE ADW Workstation (General Electric, Piscataway, New Jersey, US). Both

reoriented single plane and multiplanar reconstructions were used (Leipsic et al., 2014). From CTA we determined the severity of stenosis for each segment of main coronary arteries (LM, LAD, LCX, RCA) as well as for the side branches (D1, D2, LOM1, LOM2, LDP, LPL, RPD, RPL, IM) depending on whether they visualize or not. In addition to degree of stenosis, data of presence and plaque composition was collected. Agatson´s coronary calcium scores were calculated and recorded for each main artery separately (Agatson et al. 1990).

4.2.1.2 PET Imaging

In studies I-III myocardial blood flow was quantified with ^{15}O-water PET imaging during adenosine stress. In case of inappropriate caffeine intake within 24h prior to imaging studies, PET imaging was postponed for few days or weeks after the CTA imaging. Based on the CTA findings, patients with suspected obstructive CAD (>50% stenosis in one segment or in multiple segments) were further referred to downstream PET MPI imaging with ^{15}O-water during adenosine stress to evaluate hemodynamic significance of underlying obstructive lesions. This selective method has been previously reported. (Maaniitty et al., 2017; Kauffmann & Buechel, 2016; Saraste et al., 2012)

The tracer was injected as an intravenous bolus (mean injected activity 500–1100 MBq) over 15 s, and dynamic PET acquisition was performed (14 × 5 s, 3 × 10 s, 3 × 20 s, and 4 × 30 s). Absolute stress myocardial blood flow (sMBF) was quantified (in ml/g/min) individually for each of the 17 myocardial segments separately, excluding basal septal segments due to membranous part (standard segments 2 and 3). We considered myocardial perfusion ≥ 2.3 ml/g/min as normal (Danad et al. 2014). Perfusion ≥ 2.0 but < 2.3 ml/g/min was classified as mildly reduced, perfusion ≥ 1.5 but < 2.0 ml/g/min as moderately reduced and < 1.5 ml/g/min as severely reduced. Our routine includes stress only protocol, thus rest flows were not calculated in most patients. The quantitative PET data analysis was carried out with the Carimas software (developed at Turku PET Centre, Turku, Finland) by experienced physicians.

4.2.1.3 Genetical analysis

DNA samples were collected from patients' blood samples. Handling of the blood samples, sequencing and storing were conducted by AURIA biobank. The analysis of genetic data in study III were conducted by experienced statistician with knowledge and experience of genetic analyses at University Hospital of Eastern Finland. A total of 950 samples were genotyped using Illumina's GSAMD-24v2_0 beadchip, utilizing GenomeStudio v. 2.0.3 software. Results were checked with Plink software for sex,

identity-by-descent and Hardy-Weinberg equilibrium. One duplicate sample was found and excluded. Also, four first-degree relatives were excluded. Success rates for the genotyped samples were 98.4-99.6% (GenomeStudio software) after removing the low-quality SNPs and discarded samples. In quality control, 28904/759993 (3.8%) of SNPs were discarded. Plus/forward strand was based on information on "WRayner" – website (https://www.well.ox.ac.uk/~wrayner/strand/), and map positions were based on the genome build GRCh38.

SNPs with a 99% genotyping rate were included in imputation. SNPs in linkage disequilibrium were excluded using a window-size of 50 kb, step-size 5 and r2 threshold 0.7. Imputation was performed using Minimac4 based on the MIT server (https://imputationserver.sph.umich.edu/) using HRC (hrc-r1.1) as a reference panel. Quality control was performed prior to imputation as recommended in the server documentation by using the Will Rayner toolbox version 4.2 (https://www.well.ox.ac.uk/~wrayner/tools/index.html#Checking) and using plink. Additionally, MIT server´s extensive pre-imputation quality check on the uploaded dataset was also utilized (https://imputationserver.readthedocs.io/en/latest/pipeline/).

Derived from the genetic analyses, we calculated three known different PRS scores for each patient. MIT server was utilized in a selection progress. We consider that our selection offers a representative sample and illustrates and evaluates the topic well. We selected the following four PRS profiles to be tested: PGS000329, PGS000018 and PGS000013. These PRS are among the largest and most recognized PRS available and those have been constructed in populations genetically close to our study cohort. For clarity, we call these PRSs as PRS1, PRS2 and PRS3 respectively, in this thesis.

Genetic data was used only for research purposes. Patients or referring physicians did not receive any information of the genetic results.

4.2.2 Collection of clinical data and data analysis

Data collection for the registry has been carried out mainly retrospectively and manually by investigators. Clinical data were obtained from the electronic medical records. Clinical history included patients' prior illnesses, lab results, symptoms, and medication as well as indication for coronary imaging. Also, prior echocardiography and stress ECG results were collected. We collected the following from patients' medical history: sex, age, BMI, previous or current smoking, existence of prediabetes or diabetes 1 or 2, hypertension, dyslipidaemia, family history of premature CAD, prior myocardial infarction, or revascularisation. Symptoms were classified as typical angina, atypical angina, non-cardiac chest pain, no angina, and dyspnea or no dyspnea.

Follow-up data was obtained from the Centre for Clinical Informatics of the Turku University Hospital, the Finnish National Institute for Health and Welfare, and Statistics Finland. All adverse events and revascularisations were confirmed by

investigators from clinical records. We considered myocardial infarction, death, and unstable angina as adverse events. Death was classified as either cardiac death or non-cardiac death. Diagnostic criteria followed the ESC Guidelines (Knuuti et al., 2020). For the study I and II primary endpoints of follow-up data included all-cause death, myocardial infarction (MI) and unstable angina pectoris (UAP). Also, data on early revascularization (≤6 months), with PCI or CABG, were collected but these were not used as endpoints. The observation time was individual, and the time interval started from the CTA scan until the date of the first occurrence of an event or the date 13th May 2020, whichever happened first. In case of occurrence of multiple adverse events, the first event was considered. Study III did not include follow-up data of the progression of CAD, and analyses were based on the existence of coronary atherosclerosis and obstructive CAD.

4.3 Statistical methods

4.3.1 Study I

Continuous variables were reported as the mean and standard deviation (SD) or median [interquartile range], as appropriate. Categorical variables were expressed as count (percentage). The Chi-square test, Fisher Exact test, student t test, Mann-Whitney U test was used as appropriate. The cumulative incidence of events was based on Kaplan-Meier estimates and was compared between women and men using the log-rank test. To identify the univariable and multivariable predictors of in the whole cohort, as well as in males and females separately, cox proportional hazards models were used. Clinical variables with significant association to the composite outcome in the univariable analysis were included in the clinical multivariable model as covariates. Those covariates bearing significant association with the composite outcome in the clinical multivariable model were then further tested as covariates in multivariable models including CAC or PET-CTA findings. The prognostic value of the individual covariates as well as the multivariable models to predict the composite outcome was assessed with the use of the receiver operating characteristics (ROC) curve analysis. The interaction between sex and either the CAC or PET-CTA findings were tested using cox proportional hazard models. The annual adverse event rate was reported as % per year. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS v. 25.0 (IBM Corporation, New York, USA) statistical software.

4.3.2 Study II

Study II utilized machine learning as a tool to control greater number and complexity of variables than traditional prognostic risk assessment. Machine learning models

were trained and used to predict major adverse events by incorporating clinical cardiovascular risk factors, and CTA and PET imaging data. The data were split randomly into training and test groups with a 3:1 ratio. Each variable was checked individually to ensure similar distributions in both groups.

Patients with missing data on 66% or more of the variables from demographic and risk variables or if event occurrence information for the observation time was missing were excluded from the study. Thus, we included 2284 patients in the final ML models.

All variables went through preprocessing. The missing clinical variables were inputted as patient not having disease. We also created clinically meaningful variables derived from PET and CTA data. Those included:

- *Maximum stenosis degree:* the patient's highest stenosis degree value across all segments.
- *Maximum proximal stenosis degree*: the patient's highest stenosis degree value across segments LM, proximal LAD, proximal LCX or and proximal RCA.
- *Number of non-obstructive segments*: the number of segments with stenosis degree value 1 (i.e., non-obstructive atherosclerosis).
- *Number of obstructive segments*: the number of segments with stenosis degree value 2 (i.e., obstructive stenosis).
- *Number of atherosclerotic segments*: the number of segments with stenosis degree value 1 or 2 (i.e., either non-obstructive or obstructive atherosclerosis).

We also created the following derived PET variables from the preprocessed values:

- *Maximum PET abnormality score*: The highest PET abnormality score across all segments.
- *Maximum PET abnormality score for LAD*: The highest PET-abnormality score across segments 1, 7, 8, 13, 14, 17.
- *Maximum PET abnormality score for LCX*: The highest PET-abnormality score across segments 5, 6, 11, 12, 16.
- *Maximum PET abnormality score for RCA*: The highest PET-abnormality score across segments 4, 9, 10, 15.
- *Abnormal segment count*: Number of segments with PET-abnormality score above 0.

For the analysis we considered several ML models but Extreme Gradient Boosting, also known as XGBoost, performed the best and was chosen.

We computed feature importance vectors for 100 XGBoost models trained with all considered input variables for each observation year 1–8. Ranking vector constructed from the mean feature importance and mean occurrence vectors corresponding to a training of 100 XGBoost models per each observation year 1 – 8. Nine input variables which were ranked consistently as the most important features were selected for further analysis. In descending order of importance, those included: maximum stenosis degree, SIS, stenosis degree 1 count, age, BMI, maximum PET abnormality score for RCA, sMBF for segment 1, sMBF for segment 15, and dyspnea.

For the results, we trained two sets of XGBoost models for each observation year. The first set used clinical, CTA-based, and PET-based input variables, while the other set used only clinical and CTA-based input variables. Furthermore, we considered two sets of data for the analyses: the data set corresponding to all considered 2284 patients, and its subset of 2069 patients who did not undergo early revascularisation, in these analyses early revascularisation was not considered as adverse events. Adverse events included, MI, UAP and all-cause death.

The 95% confidence intervals were computed according to the formula

$$95\%\ \mathrm{CI} = (\mu - 1.96\,\sigma / \sqrt{k},\ \mu + 1.96\,\sigma / \sqrt{k}),$$

where k = 100 is the number of XGBoost models for each observation year. We assumed based on the central limit theorem and on the amount of samples k, that the sample means are normally distributed. Statistically significant differences between the areas under the receiver operator characteristic curves (AUCs) corresponding to all data variables and data without PET variables were assessed using the Wilcoxon signed rank test. $P < 0.05$ was considered statistically significant.

4.3.3 Study III

Statistical analyses were performed using IBM SPSS Statistics, version 27. All PRS were standardized to obtain odds ratio per SD unit. A logistic regression model was used to evaluate the association between PRS and CAD. $P < 5x10\text{-}8$ was considered statistically significant and $P<0.05$ as nominally significant. Difference between models based on the clinical risk factors, PRS alone and PRS with the clinical risk factors were generated to assess whether inclusion of PRS improves CAD risk prediction and onset CAD. The predictive accuracy between models was evaluated using AUCROC values. Furthermore, PRS was also stratified into deciles to illustrate the distribution of PRS scores in healthy patients, patients with atherosclerosis and patients with obstructive CAD.

5 Results

5.1 Sex differences in coronary artery disease characteristics and in performance of hybrid imaging (study I)

The study cohort included 1948 patients out of whom 1147 (58.9%) were women. In 1291 (66.3%) patients, obstructive CAD was excluded based on coronary CTA alone, whereas 657 (33.7%) patients were administered to ^{15}O-water PET myocardial perfusion imaging to evaluate hemodynamic significance of suspected obstructive coronary lesions. 34.0% of patients had normal coronary arteries, 32.3 % had non-obstructive CAD, 16.7% had obstructive CAD on coronary CTA and normal sMBF, and 17.0% had obstructive CAD and abnormal sMBF.

No difference in ICA referral rate was observed between women and men among the 332 patients with ischaemic CAD (60.0% vs. 55.9%, p=0.4). Similarly, equal proportion of women and men with ischaemic CAD had early revascularisation (36.2 vs. 37.4%, p=0.8) and revascularisation rate among those who underwent ICA was also similar (53.2% vs. 61.4%, p=0.2).

5.1.1 Sex difference in risk factors and disease profile

Characteristics of patients are shown in Table 3. In our cohort women were slightly older, had less frequently diabetes and more frequently family history of premature CAD than men. Smoking history was less frequently observed in women than in men. There was no difference in body mass index (BMI) or the prevalence of either dyslipidemia or hypertension.

The most common presenting symptom was atypical angina or non-anginal chest pain. Overall, women presented more symptoms than men. Typical angina and atypical angina were more common in women than in men. Women also reported dyspnea more often than men.

Despite, higher prevalence of symptoms, women had more favorable imaging findings than men. Women were more likely to have normal coronary arteries than men (42.3% vs. 22.1% p<0.001) while abnormal sMBF was less frequent in women than men (9.2% vs. 28.3% p<0.001, Table 3). Hemodynamically non-significant

CAD was present similarly in women and men (48.6% vs. 49.6%). Calcification was more extensive in men than in women as seen as in the higher CAC scores in men.

Table 3. Clinical characteristics, imaging findings and early invasive therapies of all patients, men, and women.

	Cohort (N=1948)	**Men (N=801)**	**Women (N=1147)**	**P value**
Age (years)*	62.9 ± 9.6	59.9 ± 10.6	63.4 ± 9.3	<0.001
Body mass index (kg/m^2)*	27.9 ± 6.6	27.9 ± 5.7	28.0 ± 7.3	0.6
Current or ex-smoking	625 (32.1)	338 (42.2)	287 (25.0)	<0.001
Diabetes mellitus	288 (14.8)	137 (17.1)	151 (13.2)	0.01
Hypertension	1083 (55.6)	455 (56.8)	628 (54.8)	0.3
Dyslipidemia	1221 (62.7)	497 (62.0)	724 (63.1)	0.6
Family history of CAD	918 (47.1)	301 (37.6)	617 (53.8)	<0.001
Typical chest pain	411 (22.1)	153 (20.0)	258 (23.6)	
Atypical/non-cardiac chest pain	963 (49.4)	371 (46.3)	592 (51.6)	
Dyspnea	755 (38.8)	261 (32.6)	494 (43.1)	<0.001
Coronary artery calcium score (n=1594)				<0.001
0	578 (36.3)	152 (23.2)	426 (45.3)	
1-99	467 (29.3)	185 (28.3)	282 (30.0)	
100-399	304 (19.1)	159 (24.3)	145 (15.4)	
>400	245 (15.4)	158 (24.2)	87 (9.3)	
Coronary CTA and PET findings				<0.001
No CAD	662 (34.0)	177 (22.1)	485 (42.3)	
Non-obstructive CAD	629 (32.3)	261 (32.6)	368 (32.1)	
Obstructive CAD and normal sMBF	325 (16.7)	136 (17.0)	189 (16.5)	
Obstructive CAD and abnormal sMBF	332 (17.0)	227 (28.3)	105 (9.2)	
Invasive coronary angiography	217 (11.1%)	140 (17.5)	77 (6.7)	<0.001
Early revascularisation <6 months	127 (6.5%)	86 (10.7%)	41 (3.6%)	<0.001
PCI	112 (5.7%)	74 (9.2)	38 (3.3)	<0.001
CABG	18 (0.9%)	15 (1.9)	3 (0.3)	<0.001
Follow-up events				
Death	126 (6.5)	60 (7.5)	66 (5.8)	0.1
MI	45 (2.3)	23 (2.9)	22 (1.9)	0.1
UAP	21 (1.1)	11 (1.4)	10 (0.9)	0.2
Death or MI	164 (8.4)	80 (10.0)	84 (7.3)	0.03
Death, MI or UAP	182 (9.4)	90 (11.3)	92 (8.0)	0.01

* = mean ± standard deviation (range), CAD = Coronary artery disease, CABG = Coronary artery bypass grafting, CTA = Computed tomography angiography, MI = Myocardial infarction, PCI = Percutaneous coronary intervention, PET = Positron emission tomography, sMBF = stress myocardial blood flow, UAP = Unstable angina pectoris

5.1.2 Predictors of events in women and men

Mean time of follow-up was 6.8 ± 2.5 years with a median of 6.65 years. During the follow-up, there were 126 deaths, 45 MIs and 21 UAPs. The composite outcome of death, MI or UAP occurred in 182 patients and was less frequent in women than men (8.0% vs. 11.3%, p=0.01, Table 1). There was no significant difference in the cumulative incidence of events at long-term follow-up between patients with ischaemic CAD who underwent early revascularisation (17.9%) vs. were treated conservatively (22.5%, p=0.4).

In multivariate analysis male sex remained an independent risk factor for adverse events. Age and dyspnea were associated with adverse events in women, whereas in men, age, diabetes, hypertension and typical angina predicted events (Table 5).

After adjustment for clinical risk factors and symptoms, coronary calcification was an independent predictor of events in both women and men (Tables 4 and 5). Extensive coronary calcification (CAC score ≥400) predicted events with an adjusted hazard ratio of 20.07 (95%CI 2.63-153.10) in men and 4.94 (95%CI 2.31-10.58) in women, when compared to patients without coronary calcification (CAC score 0).

PET-CT findings were independent and significant predictors of events after adjusting for clinical risk factors and symptoms both in women and men (Table 5). As compared to patients with normal coronary arteries, the presence of ischaemic CAD predicted events with an adjusted hazard ratio of 5.61 (95%CI 1.69-18.57) in men and 4.99 (95%CI 2.33-10.70) in women. Non-obstructive CAD was a predictor of adverse events in women with an adjusted hazard ratio of 3.63 (95%CI 1.83-7.20), but the effect was statistically non-significant in men p=0.2 and adjusted hazard ratio of 2.24(95% CI 0.64-7.80) (Table 5). Interestingly, non-obstructive CAD was a stronger predictor of adverse events in women than obstructive CAD with normal sMBF (HR 3.65 vs 2.99), while in men HR was systematically higher in patients' group with more advanced disease in PET-CT.

In Kaplan-Meier estimates of the cumulative incidence of the composite events. Estimates differed significantly (p>0.001) according to the extent of CAC and findings of PET-CTA in both men and women. Overall, adverse event rate was higher in men than women (Log-rank p=0.02) with the annual incidence being 1.37% in the whole cohort, 1.65% in men and 1.17% in women.

Interaction was tested in Cox proportional hazards models. There was no significant interaction between sex and either coronary calcification or hybrid PET/CT findings (p= 0.4, and p=0.2, respectively) in predicting adverse events. As in line, based on the ROC analyses the prognostic value of adjusted coronary artery calcification or hybrid PET/CT findings predicted composite outcomes was similarly in men and women (p=0.687 and p=0.999). Overall, coronary calcification and findings of coronary CTA and PET predicted events equally (p=0.477).

Table 4. Univariable predictors of adverse events (all-cause death, myocardial infarction or unstable angina pectoris) in all patients, men and women.

	All patients		Men		Women	
	HR (95% CI)	p value	HR (95% CI)2	p value	HR (95% CI)5	p value
Age	1.07 (1.05-1.09)	<0.001	1.07 (1.04-1.09)	<0.001	1.08 (1.05-1.11)	<0.001
Male Sex	1.39 (1.04-1.86)	0.02				
DM	1.68 (1.18-2.40)	0.004	2.17 (1.37-3.43)	<0.001	1.13 (0.63-2.04)	0.6
Hypertension	1.86 (1.36-2.56)	<0.001	2.11 (1.33-3.35)	0.001	1.64 (1.07-2.54)	0.02
Smoking	1.23 (0.91-1.66)	0.1	1.32 (0.87-1.99)	0.1	0.98 (0.61-1.57)	0.9
Dyslipidemia	0.97 (0.72-1.32)	0.8	1.07 (0.69-1.64)	0.7	0.90 (0.59-1.38)	0.6
Typical chest pain	1.52 (1.09-2.14)	0.01	2.00 (1.25-3.20)	0.004	1.21 (0.74-1.96)	0.4
Any chest pain	0.88 (0.63-1.24)	0.4	0.90 (0.56-1.42)	0.6	0.94 (0.56-1.56)	0.8
Dyspnea	1.88 (1.41-2.52)	<0.001	1.69 (1.11-2.56)	0.01	2.31 (1.51-3.53)	<0.001
CAC						
0	reference		reference		reference	
1 to 99	3.23 (1.74-6.01)	<0.001	11.52 (1.50-88.10)	0.01	2.68 (1.35-5.32)	<0.001
100 to 399	5.42 (2.92-10.06)	<0.001	23.24 (3.12-172.92)	0.002	3.73 (1.77-7.85)	<0.001
≥400	10.11 (5.61-18.23)	<0.001	40.48 (5.53-295.91)	<0.001	7.20 (3.55-14.59)	<0.001
Hybrid imaging finding						
No CAD	reference		reference		reference	
Non-obstructive CAD	4.57 (2.55-8.19)	<0.001	4.21 (1.24-14.30)	0.02	5.17 (2.65-10.09)	<0.001
Obstructive CAD, normal sMBF	6.01 (3.28-11.03)	<0.001	9.30 (2.76-31.31)	<0.001	4.75 (2.29-9.87)	<0.001
Obstructive CAD, abnormal sMBF	10.01 (5.63-17.79)	<0.001	13.56 (4.22-43.52)	<0.001	8.04 (3.85-16.80)	<0.001

CAC=coronary artery calcium, CAD=coronary artery disease, CI=confidence interval, DM=diabetes mellitus, HR=hazard ratio. HR for age is per 1 year.

Table 5. Multivariable predictors of adverse events (all-cause death, myocardial infarction or unstable angina pectoris) in all patients, men and women.

	All patients			Men			Women		
Model:	HR (95% CI)	p value	AUC	HR (95% CI)	p value	AUC	HR (95% CI)	p value	AUC
Clinical			0.706			0.728			0.705
Age	1.06 (1.04-1.08)	<0.001		1.06 (1.03-1.08)	<0.001		1.07 (1.04-1.10)	<0.001	
Male sex	1.81 (1.32-2.46)	<0.001							
Diabetes				1.77 (1.09-2.89)	0.03				
Hypertension	1.61 (1.15-2.25)	0.004		2.03 (1.21-3.40)	0.009				
Typical angina	1.55 (1.10-2.18)	0.01		2.01 (1.24-3.25)	0.01				
Dyspnea	1.60 (1.17-2.18)	0.003					1.88 (1.22-2.89)	0.004	
Clinical+ CAC			0.730			0.746			0.720
Age	1.03 (1.01-1.05)	0.007		1.03 (1.00-1.06)	0.04		1.03 (1.00-1.07)	0.03	
Diabetes				1.76 (1.00-3.10)	0.04				
Dyspnea	1.50 (1.05-2.04)	0.02					1.77 (1.08-2.89)	0.02	
CAC:									
0	reference			reference			reference		
1 to 99	2.75 (1.44-5.27)	0.002		7.83 (1.00-61.12)	0.04		2.11 (1.04-4.27)	0.03	
100 to 399	4.23 (2.19-8.15)	<0.001		14.11 (1.84-108.01)	0.01		2.67 (1.22-5.80)	0.01	
≥ 400	7.20 (3.80-13.65)	<0.001		20.07 (2.63-153.10)	0.004		4.94 (2.31-10.58)	<0.001	
Clinical + PET-CTA			0.743			0.753			0.753
Age	1.05 (1.03-1.07)	<0.001		1.05 (1.02-1.08)	<0.001		1.05 (1.02-1.08)	<0.001	
Hypertension				1.81 (1.08-3.03)	0.02				
Dyspnea	1.52 (1.11-2.08)	0.009					1.75 (1.13-2.69)	0.01	
PET-CT findings:									
No CAD	reference			reference			reference		
Non-obstructive CAD	3.36 (1.78-6.37)	<0.001		2.24 (0.64-7.80)	0.2		3.63 (1.83-7.20)	<0.001	
Obstructive CAD, normal sMBF	3.85 (1.97-7.52)	<0.001		4.37 (1.26-15.11)	0.02		2.99 (1.40-6.38)	0.005	
Obstructive CAD, abnormal sMBF	5.84 (3.04-11.21)	<0.001		5.61 (1.69-18.57)	0.005		4.99 (2.33-10.70)	<0.001	

The models are adjusted for significant risk factors, CAC scores and CAD groups separately. CAD=coronary artery disease, CAC=coronary calcium, CI=confidence interval, HR=hazard ratio, sMBF=stress myocardial blood flow. HR for age is per 1 year. P-values for comparing AUC values between models (Clinical + CAC and Clinical + PET-CTA) 0.477 for all patients, 0.625 for men and 0.394 for women.

5.2 Incremental prognostic value of downstream PET perfusion imaging after coronary CT angiography: A study using machine learning (study II)

Study II cohort consisted of 2284 patients who underwent coronary CTA due to suspected CAD. Following our protocol, 891 (39%) underwent downstream PET MPI for evaluation of hemodynamic significance of coronary stenosis. 31.4% of patients had normal coronary arteries, 27.8% had non-obstructive CAD, 17.6% had obstructive CAD on CTA but normal sMBF on PET, and 17.5% had obstructive CAD and abnormal sMBF (Table 6).

Table 6. Demographics Table.

	All patients (n=2284)	**Patients with no early revascularisation (n=2069)**
Age (years)	[23.0 – 88.0], 62.2 ± 9.7	[23.0 – 88.0], 62.0 ± 9.8
Women	1317 (57.7%)	1249 (60.4%)
BMI	[15.4 – 54.3], 28.3 ± 5.2	[15.4 – 54.3], 28.3 ± 5.2
Smoking	757 (33.1%)	657 (31.8%)
Diabetes	341 (14.9%)	289 (14.0%)
Hypertension	1292 (56.6%)	1155 (55.8%)
Dyslipidemia	1456 (63.7%)	1287 (62.2%)
Dyspnea	877 (38.4%)	791 (38.2%)
Atypical angina or non-anginal chest pain	1083 (47.4%)	1004 (48.5%)
Typical angina	528 (23.1%)	432 (20.9%)
CCTA and PET findings		
Normal coronary arteries	718 (31.4%)	718(34.7%)
Non-obstructive CAD	636 (27.8%)	636 (30.7%)
Obstructive CAD and normal sMBF	403 (17.6%)	392 (18.9%)
Obstructive CAD and abnormal sMBF	400 (17.5%)	233 (11.3%)
Endpoints	210 (9.2%)	172 (8.3%)
MI	59 (2.6%) (28.1% of all endpoints)	46 (2.2%) (26.7% of all endpoints)
UAP	35 (1.5%) (16.7% of all endpoints)	19 (0.9%) (11.0% of all endpoints)
Death	116 (5.1%) (55.2% of all endpoints)	107 (5.2%) (62.2% of all endpoints)
Early revascularisation		
PCI	180 (7.9%)	0 (0%)
CABG	40 (1.8%)	0 (0%)

Clinical characteristics, imaging findings, endpoints and early invasive therapies of all patients and patients with no early revascularisation separately.CAD = Coronary artery disease, CABG = Coronary artery bypass grafting, CCTA = Coronary computed tomography angiography, MI = Myocardial infarction, PCI = Percutaneous coronary intervention, PET = Positron emission tomography, sMBF = stress myocardial blood flow, UAP = Unstable angina pectoris

During 8-year follow-up period major adverse event occurred in 210 patients, out of which 28.1% were MI, 16.7% were UAP and 55.2% all-cause death. Events distributed evenly in the follow-up period; year 1: 33 events, year 2: 18 events, year 3: 30 events, year 4: 28 events, year 5: 31 events, year 6: 32 events, year 7: 21 events, year 8: 17 events. In total, 215 patients had early revascularisation (within 6 months) after CTA, with 180 percutaneous coronary intervention (PCI), 40 coronary artery bypass graft (CABG) surgery and 5 patients underwent both. Early revascularisation was not considered as a major adverse event.

Figure 1 shows the results of the XGBoost ML models in the prediction of composite outcomes (event or no event) for each observation time point (1 – 8 years) using the random and independent test sets (N = 571). In Figure 1, AUCs and their 95% confidence intervals are represented of Xboost models with all input variables and without PET variables.

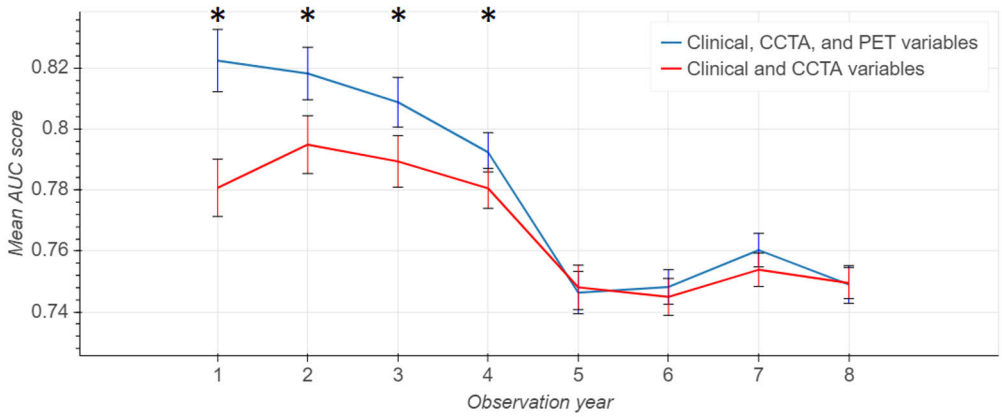

Figure 1. Mean AUCs and 95% confidence intervals for the XGBoost models trained with all variables, and without PET variables in the test sets randomly selected from the whole cohort. Per each observation year, 100 XGBoost models were trained and tested for each input variable set; an independent and random training and test data split was performed for each of these models. The AUCs are calculated using the test sets (N = 571). The difference between these curves is statistically significant for the first four observation years; this is highlighted by the black asterisks.

The results show that the predictive power decreases over time. The highest AUC 0.82 (95% CI 0.812 – 0.833) is observed at 1 year and decreases within the observation time of 8 years to AUC of 0.75 (95% CI 0.742 – 0.755) in the ML model with all variables. The highest AUC for ML model trained with clinical data and CTA data alone shows highest AUC at 2 years and after that decreases within the observation time. The predictive accuracy of the models differed significantly, AUCs were higher in the ML model which included all the variables than the model

with clinical and CTA variables only ($p < 0.05$). However, the significant difference was detected only during the first 4 years of observation time.

As revascularisation is used to treat ischaemia and is strongly linked with the ischaemic imaging findings in the noninvasive imaging, we performed a separate analysis restricted to patients without early revascularisation. In this analysis, there was no statistically significant difference between the mean AUCs of the groups with all variables and CTA + clinical variables only. This is depicted in Figure 3 in the manuscript re-print.

5.3 Polygenic risk scores in prediction of atherosclerosis and obstructive CAD (study III)

The study cohort consisted of 943 patients (59.7% women). The mean age of patients was 64 years (SD 8.6 years). Family history of premature CAD was reported by 49.5% (n=467) participants. Based on the imaging, 273 (29.0%) individuals had no coronary atherosclerosis, 465 (49.3%) had non-obstructive CAD and 205 (21.6%) had obstructive CAD. Patient characteristics are shown in Table 6.

For the analysis of predictive power of PRS for CAD we performed two comparisons. The first analysis compared patients with any degree of coronary atherosclerosis (i.e., either non-obstructive or obstructive CAD) (n=670) to those without atherosclerosis (n=273). The second analysis compared patients with obstructive CAD (n=205) to patients with either non-obstructive CAD or no atherosclerosis (n=738).

We chose three yet most promising PRS to conduct the analyses. The selection process of PRS are described in more detail in the methods section. In table 7 we are presenting the PRS scores as predictors by original studies for comparison.

Table 7. PRS as predictors by original study. CI= confidence interval, OR = Odds ratio, [a] = Coverage of the SNPs in current study. CAD= coronary artery disease, PRS = polygenic risk score, SNP = single nucleotide polymorphism

Polygenic Risk Scores	OR / C-statistic	95% C.I.		N	Coverage[a]
		Lower	Upper		
PGS000329 – Coronary heart disease	1,31	1,29	1,33	20,165	99.99%
PGS000018 – Coronary artery disease	1,71	1,68	1,73	480,000	100%
PGS000013 – Coronary artery disease	2,55	2,43	2,67	184,305	94.46%

Table 8. Clinical characteristics and imaging findings for the cohort.

	(n=943)
Age (years)[a]	64±8.6
Body mass index (kg/m2)[a]	27.5±4.9
Male sex	380(40.3)
Current smoking	95 (10.0)
Diabetes	130 (13.8)
Hypertension	542 (57.5)
Dyslipidemia	611 (64.8)
Family history of premature CAD	467 (49.5)
Symptoms	
Typical angina	250(26.5)
Atypical/non-anginal pain/dyspnea	594(63.0)
No chest pain nor dyspnea	99(10.5)
Imaging findings	
No coronary atherosclerosis	273 (29.0)
Non-obstructive CAD	465 (49.3)
Obstructive CAD	205 (21.7)

[a] mean ± standard deviation (range), CAD=Coronary artery disease

5.3.1 Predictive power of PRS

All three different PRS were predicting coronary atherosclerosis (Table 9) and obstructive CAD (Table 10) statistically significantly and the ORs varied between 1.25 (95% CI 1.07-1.46) - 1.70 (95% CI 1.44-2.01). The highest OR was found with PRS3 in predicting obstructive CAD. The ORs of clinical data alone were 3.13 (95% CI 2.63-3.72) and 2.53 (95% CI 2.15-2.98) for predicting coronary atherosclerosis or obstructive CAD, respectively. The ORs of models combining clinical data and different PRS varied between 2.59 (95% CI 2.20-3.05) and 3.01(95% CI 2.53-3.57) in predicting obstructive CAD and between 3.35 (95% CI 2.81-4.00) and 3.59 (95% CI 3.00-4.30) for predicting coronary atherosclerosis (Table 9 and 10).

Table 9. The prediction of coronary atherosclerosis by clinical data and three PRS.

Model	OR	95% CI	p-value
PRS1 (PGS000329)	1.40	1.21-1.62	<0.001
PRS2 (PGS000018)	1.62	1.39-1.89	<0.001
PRS3 (PGS000013)	1.62	1.39-1.88	<0.001
Clinical data[a]	3.13	2.63-3.72	<0.001
Clinical data[a] + PRS1	3.35	2.81-4.00	<0.001
Clinical data[a] + PRS2	3.59	3.00-4.30	<0.001
Clinical data[a] + PRS3	3.59	3.00-4.30	<0.001

Patients with coronary atherosclerosis (n=670) are compared against patients without coronary atherosclerosis (n=273).

[a]The clinical model includes age, sex, hypertension, diabetes, smoking, dyslipidemia, family history of premature CAD and symptoms. OR=odds ratio, CI=confidence interval.

Table 10. The prediction of obstructive CAD by clinical data and three PRS.

Model	OR	95% CI	p-value
PRS1 (PGS000329)	1.25	1.07-1.46	0.006
PRS2 (PGS000018)	1.68	1.42-1.97	<0.001
PRS3 (PGS000013)	1.70	1.44-2.01	<0.001
Clinical data[a]	2.53	2.15-2.98	<0.001
Clinical data[a] + PRS1	2.59	2.20-3.05	<0.001
Clinical data[a] + PRS2	2.97	2.50-3.53	<0.001
Clinical data[a] + PRS3	3.01	2.53-3.57	<0.001

Patients with obstructive CAD (n=205) are compared against patients without obstructive CAD (n=738).

[a]The clinical model includes age, sex, hypertension, diabetes, smoking, dyslipidemia, family history of premature CAD and symptoms. OR=odds ratio, CI=confidence interval.

The PRS appeared to perform better in predicting obstructive CAD than coronary atherosclerosis while clinical data alone predicted atherosclerosis better. The areas under the curve of the PRS models predicting obstructive CAD vary between 0.561-0.640, suggesting rather low predictive power. In contrast, The AUCs of ROC curves in models predicting atherosclerosis vary between 0.589-0.630, indicating mildly lower predictive value than when predicting obstructive CAD.

The ROC curves for combined models (clinical data + PRS) in predicting obstructive CAD are illustrated in Figure 2. The predictive accuracy of the models varies between 0.778-0.805 (in terms of AUROC), being close to the model including only clinical variables (AUROC 0.769) and yielding an improvement of only 0.9-3.6 percentage points over clinical data alone. No statistically significant difference between clinical model and combined model was observed for PRS1

(p=0.627) and PRS3 (p=0.061), respectively. Only PRS2 slightly (AUC change 0.036) but nominally significantly (without Bonferroni correction for four independent tests) improved the predictive power of the model (p=0.04).

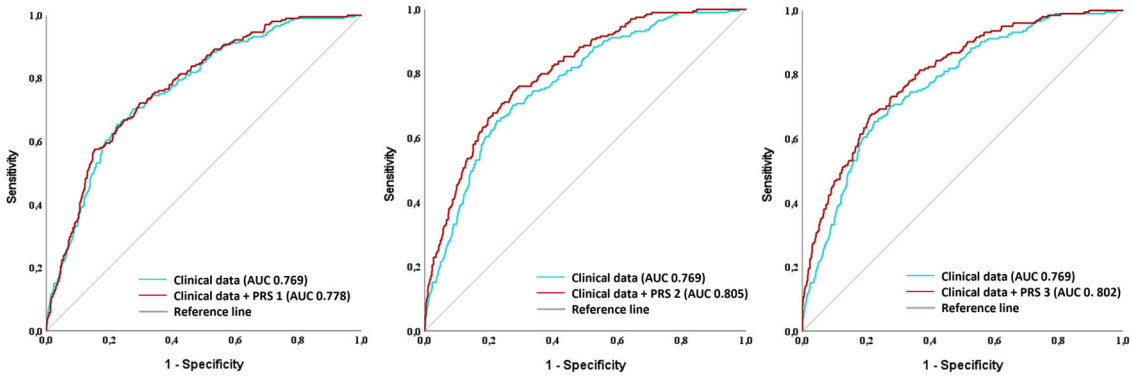

Figure 2. Receiver operating characteristic (ROC) curves of clinical data and PRS in predicting obstructive CAD.

5.3.2 Distribution of PRS

The distribution of the PRS values in patients with and without obstructive CAD are illustrated in Figure 3. In visual observation it is clearly seen that the distribution of the PRS in the two groups were completely overlapping with only minor shift towards higher PRS values in patients with obstructive CAD as compared with those without obstructive CAD. Complete overlap was also seen in distribution plots in the model's predicting atherosclerosis.

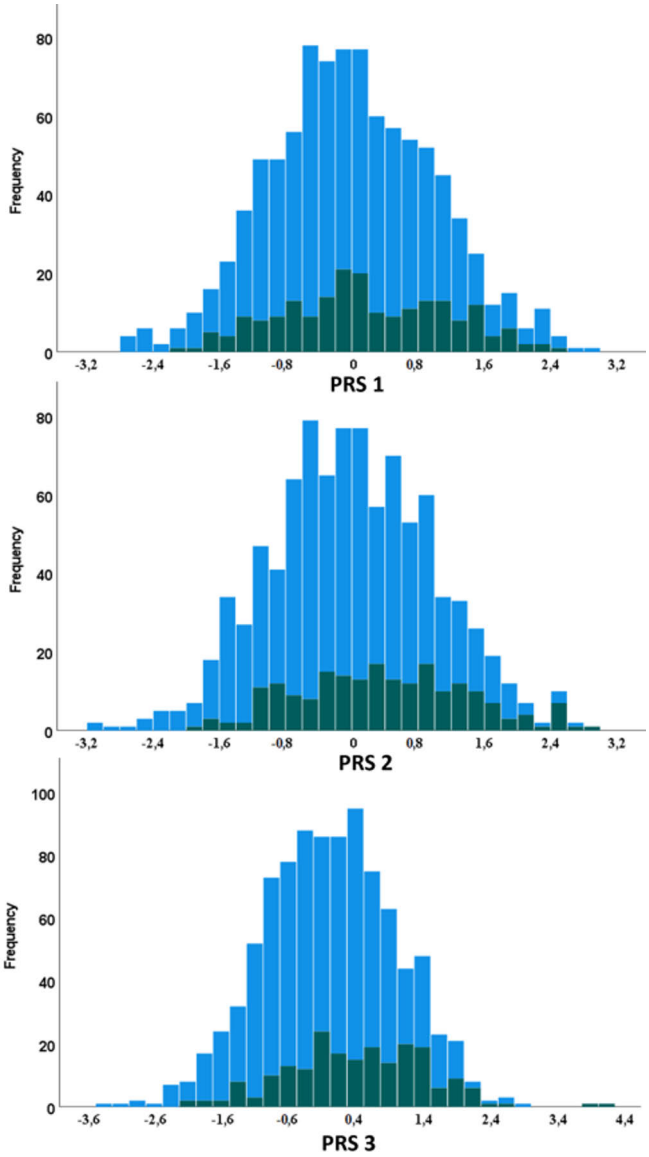

Figure 3. Distribution charts of PRS per standard deviation (SD). Distribution charts demonstrating distribution of PRS per SD for each three PRS separately in patients with (in green) and without (in blue) obstructive CAD.

In Figure 4, the PRS values are categorized to deciles to visualise the relative risk in each PRS category. The prevalence of obstructive CAD was higher in higher PRS deciles but still a large proportion of the patients classified as having high risk by PRS did not have obstructive CAD based on imaging. On the other hand, most of the patients with obstructive CAD had a lower PRS than the highest deciles. Same

results were observed in when predicting atherosclerosis. However, there was relatively less control patients in the highest deciles but as when predicting obstructive CAD, majority of patients with atherosclerosis was not classified in the highest deciles.

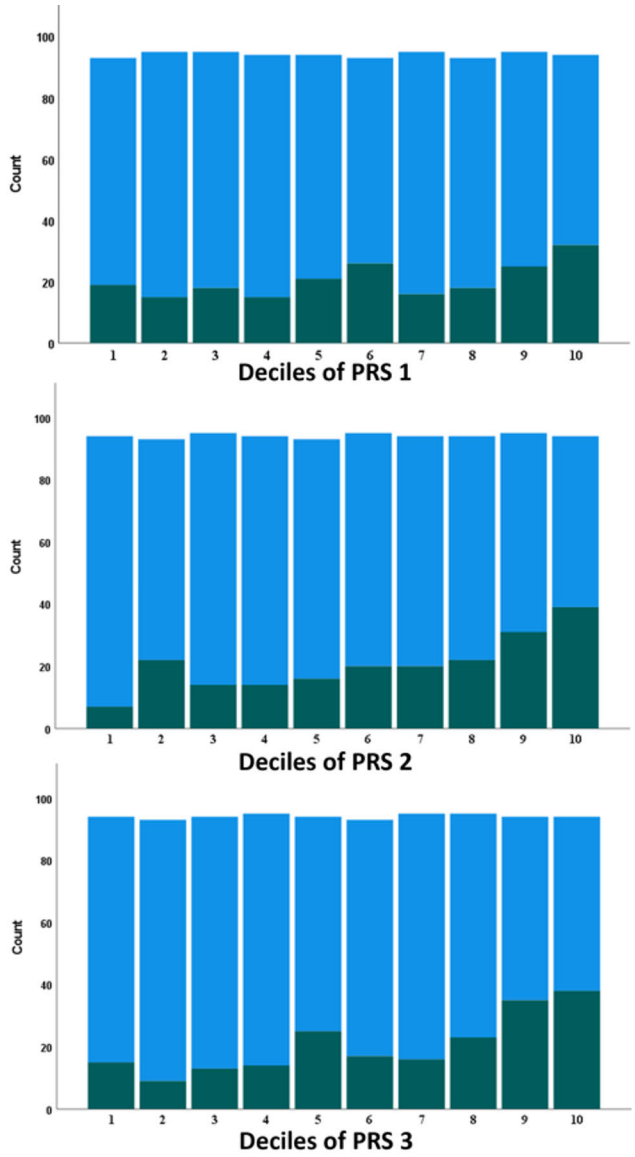

Figure 4. Distribution of PRS in deciles. The distribution of PRSs (in deciles) in participants with (in green) and without (in blue) obstructive CAD.

6 Discussion

6.1 The prognostic value of hybrid imaging in men and women

In this thesis we evaluated the prognostic value of combined CTA and PET MPI (hybrid imaging) in men and women separately and the incremental prognostic value of PET perfusion imaging over coronary CTA**.** In sex specific analyses, we also assessed the differences in CAD characteristics in both sexes.

In line with previous studies, in our cohort, men have more extensive coronary atherosclerosis, higher prevalence of obstructive CAD, and lower event-free survival than women (Pagidipati et al., 2016; Mangion et al., 2020; Lubbers et al., 2017). Our results suggest that the higher event rate in men is due to more extensive CAD. However, it needs to be noted that we did not have data on used medical therapy and adherence to secondary preventative medications. In contrast to recent analyses, our results do not show differences in referral for early invasive management between women and men with myocardial ischaemia (Pagidipati et al., 2016; Walli-Attaei et al., 2020).

In Study I it was shown that CAC and severity of CAD in hybrid imaging predicts the adverse events increasingly as the severity of calcification or atherosclerosis increase in men. However, in a subgroup of patients in whom obstructive CAD was excluded by coronary CTA alone, the presence of non-obstructive CAD was associated with an increased event rate only in women. The higher rate of adverse events could be explained with higher prevalence of CMD in women, which cannot be diagnosed with CTA only. However, it needs to be noted that the number of patients was relatively small, and degree of atherosclerosis was low (77% of patients having CAC score <100) in this subgroup.

Risk stratification is an important goal of diagnostic testing for CAD. Previous analyses have pointed out that there is limited data on differences in prognostic value of noninvasive diagnostic tests for CAD according to sex (Dolor et al., 2012). More recently, retrospective studies have reported equal or even greater prognostic value of coronary CTA in women than in men (Schulman-Marcus et al., 2016, Mangion et al., 2020, Pagidipati et al., 2019). Research have also reported similar prognostic value of myocardial perfusion abnormalities detected by ^{82}Rb or ^{13}N-ammonia PET

perfusion imaging in women and men (Gebhard et al., 2018; Kay et al., 2013). Instead, the prognostic value of hybrid imaging has been widely unexplored. Two previous studies have suggested, that in patients with intermediate coronary plaques, evidence of ischaemia is associated with an increased event risk after hybrid imaging (Maaniitty et al.,2017; Pazhenkottil et al., 2011). However, possible differences in the prognostic value of combined imaging between women and men have remained unexplored.

Study I included a large cohort of patients evaluated for clinically suspected obstructive CAD by selective hybrid imaging in coronary CTA and PET MPI. Abnormal sMBF was observed more often in men than in women in ^{15}O-water PET (63% vs. 36%). Abnormal sMBF was associated with increased risk of death, MI or UAP in both women and men (HR 5.0 and 5.6, respectively). Our study extends previous findings that there was no interaction between sex and the prognostic value of imaging findings based on a hybrid imaging strategy using coronary CTA combined with selective PET perfusion imaging.

In study II we used machine learning approaches (ML) to include large number of parameters, both clinical and imaging variables, in the statistical model. It has been shown that ML is feasible and can improve risk stratification over traditional statistical methods of patients with suspected or known CAD (Juarez-Orozco et al., 2020; Haro etl al., 2019). In this study we further evaluated the incremental value of PET MPI over coronary CTA. It has been previously suggested that combining data from anatomical imaging and functional imaging may further improve the predictive power (Maaniitty et al., 2017). We hypothesized that the presence of ischaemia as a sign of more severe CAD could be stronger in predicting short-term outcome while anatomical findings, reflecting also earlier phases of development of CAD might be better in predicting events in long term. Our findings in Study II supported this hypothesis and illustrated well the complementary nature of these two imaging modalities. In our analyses, the predictive power increased with addition of PET MPI parameters on top of the clinical and coronary CTA variables in the first 4 years of follow-up period, whereas after this the incremental value diminished over the 8 years of follow-up.

In contrast to our study, in a recent study utilizing SPECT/CCTA, hybrid imaging provided independent prognostic information in both short and long-term risk prediction (Pazhenkottil et al., 2018; Pazhenkottil et al., 2011). Unlike in our work, the prognostic benefit of hybrid imaging lasted for 10 years (median 6.8) follow-up (Pazhenkottil et al., 2018). However, in Pazhenkottil et al study, the patient population was rather small (n=357) and included only 46 patients with matched anatomical stenosis and perfusion deficit (Pazhenkottil et al., 2018).

Interestingly, incremental prognostic value of perfusion imaging was not detected when the patients with early revascularisation were excluded from the

analysis. Off note, early revascularisation was not used as an endpoint. In partly overlapping patient population, we have previously shown that the adherence of early revascularisation to myocardial ischaemia detected by PET perfusion imaging is very high (Stenström et al., 2019). As revascularisation is triggered by ischaemic finding, a large proportion of the high-risk patients based on the perfusion findings were revascularised and thus, were excluded from the secondary analysis. These findings further suggest that the increased event risk is associated with abnormal perfusion and the residual risk for adverse events after revascularisation is still high, as a major part of adverse events occurred in this subgroup. This risk is likely associated with generally more severe CAD in patients with detected ischaemia at the time of diagnosis. These findings are consistent with previous studies showing an interplay between the severity of ischaemia, myocardial revascularisation, and outcomes (Taqueti et al., 2015; Gould et al., 2020; Patel et al., 2020; Kumar et al., 2021).

6.2 Clinical utility of PRS

Many previous studies have reported that PRS are feasible in assessing the risk of developing various diseases, including CAD. Different cohort studies have shown that PRS are independently and incrementally associated with CAD in addition to conventional cardiovascular risk factors (Agbaedeng et al., 2021; Elliot et al., 2020; Mars et al., 2020; Inouye et al., 2018; Newman et al., 2022). It has been suggested that PRS can be incorporated into CAD risk prediction (Agbaedeng et al., 2021). However, the clinical utility has not been previously evaluated at individual level, and a validation of various PRS in independent population was not yet available (Agbaedeng et al., 2021)

PRS are typically constructed, and their predictive power is evaluated in large-scale cohorts including hundreds of thousands of patients with healthy individuals and individuals with CAD. However, in this kind of a setting, phenotype is typically very weakly characterized as the diagnoses of CAD are based only on general previous medical reports of having or not having CAD. Therefore, the validation of the performance of the PRS was needed in real-life clinical patients with accurately characterized phenotype. Our unique setting provided valuable information of the usability of PRS in risk stratification on the individual level and enabled comparison against conventional risk factors.

Our analysis elucidated that, although PRS carry some predictive power, it is small and does not appear to provide clinically useful information over routine conventional cardiovascular risk factors in evaluation of incident CAD. The predictive accuracy improved only by 0.9-3.6 percentage points over clinical data when PRS were added. This is in line with a very recent review carried out in

symptomatic primary prevention population. Groenendyk et al. study used five different PRS, two of which were the same as in our study. In that review, the PRS were also significantly associated with the risk of CAD, but the improvement over traditional risk scores ranged from negligible to modest (Groenendyk et al.,2022)

The results illustrate the dilemma of the proposed use of PRS in risk stratification or as a screening test. Odds ratios indicating moderate risk can have significance in population studies in determining causes of the disease, but they are not applicable in risk prediction at the individual level as they do not directly indicate the discriminatory value of a screening test (Wald & Old, 2019; Wald et al., 1999). Despite these facts, there have been big expectations for PRS in the hope of using them in risk stratification in the future.

The challenge of using PRS in clinical work is illustrated in Study III distribution plots. The range of PRS scores are overlapping in healthy, atherosclerotic, and obstructive CAD patients. This demonstrates that these PRS cannot be used to discriminate patients with and without obstructive CAD or atherosclerosis in clinical practice. Furthermore, observing the top deciles (which should stand for the highest genetical risk for CAD), it becomes evident that even in these deciles, most patients did not have CAD, rendering low specificity. In addition, most of the patients with CAD had low PRS making the sensitivity very low even whatever PRS cut-off is applied.

6.3 Future directions

In study III the results illustrated the dilemma of the proposed use of PRS in individual risk stratification. We found that the discriminatory value is very low as well as PRS doesn´t seem to provide clinically useful information over routine cardiovascular risk factors. In addition to this, as PRS can be constructed in different ways (Clifton et al., 2022), the different PRS can give quite varying risk estimates for an individual with minimal concordance (Clifton et al.,2022; Ding et al., 2022). This needs to be further tested in our cohort and can further hamper the usability of PRS as a part of clinical evaluation of patients.

7 Limitations

Study I

Our study is subject to limitations of a retrospective analysis conducted in a cohort where imaging findings were reported to treating physicians. While previous research has indicated gender-related differences in the intensity of secondary prevention efforts (as noted in studies by Pagidipati et al., 2016, and Walli-Attei et al., 2020), our retrospective design did not allow for the assessment of changes in medication following imaging. In our study, we defined abnormal perfusion as any segment with sMBF below the ischemic threshold, a criterion supported by prior research demonstrating the predictive value of this parameter (Danad et al., 2014, and Harjulahti et al., 2021). While the severity and extent of perfusion abnormalities may offer prognostic insights, it's important to note that specific thresholds have not been established for 15O-water PET (Harjulahti et al., 2021, and Van Diemen Pa et al., 2021). Moreover, our patient cohort size was insufficient for detailed subgroup analyses. Reduced coronary flow reserve (CFR) has been linked to an elevated risk of cardiovascular events, particularly in women, even in the absence of obstructive coronary artery disease as demonstrated in studies by Taqueti et al., 2017, and Gupta et al., 2017. In our patient cohort, PET perfusion imaging was primarily performed to assess the hemodynamic significance of suspected obstructive lesions identified in coronary CTA scans. Consequently, we evaluated only sMBF, and not CFR. This means that microvascular dysfunction in cases lacking epicardial CAD and reduced CFR, despite preserved sMBF, could not be detected. Nonetheless, it's worth noting that we have previously published findings suggesting that pure microvascular dysfunction is relatively infrequent in cohorts like the one in our present study (Stenström et al., 2017).

Study II

Patients underwent perfusion imaging based on coronary CTA findings. Thus, it needs to be noted that our results can be only transferred to similar situations than in the present study. It's important to clarify that our machine learning model incorporated all patients referred for coronary CTA, regardless of whether they

subsequently underwent PET perfusion imaging. Patients who had obstructive CAD ruled out by coronary CTA alone, were categorized as having non-obstructive CAD. We believe that this approach mirrors the real-world clinical scenario in which a patient's CAD status is determined by coronary CTA, and no further testing is pursued. However, we acknowledge that conditions like coronary microvascular dysfunction can affect myocardial blood flow even in the absence of obstructive epicardial CAD. Another potential limitation is the lack of detailed coronary CTA characteristics to inform prognostic assessments, such as quantitative plaque volumes. On the other hand, it's worth noting that coronary CTA variables used in our study are standard parameters widely employed in clinical practice (Leipsic et al., 2014).

Study III

This study has several limitations. Firstly, we lack follow-up data regarding the progression of CAD and information about medications used by participants. Secondly, our research focused on three specific PRS, recognizing that there are numerous other PRS options to consider. However, we believe that our selected PRS provide a representative sample that effectively illustrates and assesses the topic at hand. Third, it's essential to note that the ethnicity of our study participants, who are primarily Caucasian Northern Europeans descent in Finland, can significantly influence the discriminatory power of the PRS. Given that the PRS we used were developed based on a similar European ancestry population (as seen in the study by Mars et al., which also included a Finnish population), it is reasonable to assume their applicability to our cohort. Fourth, in our investigation, one of the clinical risk factors under consideration was a family history of premature CAD. It's worth mentioning that this risk factor may encompass certain genetic information, as indicated in the study by Mars et al., where PRS did not offer incremental diagnostic benefit beyond reported family history. Nevertheless, in clinical practice, inquiring about family history is a common, cost-free, and minimally effort-intensive approach, in contrast to the use of PRS. Lastly, it's important to highlight that our analysis did not include access to patient clinical outcomes. Consequently, further research is needed to delve deeper into this topic and its implications.

8 Conclusions

The major conclusions of Studies I-III are as follows:

Study I

Women present more often normal coronary anatomy and less frequently obstructive ischaemic CAD in combined coronary CTA and PET MPI imaging than men. Adverse events (death, MI and UAP) occur in lower rate in women than in men after hybrid imaging. Findings of hybrid imaging independently predict adverse events equally in women and men.

Study II

PET MPI improves risk prediction of adverse events beyond coronary CTA alone for the first 4 years of follow-up, after that no incremental benefit was observed. The results illustrate the differences and complementary nature of anatomic and functional imaging in predicting adverse events of patients with chest pain and suspected obstructive CAD.

Study III

The current PRS predicts the presence of coronary atherosclerosis and obstructive CAD statistically significantly. However, the additional value in risk prediction is limited and appears not to provide clinically useful information beyond conventional cardiovascular risk factors. The results of this study suggest that current PRS are not justified for clinical routine in symptomatic patients referred for evaluation of suspected CAD.

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Original Publications

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ORIGINAL PAPER

Prognostic value of combined coronary CT angiography and myocardial perfusion imaging in women and men

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Aims Combined anatomical and functional imaging enables detection of non-obstructive and obstructive coronary artery disease (CAD) as well as myocardial ischaemia. We evaluated sex differences in disease profile and outcomes after combined computed tomography angiography (CTA) and positron emission tomography (PET) perfusion imaging in patients with suspected obstructive CAD.

Methods and results We retrospectively evaluated 1948 patients (59% women) referred for coronary CTA due to suspected CAD during the years 2008–2016. Patients with a suspected obstructive lesion on coronary CTA ($n = 657$) underwent ^{15}O-water PET to assess stress myocardial blood flow (MBF). During a mean follow-up of 6.8 years, 182 adverse events (all-cause death, myocardial infarction, or unstable angina) occurred. Women had more often normal coronary arteries (42% vs. 22%, $P < 0.001$) and less often abnormal stress MBF (9% vs. 28%, $P < 0.001$) than men. The annual adverse event rate was lower in women vs. men (1.2% vs. 1.7%, $P = 0.02$). Both in women and men, coronary calcification, non-obstructive CAD, and abnormal stress MBF were independent predictors of events. Abnormal stress MBF was associated with 5.0- and 5.6-fold adverse event rates in women and men, respectively. There was no interaction between sex and coronary calcification, non-obstructive CAD, or abnormal stress MBF in terms of predicting adverse events.

Conclusion Among patients evaluated for chronic chest pain, women have a lower prevalence of ischaemic CAD and a lower rate of adverse events. Combined coronary CTA and PET myocardial perfusion imaging predict outcomes equally in women and men.

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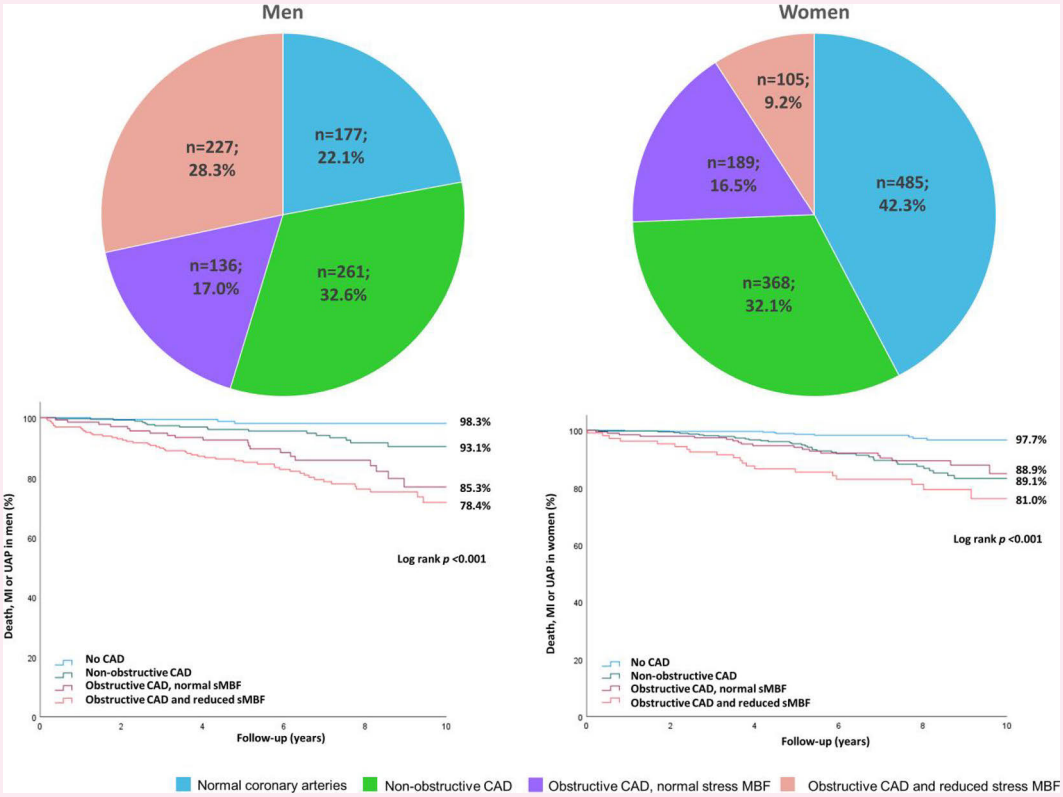
Graphical Abstract



Keywords chronic chest pain • computed tomography angiography • coronary artery disease • positron emission tomography • hybrid imaging • sex

Introduction

There are differences in the diagnostic accuracy of non-invasive tests to assess obstructive coronary artery disease (CAD) between women and men.[1–3] Recent comparative studies indicate that in women anatomic evaluation by coronary computed tomography angiography (CTA) is associated with a lower rate of CAD diagnosis than noninvasive functional tests as compared to men.[4–6] Furthermore, women may gain similar or even more prognostic information from coronary CTA than stress testing for ischaemia, whereas men benefit equally from both of these testing modalities.[4–6]

Hybrid or combined imaging using coronary CTA and functional testing for ischaemia enables accurate detection of non-obstructive and obstructive CAD as well as assessment of the haemodynamic significance of coronary stenosis.[7,8] Clinical practice guidelines recommend functional evaluation of CAD before revascularization decisions.[9] We have constructed an observational registry of patients in whom the haemodynamic significance of any suspected obstructive stenosis detected by coronary CTA was routinely evaluated by ^{15}O-water positron emission tomography (PET) myocardial perfusion imaging.[10] We previously demonstrated that normal stress myocardial blood flow (sMBF) despite obstructive CAD on CTA, is associated with favourable prognosis.[10] However, the prognostic value of combined anatomical and functional imaging in men vs. women remains unknown.

We hypothesized that a detailed assessment of CAD by combining anatomical and functional imaging reveals differences in characteristics of CAD, while the prognostic value of diagnostic testing in women vs. men with chronic chest pain would be the same. For this purpose, we compared the findings and prognostic value of combined coronary CTA and ^{15}O-water PET perfusion imaging among women and men with suspected CAD.

Methods

Study cohort

We retrospectively identified all consecutive patients referred for coronary CTA in the Turku PET Centre due to suspected obstructive CAD in the period of 2008–2016. We did not include patients with known CAD (previous coronary revascularization or ≥50% diameter stenosis on invasive coronary angiography, ICA) or those referred primarily for reasons other than suspected symptomatic CAD, such as aetiological evaluation of heart

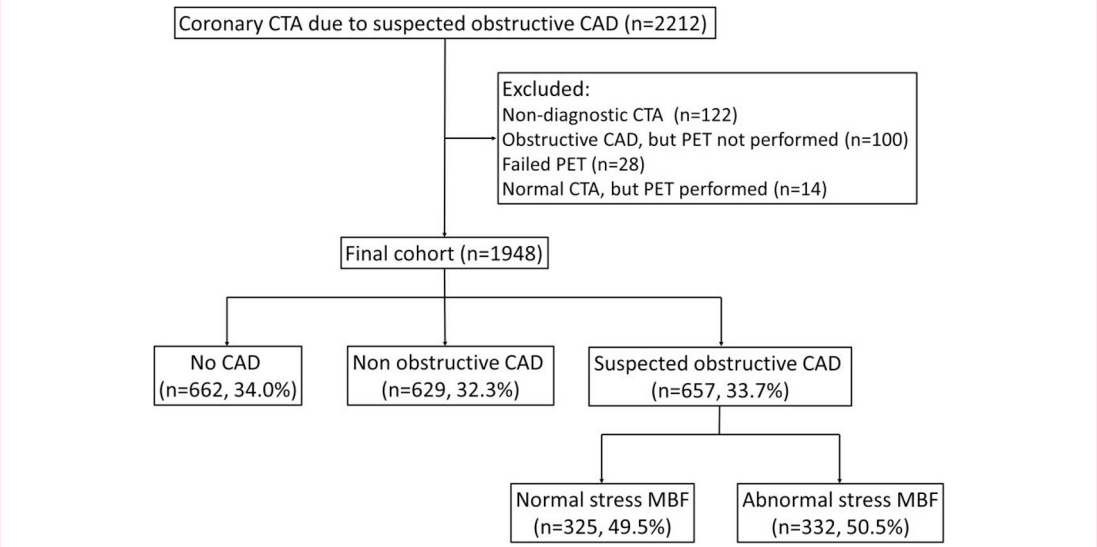


Figure 1 Flow chart of patient selection and classification according to imaging findings.

failure or pre-operative evaluation. In cases of repeat tests during the study period, only the earliest test was included in the current analysis.

As previously described,[10,11] it is routine practice in our hospital that patients initially undergo coronary CTA using a hybrid PET-CT scanner, and immediately after coronary CTA, the attending physician evaluates the CTA scan to decide whether to perform PET myocardial perfusion imaging during the same visit. If the coronary CTA reveals obstructive CAD (diameter stenosis ≥50%), the haemodynamic significance of lesions is evaluated using ^{15}O-water PET during adenosine stress. Out of the 2212 patients identified, we excluded 122 patients who did not complete the combined imaging protocol for reasons outlined in *Figure 1*, including 61 patients referred directly for ICA without PET. Thus, the final analysis included 1948 patients.

The study complies with the Declaration of Helsinki. The Ethics Committee of the Hospital District of Southwest Finland approved the study protocol and waived the need for written informed consent by patients. The Finnish Institute for Health and Welfare gave permission to the retrospective collection of clinical data.

Coronary CTA and PET image acquisition and interpretation

Coronary CTA and PET scans were performed using a 64-row hybrid PET-CT scanner (GE Discovery VCT or GE D690, General Electric Medical Systems, Waukesha, Wisconsin) using previously described procedures.[7,10]

Coronary artery calcium (CAC) scans were performed before coronary CTA in the majority of patients. Based on the CAC score, patients were categorized into those with no coronary calcification (CAC score = 0), mild calcification (CAC score = 1–99), moderate calcification (CAC score = 100–399), and extensive calcification (CAC score ≥400).

Before coronary CTA, metoprolol was given intravenously to achieve a target heart rate of <60 beats/min. Isosorbide dinitrate aerosol (1.25 mg) was administered for coronary artery vasodilation. Coronary CTA was performed using an intravenously administered low-osmolal iodine contrast agent followed by a saline flush. The prospectively triggered acquisition was applied whenever feasible. Collimation was set at 64 × 0.625 mm, gantry rotation time was 350 ms, tube current 600–750 mA, and voltage 100–120 kV, depending on patient size. Coronary CTA scans were analysed in a standardized fashion, reporting the presence of atherosclerotic plaque and obstructive stenosis (≥50% in diameter) for each coronary artery segment.

Before the PET scan, patients fasted overnight and abstained from alcohol and caffeine for 24 h. Adenosine infusion started 2 min before the stress PET scan and continued at a rate of 140 μg/kg/min until the scan was complete. ^{15}O-water (Radiowater Generator, Hidex Oy, Turku, Finland) was injected as an intravenous bolus (mean injected activity 900–1100 MBq) over 15 s followed by dynamic PET acquisition (14 × 5 s, 3 × 10 s, 3 × 20 s, and 4 × 30 s).

The PET data were analysed quantitatively using Carimas software (developed at Turku PET Centre, Turku, Finland). Absolute sMBF (mL/g/min) was quantified individually for each of the standard 17 myocardial segments. Basal septal segments (segments 2 and 3) were excluded from the analysis. Abnormal stress MBF was defined as ≤2.3 mL/g/min in ≥1 segment based on previous research.[12]

Based on both CTA and PET findings we classified patients into four categories: (i) normal coronary arteries; (ii) non-obstructive CAD based on CTA; (iii) obstructive CAD by CTA and normal sMBF; and (iv) obstructive CAD by CTA and abnormal sMBF.

Clinical and follow-up data collection

Data on traditional cardiovascular risk factors (smoking, diabetes mellitus, hypertension, dyslipidemia, and family history of CAD), symptoms, and medications were retrospectively collected from electronic medical records.

The primary outcome was a composite of all-cause death, non-fatal myocardial infarction (MI), or unstable angina pectoris (UAP) based on comprehensive data until May 2020 in the registries of the Finnish Institute for Health and Welfare and the Centre for Clinical Informatics of the Turku University Hospital. Investigators used electronic medical records to validate all adverse events. Data of early revascularization within 6 months after coronary CTA, with either percutaneous coronary intervention (PCI) or

Table 1 Clinical characteristics, imaging findings, and early invasive therapies of all patients, men, and women

	Cohort ($n = 1948$)	Men ($n = 801$)	Women ($n = 1147$)	*P* value
Age (years)[a]	62.9 ± 9.6	59.9 ± 10.6	63.4 ± 9.3	<0.001
Body mass index (kg/m^2)[a]	27.9 ± 6.6	27.9 ± 5.7	28.0 ± 7.3	0.6
Current or ex-smoking	625 (32.1)	338 (42.2)	287 (25.0)	<0.001
Diabetes mellitus	288 (14.8)	137 (17.1)	151 (13.2)	0.01
Hypertension	1083 (55.6)	455 (56.8)	628 (54.8)	0.3
Dyslipidemia	1221 (62.7)	497 (62.0)	724 (63.1)	0.6
Family history of CAD	918 (47.1)	301 (37.6)	617 (53.8)	<0.001
Typical chest pain	411 (22.1)	153 (20.0)	258 (23.6)	
Atypical/non-cardiac chest pain	963 (49.4)	371 (46.3)	592 (51.6)	
Dyspnoea	755 (38.8)	261 (32.6)	494 (43.1)	<0.001
Coronary artery calcium score ($n = 1594$)				<0.001
0	578 (36.3)	152 (23.2)	426 (45.3)	
1–99	467 (29.3)	185 (28.3)	282 (30.0)	
100–399	304 (19.1)	159 (24.3)	145 (15.4)	
> 400	245 (15.4)	158 (24.2)	87 (9.3)	
Coronary CTA and PET findings				<0.001
No CAD	662 (34.0)	177 (22.1)	485 (42.3)	
Non-obstructive CAD	629 (32.3)	261 (32.6)	368 (32.1)	
Obstructive CAD and normal sMBF	325 (16.7)	136 (17.0)	189 (16.5)	
Obstructive CAD and abnormal sMBF	332 (17.0)	227 (28.3)	105 (9.2)	
Invasive coronary angiography	217 (11.1%)	140 (17.5)	77 (6.7)	<0.001
Early revascularization (6 months)	127 (6.5%)	86 (10.7%)	41 (3.6%)	<0.001
PCI	112 (5.7%)	74 (9.2)	38 (3.3)	<0.001
CABG	18 (0.9%)	15 (1.9)	3 (0.3)	<0.001
Follow-up events				
Death	126 (6.5)	60 (7.5)	66 (5.8)	0.1
MI	45 (2.3)	23 (2.9)	22 (1.9)	0.1
UAP	21 (1.1)	11 (1.4)	10 (0.9)	0.2
Death or MI	164 (8.4)	80 (10.0)	84 (7.3)	0.03
Death, MI or UAP	182 (9.4)	90 (11.3)	92 (8.0)	0.01

[a]mean ± standard deviation (range).
CAD, coronary artery disease; CABG, coronary artery bypass grafting; CTA, computed tomography angiography; MI, myocardial infarction; PCI, percutaneous coronary intervention; PET, positron emission tomography; sMBF, stress myocardial blood flow; UAP, unstable angina pectoris.

coronary artery bypass graft (CABG) surgery, were also collected, but not used as endpoints. In case of the occurrence of multiple adverse events, the first event was considered.

Statistical analysis

Continuous variables are reported as mean and standard deviation (SD) or median [interquartile range], as appropriate. Categorical variables are shown as count (percentage). The χ^2 test, Fisher Exact test, student *t*-test, and Mann–Whitney U test were used as appropriate. The cumulative incidence of events was based on Kaplan–Meier estimates and was compared between women and men using the log-rank test. Cox proportional hazards models were used to identify the univariable and multivariable predictors in the whole cohort, men, and women. The clinical variables bearing significant association with the composite outcome in the univariable analysis were added to the clinical multivariable model as covariates. Those covariates bearing significant association with the composite outcome in the clinical multivariable model were then transferred as covariates to multivariable models including CAC or PET-CTA findings. The prognostic value of the multivariable models to predict the composite outcome was tested using the receiver operating characteristics (ROC) curve analysis and time-dependent ROC as implemented in the R package time ROC.[13] The statistical difference between the ROC curves was determined using the DeLong method[14] using R package pROC.[15] Furthermore, the interaction between sex on one hand and either the CAC or PET-CTA findings, on the other hand, was tested using Cox proportional hazards models. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using SPSS v. 25.0 (IBM Corporation, New York, USA) statistical software and R statistical computing environment version 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/).

Results

The cohort consisted of 1948 patients including 1147 (58.9%) women with suspected obstructive CAD who underwent coronary CTA. In

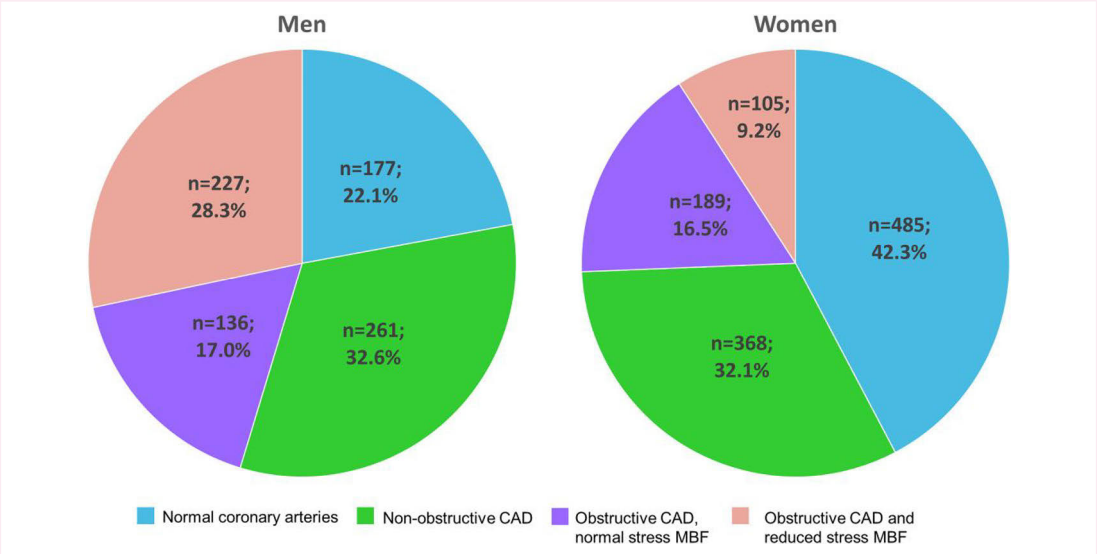


Figure 2 Graphs showing proportions of women and men with normal coronary arteries, non-obstructive CAD, obstructive CAD and normal sMBF, and obstructive CAD and abnormal sMBF.

1291 (66.3%) patients, obstructive CAD was excluded based on coronary CTA alone, whereas 657 (33.7%) had ^{15}O-water PET myocardial perfusion imaging to evaluate haemodynamic significance of suspected obstructive coronary stenosis. As shown in *Figure 1*, 34.0% of patients had normal coronary arteries, 32.3% had non-obstructive CAD, 16.7% had obstructive CAD on coronary CTA and normal sMBF, and 17.0% had obstructive CAD and abnormal sMBF. Supplementary data online, *Figure S1* shows an example of findings.

Baseline characteristics of men vs. women

Characteristics of patients are shown in *Table 1*. Women were slightly older, and had less frequently diabetes, and more frequent family history of premature CAD than men. Women were less likely to have a smoking history than men, but there was no difference in body mass index or the prevalence of either dyslipidemia or hypertension. There were only four women and eight men with known left ventricular systolic dysfunction (ejection fraction <40%).

The most common presenting symptom was atypical angina or non-anginal chest pain. Typical angina was more common in women than men. Women also reported dyspnoea more often than men.

Sex differences in coronary CTA and PET perfusion findings

Women were more likely to have normal coronary arteries than men (42.3% vs. 22.1% $P < 0.001$) while abnormal sMBF was less frequent in women than men (9.2% vs. 28.3% $P < 0.001$, *Table 1* and *Figure 2*). Hemodynamically non-significant CAD was present similarly in women and men (48.6% vs. 49.6%). However, the CAC score was higher in men than women.

Follow-up

The mean time of follow-up was 6.8 ± 2.5 years with a median of 6.65 years. During the follow-up, there were 126 deaths, 45 MIs, and 21 UAPs. The composite outcome of death, MI, or UAP occurred in 182 patients and was less frequent in women than men (8.0% vs. 11.3%, $P = 0.01$, *Table 1*).

Most (190 out of 217) early ICAs were performed in patients with ischaemic CAD (obstructive CAD on CTA and abnormal sMBF). Similarly, most revascularizations were performed in patients with ischaemic CAD (123 out of 127). Among 332 patients with ischaemic CAD, there was no difference in the ICA referral rate between women and men (60.0% vs. 55.9%, $P = 0.4$). A similar proportion of women and men with ischaemic CAD had early revascularization (36.2 vs. 37.4%, $P = 0.8$), and the revascularization rate among those undergoing ICA was similar (53.2% vs. 61.4%, $P = 0.2$). Among patients with ischaemic CAD, there was no significant difference in the cumulative incidence of events at long-term follow-up between patients who underwent early revascularization (17.9%) vs. were treated conservatively (22.5%, $P = 0.4$).

Predictors of events in women and men

Male sex was an independent risk factor for adverse events. Age and dyspnoea were associated with adverse events in women, whereas in men, age, diabetes, hypertension, and typical angina predicted events (*Table 3*).

Coronary calcification was an independent predictor of events after adjustment for age, clinical risk factors, and symptoms both in women and men (*Tables 2* and *3*). Extensive coronary calcification (CAC score ≥400) predicted events with an adjusted hazard ratio of 20.07 (95%CI 2.63–153.10) in men and 4.94 (95%CI 2.31–10.58) in women, when compared to patients without coronary calcification (CAC score 0).

Findings of PET-CTA were predictors of events after adjusting for age, clinical risk factors, and symptoms both in women and men (*Table 3*). As compared to patients with normal coronary arteries, the presence of abnormal sMBF predicted events with an adjusted hazard ratio of 5.61 (95%CI 1.69–18.57) in men and 4.99 (95%CI 2.33–10.70) in women. Non-obstructive CAD predicted adverse events in women, but the effect was statistically non-significant in men (*Table 3*).

Table 2 Univariable predictors of adverse events (all-cause death, myocardial infarction, or unstable angina pectoris) in all patients, men and women

	All patients		Men		Women	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age	1.07 (1.05–1.09)	<0.001	1.07 (1.04–1.09)	<0.001	1.08 (1.05–1.11)	<0.001
Male sex	1.39 (1.04–1.86)	0.02				
DM	1.68 (1.18–2.40)	0.004	2.17 (1.37–3.43)	<0.001	1.13 (0.63–2.04)	0.6
Hypertension	1.86 (1.36–2.56)	<0.001	2.11 (1.33–3.35)	0.001	1.64 (1.07–2.54)	0.02
Smoking	1.23 (0.91–1.66)	0.1	1.32 (0.87–1.99)	0.1	0.98 (0.61–1.57)	0.9
Dyslipidemia	0.97 (0.72–1.32)	0.8	1.07 (0.69–1.64)	0.7	0.90 (0.59–1.38)	0.6
Typical chest pain	1.52 (1.09–2.14)	0.01	2.00 (1.25–3.20)	0.004	1.21 (0.74–1.96)	0.4
Any chest pain	0.88 (0.63–1.24)	0.4	0.90 (0.56–1.42)	0.6	0.94 (0.56–1.56)	0.8
Dyspnoea	1.88 (1.41–2.52)	<0.001	1.69 (1.11–2.56)	0.01	2.31 (1.51–3.53)	<0.001
CAC						
0	Reference		Reference		Reference	
1–99	3.23 (1.74–6.01)	<0.001	11.52 (1.50–88.10)	0.01	2.68 (1.35–5.32)	<0.001
100–399	5.42 (2.92–10.06)	<0.001	23.24 (3.12–172.92)	0.002	3.73 (1.77–7.85)	<0.001
≥400	10.11 (5.61–18.23)	<0.001	40.48 (5.53–295.91)	<0.001	7.20 (3.55–14.59)	<0.001
PET-CTA						
No CAD	Reference		Reference		Reference	
Non-obstructive CAD	4.57 (2.55–8.19)	<0.001	4.21 (1.24–14.30)	0.02	5.17 (2.65–10.09)	<0.001
Obstructive CAD, normal sMBF	6.01 (3.28–11.03)	<0.001	9.30 (2.76–31.31)	<0.001	4.75 (2.29–9.87)	<0.001
Obstructive CAD, abnormal sMBF	10.01 (5.63–17.79)	<0.001	13.56 (4.22–43.52)	<0.001	8.04 (3.85–16.80)	<0.001

HR for age is per 1 year.
CAC, coronary artery calcium, CAD, coronary artery disease, CI, confidence interval, DM, diabetes mellitus, HR, hazard ratio.

Based on Cox proportional hazards models, there was no significant interaction between sex and either coronary calcification or combined coronary CTA and PET findings ($P = 0.4$, and $P = 0.2$, respectively) in predicting adverse events. Similarly, based on the ROC analyses the prognostic value of adjusted coronary artery calcification or combined coronary CTA and PET to predict composite outcome was similar in males and females ($P = 0.687$ and $P = 0.999$, *Figure 3* and Supplementary data online, *Figure S2*). Overall, coronary calcification and findings of coronary CTA and PET predicted events equally ($P = 0.477$).

Figure 4 shows Kaplan–Meier estimates of the cumulative incidence of the composite events that were significantly different ($P > 0.001$) according to the extent of CAC and findings of PET-CTA in both men and women. Overall, the event rate was higher in men than women (Log-rank $P = 0.02$) with the annual adverse event rate being 1.37% in the whole cohort, 1.65% in men, and 1.17% in women.

Discussion

This study compared the findings and prognostic value of combined coronary CTA and PET myocardial perfusion imaging between women and men referred to diagnostic testing for suspected obstructive CAD. The main finding of our study is that coronary CTA combined with ^{15}O-water PET myocardial perfusion imaging predicts adverse events equally well in both women and men.

Our results are in line with recent studies showing that among patients evaluated for suspected CAD, men have more coronary atherosclerosis, a higher prevalence of obstructive CAD, and lower event-free survival than women.[4,5,6] Unlike some recent analyses,[4,16] our results do not show differences in referral for ICA or early invasive management between women and men with myocardial ischaemia. The observed revascularization rate is in line with previous studies.[11] Although we do not have data on medical management and adherence to preventive therapies, our results suggest that the higher event rate in men is due to more extensive CAD.

Risk stratification is an important goal of diagnostic testing for CAD. Previous analyses have pointed out that there is limited data on differences in the prognostic value of noninvasive diagnostic tests for CAD, particularly coronary CTA, according to sex.[1] More recently, retrospective studies have reported equal predictive value of per vessel extent of CAD by coronary CTA in women and men.[17] In the Scottish Computed Tomography of the Heart (SCOT-HEART) trial, the addition of coronary CTA to a standard evaluation of patients with chest pain showed similar benefits in women and men.[5] In the Prospective Multicenter Imaging Study for Evaluation of Chest Pain (PROMISE) trial, women appeared to derive even more prognostic information from CTA than men who tend to derive similar prognostic value from both anatomical and functional tests.[18] Our results are in line with these previous studies demonstrating the prognostic value of a diagnostic strategy based on initial coronary CTA in both women and men.

The finding that CAC and non-obstructive CAD were predictive of events is consistent with the prognostic significance of atherosclerotic plaque.[19] In a subgroup of patients in whom obstructive CAD was excluded by coronary CTA alone, the presence of non-obstructive CAD was associated with an increased event rate only in women. However, the number of patients was relatively small and the degree of atherosclerosis was small (77% of patients having CAC score <100) in this subgroup.

Table 3 Multivariable predictors of adverse events (all-cause death, myocardial infarction, or unstable angina pectoris) in all patients, men and women

	All patients			Men			Women		
Model	**HR (95% CI)**	***P* value**	**AUC**	**HR (95% CI)**	***P* value**	**AUC**	**HR (95% CI)**	***P* value**	**AUC**
Clinical			0.706			0.728			0.705
Age	1.06 (1.04–1.08)	<0.001		1.06 (1.03–1.08)	<0.001		1.07 (1.04–1.10)	<0.001	
Male sex	1.81 (1.32–2.46)	<0.001							
Diabetes				1.77 (1.09–2.89)	0.03				
Hypertension	1.61 (1.15–2.25)	0.004		2.03 (1.21–3.40)	0.009				
Typical angina	1.55 (1.10–2.18)	0.01		2.01 (1.24–3.25)	0.01				
Dyspnoea	1.60 (1.17–2.18)	0.003					1.88 (1.22–2.89)	0.004	
Clinical + CAC			0.730			0.746			0.720
Age	1.03 (1.01–1.05)	0.007		1.03 (1.00–1.06)	0.04		1.03 (1.00–1.07)	0.03	
Diabetes				1.76 (1.00–3.10)	0.04				
Dyspnoea	1.50 (1.05–2.04)	0.02					1.77 (1.08–2.89)	0.02	
CAC									
0	reference			reference			reference		
1–99	2.75 (1.44–5.27)	0.002		7.83 (1.00–61.12)	0.04		2.11 (1.04–4.27)	0.03	
100–399	4.23 (2.19–8.15)	<0.001		14.11 (1.84–108.01)	0.01		2.67 (1.22–5.80)	0.01	
≥ 400	7.20 (3.80–13.65)	<0.001		20.07 (2.63–153.10)	0.004		4.94 (2.31–10.58)	<0.001	
Clinical + PET-CTA			0.743			0.753			0.753
Age	1.05 (1.03–1.07)	<0.001		1.05 (1.02–1.08)	<0.001		1.05 (1.02–1.08)	<0.001	
Hypertension				1.81 (1.08–3.03)	0.02				
Dyspnoea	1.52 (1.11–2.08)	0.009					1.75 (1.13–2.69)	0.01	
PET-CT findings									
No CAD	Reference			Reference			Reference		
Non-obstructive CAD	3.36 (1.78–6.37)	<0.001		2.24 (0.64–7.80)	0.2		3.63 (1.83–7.20)	<0.001	
Obstructive CAD, normal sMBF	3.85 (1.97–7.52)	<0.001		4.37 (1.26–15.11)	0.02		2.99 (1.40–6.38)	0.005	
Obstructive CAD, abnormal sMBF	5.84 (3.04–11.21)	<0.001		5.61 (1.69–18.57)	0.005		4.99 (2.33–10.70)	<0.001	

The models are adjusted for significant clinical factors, CAC scores, and PET-CTA findings separately.
HR for age is per 1 year. *P*-values for comparing AUC values between models (clinical + CAC and clinical + PET-CTA) were 0.477 for all patients, 0.625 for men and 0.394 for women.
CAD, coronary artery disease; CAC, coronary calcium; CI, confidence interval; HR, hazard ratio; sMBF, stress myocardial blood flow.

Combined or hybrid imaging enables the evaluation of both coronary anatomy by CTA and the detection of myocardial ischaemia by perfusion imaging. Previous evidence supports the diagnostic and prognostic value of SPECT perfusion imaging in women and men.[1,3,20,21] Studies have also reported the similar prognostic value of myocardial perfusion abnormalities detected by ^{82}Rb or ^{13}N-ammonia PET perfusion imaging in women and men.[20,21] Instead, there is limited evidence on the prognostic value of hybrid imaging. Two studies have shown that in patients with intermediate coronary lesions, evidence of ischaemia is associated with an increased event risk after hybrid imaging.[10,22] However, possible differences in the prognostic value of combined imaging between women and men have remained unexplored. Our study included a large cohort of patients evaluated for suspected obstructive CAD by coronary CTA followed by ^{15}O-water PET. Instead of all patients, stress MBF was evaluated by ^{15}O-water PET in 657 patients (45% women) who had suspected stenosis detected by coronary CTA. ^{15}O-water PET showed abnormal sMBF more often in men than in women (63% vs. 36%). Abnormal sMBF was associated with an increased risk of death, MI, or UAP in both women and men (HR 5.0 and 5.6, respectively). Our study extends previous findings in that there was no interaction between sex and the prognostic value of imaging findings based on a hybrid imaging strategy using coronary CTA combined with selective PET perfusion imaging.

Study limitations

Our study is associated with all limitations of retrospective analysis in a cohort where imaging findings were reported to treating physicians. Previous studies suggest differences in the intensity of secondary prevention in men and women,[4,16] but in this retrospective study, it was not feasible to evaluate changes in medication after imaging. Furthermore, since results of laboratory tests were available for a limited number of patients, some potentially relevant prognostic factors, such as anaemia, blood cholesterol or glucose levels, or renal dysfunction could not be included in the analyses. We defined abnormal perfusion as any segment with sMBF below the ischaemic threshold based on previous studies showing the predictive value of this parameter.[12,23] The severity and extent of perfusion abnormalities may add prognostic information, but thresholds have not been established for ^{15}O-water PET[23,24] and our patient cohort was small for subgroup analyses. Reduced coronary flow reserve (CFR) has been associated with an excess risk of cardiovascular events in the absence of obstructive CAD,

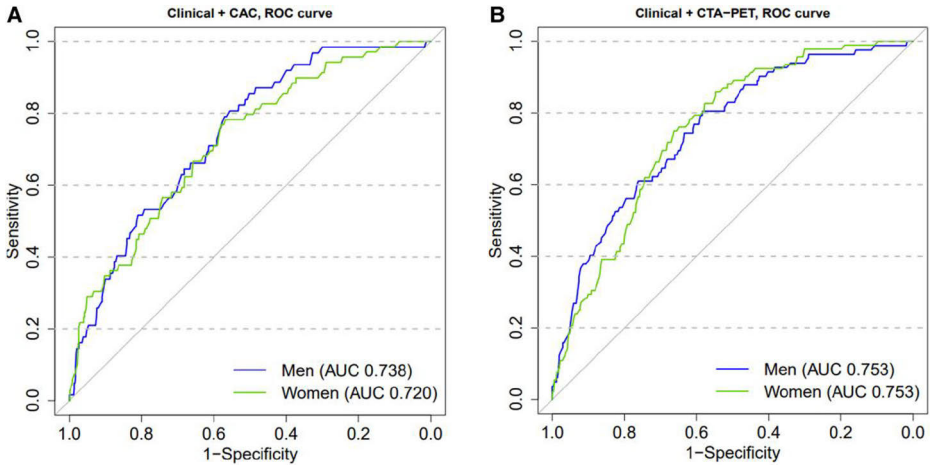


Figure 3 Receiver operating characteristic (ROC) curves showing no difference in the performance of coronary artery calcium (CAC) score ($P = 0.687$) (*A*) or combined coronary CTA and ^{15}O-water PET ($P = 0.999$) (*B*) in predicting composite end-point of death, non-fatal MI or UAP between men and women. Analyses were adjusted for age, hypertension, and symptom status (typical angina and dyspnoea).

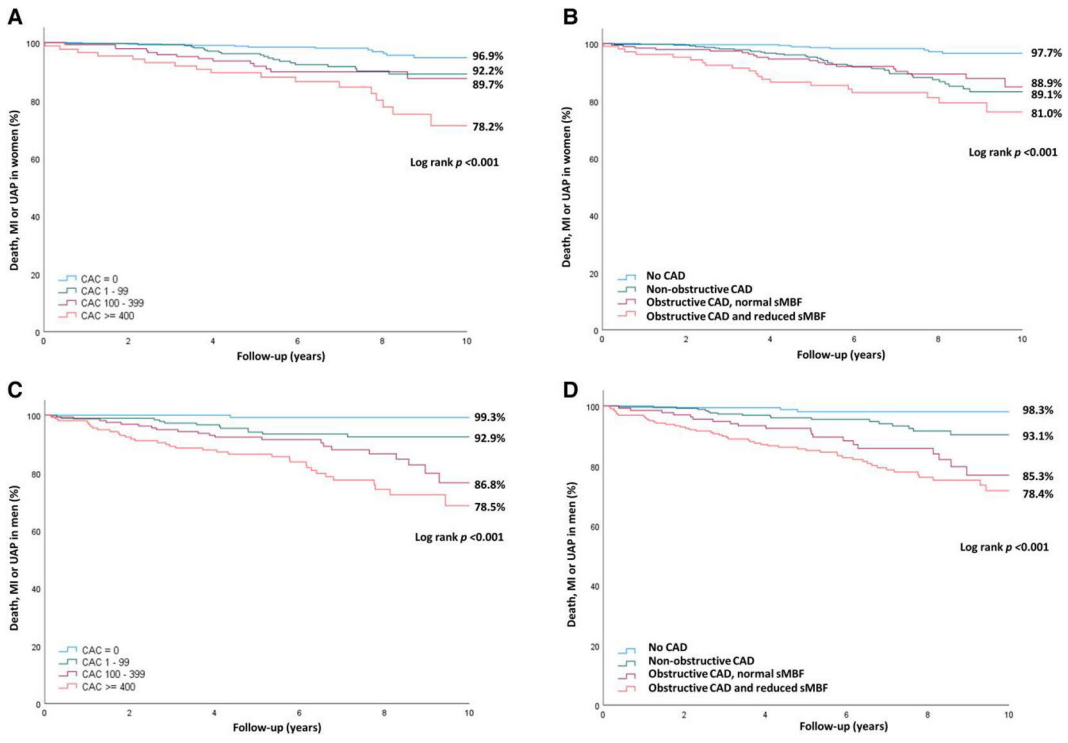


Figure 4 Kaplan–Meier curves comparing the occurrence of death, non-fatal MI or UAP according to coronary artery calcium (CAC) score (*A* and *C*) or combined coronary CTA and ^{15}O-water PET (*B* and *D*) in women (*A* and *B*) and men (*C* and *D*).

particularly in women.[25,26] In our patient cohort, PET perfusion imaging was performed only for evaluation of haemodynamic significance of suspected obstructive lesions on coronary CTA and instead of CFR, only sMBF was assessed.[12] Therefore, microvascular dysfunction in the absence of epicardial CAD as well as reduced CFR despite preserved sMBF remains undetected. However, we have previously published that the frequency of pure microvascular dysfunction is low in a similar cohort as in this study.[27] Our study provides novel prognostic information about hybrid or combined imaging in women and men evaluated for suspected CAD.

Conclusion

Combined anatomical and functional imaging shows more often normal coronary anatomy and less frequently ischaemic CAD in women than in men. Women have a lower rate of adverse events including death, MI, or UAP, after diagnostic testing. Findings of coronary CTA combined with PET myocardial perfusion imaging independently predict adverse events equally in women and men.

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Supplementary data

Supplementary data is available at *European Heart Journal - Cardiovascular Imaging* online.

Conflict of interest: Dr. Knuuti received consultancy fees from GE Healthcare and speaker fees from GE Healthcare, Bayer, Lundbeck, Boehringer-Ingelheim, Pfizer, Siemens, and Merck, outside of the submitted work. Dr. Saraste received consultancy fees from Amgen and Astra Zeneca, Boehringer Ingelheim and Pfizer, and speaker fees from Abbott, Astra Zeneca, and Bayer outside of the submitted work. Dr. Bax received speaker fees from Abbot Vascular. The Department of Cardiology, Leiden University Medical Center, Leiden, The Netherlands has received unrestricted research grants from Bayer, Abbott Vascular, Medtronic, Biotronik, Boston Scientific, GE Healthcare, and Edwards Lifesciences. Other authors have no conflicts of interest to declare.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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ORIGINAL PAPER

Incremental prognostic value of downstream positron emission tomography perfusion imaging after coronary computed tomography angiography: a study using machine learning

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Aims To evaluate the incremental value of positron emission tomography (PET) myocardial perfusion imaging (MPI) over coronary computed tomography angiography (CCTA) in predicting short- and long-term outcome using machine learning (ML) approaches.

Methods and results A total of 2411 patients with clinically suspected coronary artery disease (CAD) underwent CCTA, out of whom 891 patients were admitted to downstream PET MPI for haemodynamic evaluation of obstructive coronary stenosis. Two sets of Extreme Gradient Boosting (XGBoost) ML models were trained, one with all the clinical and imaging variables (including PET) and the other with only clinical and CCTA-based variables. Difference in the performance of the two sets was analysed by means of area under the receiver operating characteristic curve (AUC). After the removal of incomplete data entries, 2284 patients remained for further analysis. During the 8-year follow-up, 210 adverse events occurred including 59 myocardial infarctions, 35 unstable angina pectoris, and 116 deaths. The PET MPI data improved the outcome prediction over CCTA during the first 4 years of the observation time and the highest AUC was at the observation time of Year 1 (0.82, 95% confidence interval 0.804–0.827). After that, there was no significant incremental prognostic value by PET MPI.

Conclusion PET MPI variables improve the prediction of adverse events beyond CCTA imaging alone for the first 4 years of follow-up. This illustrates the complementary nature of anatomic and functional information in predicting the outcome of patients with suspected CAD.

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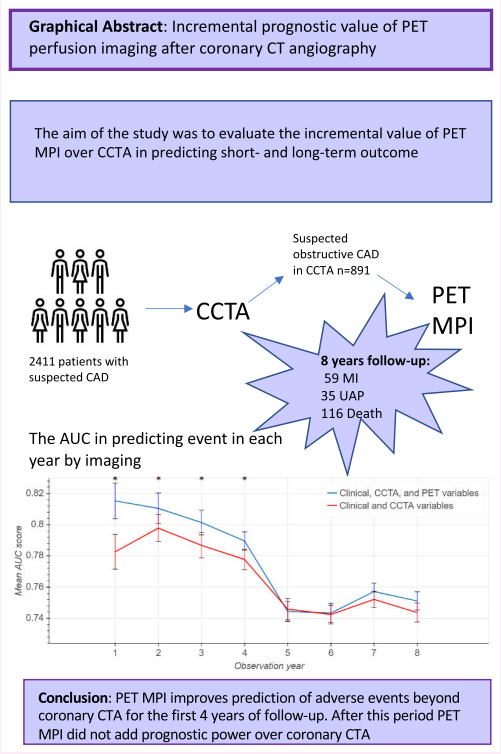
Graphical Abstract



Keywords positron emission tomography • myocardial perfusion imaging • coronary computed tomography angiography • machine learning • extreme gradient boosting

Introduction

Coronary artery disease (CAD) is a leading cause of death globally. Coronary computed tomography angiography (CCTA) is an accurate non-invasive imaging method to diagnose or exclude coronary atherosclerosis and obstructive CAD.[1] CCTA gives information on the extent of atherosclerotic findings, their distribution, and severity. On the other hand, myocardial perfusion imaging (MPI) enables the detection of functionally significant coronary artery stenoses and myocardial ischaemia. In hybrid imaging, CCTA and MPI are combined to accurately assess anatomical changes as well as ischaemia, both at the patient level and regionally from each coronary artery.[2]

Hybrid imaging may be useful for identification of patients who are at high risk of developing acute coronary syndrome (ACS). In patients with intermediate coronary lesions, evidence of ischaemia at an anatomically appropriate location by hybrid imaging is associated with an increased rate of subsequent death or cardiac adverse events.[3–5] Although the complementary nature of the anatomical and functional imaging is well established, there is only limited evidence of how each method can predict events in the short and the long term. We hypothesize that ischaemia as a sign of more severe CAD is stronger in predicting short-term outcome while anatomical findings, reflecting also earlier phases of the development of CAD, may be better in predicting adverse events in the long term.

Previous studies suggest that machine learning (ML) is feasible and can improve the risk stratification of patients with suspected or established CAD.[6,7] Previous studies have shown that the risk of the cardiac event is determined by both clinical risk factors and imaging-derived findings.[8] Combining both CCTA and clinical risk factors into ML models seems to predict all-cause mortality significantly better than either alone.[9] Recently, an XGBoost ML model trained on clinical data, CT quantitative plaque measures, and ^{18}F-NaF uptake was used for optimized risk stratification in patients with CAD.[8] Substantial improvement over traditional methods for risk stratification was achieved when all available factors (clinical and imaging) were included in the ML model.

The goal of the presented work was to evaluate the incremental value of positron emission tomography (PET) MPI over CCTA in predicting major adverse events. To achieve this, ML was used as a tool to assess the predictive power of CCTA and PET MPI both in the short and long term.

Methods

Cohort

We retrospectively collected data from symptomatic (chest pain and/or dyspnoea) patients who have undergone selective hybrid PET/CCTA in

Turku PET Centre due to clinically suspected CAD between February 2007 and December 2016. Patients with prior known CAD were not included. Our cohort consists of a total of 2411 patients. Out of these, 864 patients have been previously reported in Maaniitty *et al.*,[10] 670 patients in Stenström *et al.*,[11] 530 patients in Harjulahti *et al.*,[12] and 830 patients in Benjamins *et al.*[13] The novelty of the present manuscript is the application of ML methods as well as the inclusion of more patients and longer follow-up time.

After removal of incomplete data entries, data from 2284 patients (42% male) were retained for further analysis. Following our clinical routine, patients first undergo CCTA using a hybrid PET–CT scanner, and immediately after that, the attending physician performs an initial evaluation of the CCTA scan. If obstructive CAD is excluded by CCTA, no further imaging procedures are performed. In case of the presence of obstructive CAD (diameter stenosis ≥50%) or if obstructive CAD cannot be excluded by CCTA, PET MPI is performed.

Data collection and follow-up

Imaging results, symptoms, and cardiovascular risk factors were retrospectively collected from electronic medical records and saved to the database by investigators. CCTA and perfusion imaging results were obtained from the institutional imaging database. Primary endpoints of follow-up data included all-cause death, myocardial infarction (MI), and unstable angina pectoris (UAP). Comprehensive data on the occurrence of all-cause death, MI, and UAP until May 2020 were obtained from the registries of the Finnish Institute for Health and Welfare and the Centre for Clinical Informatics of the Turku University Hospital. Investigators used electronic medical records to validate all adverse events. If an event occurred, the time difference in years between the CCTA scan and the event was recorded. The observation time is the time interval starting from the CCTA scan until the date of the first occurrence of an event or the end date of follow-up (13 May 2020), whichever happened first. In the analyses with limited observation time, we considered only the events that took place within the limited time. Data on early revascularization (≤6 months), with either percutaneous coronary intervention (PCI) or coronary artery bypass graft surgery (CABG), were collected but were not used as endpoints.

Imaging methods and metrics

The CCTA and PET MPI imaging methods have been previously described.[10,14] CCTA scans were performed using a 64-row hybrid PET–CT scanner (GE Discovery VCT or GE D690, General Electric Medical Systems, Waukesha, WI, USA). Collimation was set at 64 × 0.625 mm, gantry rotation time was 350 ms, tube current 500–750 mA, and voltage 100–1x20 kV, depending on patient size. Before CCTA, beta-blocker (metoprolol, 0–30 mg) was given intravenously to achieve a target heart rate of <60 beats/min. Isosorbide dinitrate aerosol (1.25 mg) was administered to achieve coronary dilation. CCTA was performed using intravenously administered low-osmolal iodine contrast agent followed by saline flush. Prospectively triggered acquisition was applied whenever feasible. From CCTA, we determined the severity of stenosis for each segment of the main coronary arteries (LM, LAD, LCX, RCA) and side branches (D1, D2, LOM1, LOM2, LDP, LPL, RPD, RPL, IM). The presence, extent, and severity of coronary atherosclerosis were evaluated by defining the number of coronary artery segments with any atherosclerosis (also known as segment involvement score), the number of segments with non-obstructive (<50%) plaque, and the number of segments with obstructive (≥50%) plaque (details in Supplementary data online).

Based on the CCTA findings, patients with suspected obstructive CAD (≥50% diameter stenosis in ≥1 coronary artery segment) underwent PET MPI with ^{15}O–water during adenosine stress, as previously described.[10,14] The tracer was injected as an intravenous bolus (mean injected activity 900–1100 MBq) over 15 s, and dynamic PET acquisition was performed (14 × 5, 3 × 10, 3 × 20, and 4 × 30 s). Absolute stress myocardial blood flow (asMBF) was quantified (in mL/g/min) individually for each of the 17 myocardial segments separately, excluding basal septal segments due to membranous part (standard Segments 2 and 3). Myocardial perfusion ≥ 2.3 mL/g/min is considered as normal.[15] Rest myocardial blood flow was not included in analysis as rest perfusion imaging is not routinely performed in our institution (stress-only protocol).[16] We define the stress myocardial blood flow (sMBF) abnormality score as

$$\mathrm{sMBF} = \max(0, 2.3 - \mathrm{asMBF}),$$

so that the zero score corresponds to normal flow. Zero imputation was applied for the sMBF scores for patients who did not undergo PET MPI (or for whom the PET MPI failed) for the whole study cohort.

Clinical data

Data on traditional risk factors [smoking, sex, body mass index (BMI), diabetes mellitus, hypertension, dyslipidaemia, and family history of CAD] were collected from electronic medical records. Symptoms were classified as either typical angina pectoris, atypical (atypical angina pectoris or non-anginal chest pain), or no chest pain. Also, a history of dyspnoea was collected. A summary of the clinical data is presented in *Table 1* in Results.

Machine learning, XGBoost

ML models were trained and used to predict major adverse events by incorporating clinical cardiovascular risk factors and CCTA and PET imaging data. For the training of each model, data were split randomly and independently into training and test groups with a 3:1 ratio. Each variable was checked individually to ensure similar distributions in both groups as described in the Supplementary data online.

For this study, we considered several models, including linear regression, lasso,[17] support vector machine,[18] ridge,[19] elastic net,[20] logistic regression,[21] random forest,[22] and Extreme Gradient Boosting,[23] also known as XGBoost. Of these, XGBoost consistently performed the best in the preliminary analysis and was selected as the ML method to generate the results of this paper.

To simplify the XGBoost models presented in Results, we computed feature importance vectors for 100 XGBoost models trained with all considered input variables—in total 53 variables containing 20 PET-based variables—for each observation year 1–8 and selected nine input variables which were ranked consistently as the most important features. An independent random training and test data split, as described in the Supplementary data online, was performed for each of the 100 XGBoost models. Supplementary data online, *Table S1* presents the feature ranking vector used for the selection of variables of the XGBoost models. These selected nine input variables in the descending order of importance are maximum stenosis degree (a CCTA-based variable), number of atherosclerotic segments (CCTA), number of non-obstructive segments (CCTA), age, maximum PET abnormality score for RCA (a PET-based variable), BMI, sMBF for Segment 10 (PET), sMBF for Segment 15 (PET), and dyspnoea. We define the maximum PET abnormality score for RCA as:

$$s_{\mathrm{maxRCA}} = \text{maximum sMBF across segments 4, 9, 10 and 15.}$$

The preprocessing of variables is described in detail in the Supplementary data online, as are the hyperparameter selection for the considered XGBoost model, and the training and test set division. For the results presented in this work, we trained two sets of XGBoost models for each observation year: the first set uses clinical, CCTA-based, and PET-based input variables, while the other set uses only clinical and CCTA-based variables. Per each observation year, each set consists of $k = 100$ XGBoost models, each trained and tested using an independent and random training and test data split. Furthermore, we considered 2 sets of data for the analyses: the data set corresponding to all considered 2284 patients, and its subset of 2069 patients who did not undergo early revascularization, as explained in more detail in Results. For the set of all considered 2284 patients, each

Table 1 Demographics table

	All patients ($n = 2284$)	Patients with no early revascularization ($n = 2069$)
Age (years)	(23.0–88.0), 62.2 ± 9.7	(23.0–88.0), 62.0 ± 9.8
Women	1317 (57.7%)	1249 (60.4%)
BMI	(15.4–54.3), 28.3 ± 5.2	(15.4–54.3), 28.3 ± 5.2
Smoking	757 (33.1%)	657 (31.8%)
Diabetes	341 (14.9%)	289 (14.0%)
Hypertension	1292 (56.6%)	1155 (55.8%)
Dyslipidaemia	1456 (63.7%)	1287 (62.2%)
Dyspnoea	877 (38.4%)	791 (38.2%)
Atypical angina or non-anginal chest pain	1083 (47.4%)	1004 (48.5%)
Typical angina	528 (23.1%)	432 (20.9%)
CCTA and PET findings		
Normal coronary arteries	718 (31.4%)	718 (34.7%)
Non-obstructive CAD	636 (27.8%)	636 (30.7%)
Obstructive CAD and normal sMBF	403 (17.6%)	392 (18.9%)
Obstructive CAD and abnormal sMBF	400 (17.5%)	233 (11.3%)
Endpoints	210 (9.2%)	172 (8.3%)
MI	59 (2.6%) (28.1% of all endpoints)	46 (2.2%) (26.7% of all endpoints)
UAP	35 (1.5%) (16.7% of all endpoints)	19 (0.9%) (11.0% of all endpoints)
Death	116 (5.1%) (55.2% of all endpoints)	107 (5.2%) (62.2% of all endpoints)
Early revascularization		
PCI	180 (7.9%)	0 (0%)
CABG	40 (1.8%)	0 (0%)

Clinical characteristics, imaging findings, endpoints, and early invasive therapies of all patients and patients with no early revascularization separately.
CAD, coronary artery disease; CABG, coronary artery bypass grafting; CCTA, coronary computed tomography angiography; MI, myocardial infarction; PCI, percutaneous coronary intervention; PET, positron emission tomography; sMBF, stress myocardial blood flow; UAP, unstable angina pectoris.

training set contains 1713 patients (75%) and each test set contains 571 patients (25%). The same XGBoost models—and hence training and test sets—were used for analysing the results corresponding to all the 2284 patients and corresponding to the subset of 2069 patients by restricting the result analysis to only those patients who did not undergo revascularization. A flow diagram of this work is illustrated in *Figure 1*.

Statistical analyses

Statistically significant differences between the areas under the receiver operator characteristic curves (AUCs) corresponding to all data variables and data without PET variables were assessed using the two-sided Wilcoxon signed-rank test with $P = 0.05$. The representation of variables is described in the Supplementary data online in the Preprocessing subsection.

Results

The study cohort consisted of 2284 patients who underwent CCTA due to suspected CAD, of whom 891 (39%) underwent downstream PET MPI for evaluation of haemodynamic significance of coronary stenosis. As shown in *Table 1*, 31.4% of patients had normal coronary arteries, 27.8% had non-obstructive CAD, 17.6% had obstructive CAD on CCTA but normal myocardial perfusion on PET, and 17.5% had obstructive CAD and abnormal myocardial perfusion. The remaining 5.7% of the patients consist of those who did not undergo PET MPI despite suspected CAD, and of those whose PET perfusion imaging study was unsuccessful or whose PET MPI data were missing. The clinical characteristics of patients are presented in *Table 1*.

During the 8-year follow-up period, 210 patients had major adverse event (Year 1: 33 events, Year 2: 18 events, Year 3: 30 events, Year 4: 28 events, Year 5: 31 events, Year 6: 32 events, Year 7: 21 events, Year 8: 17 events). Of these endpoints, 28.1% correspond to MI, 16.7% to UAP, and 55.2% to death. In total, 215 patients had early revascularization within 6 months after CCTA, with percutaneous coronary intervention (PCI) ($n = 180$), coronary artery bypass graft (CABG) surgery ($n = 40$), or both ($n = 5$). Of note, early revascularization was not considered as a major adverse event.

Figure 2 shows the results of the XGBoost ML models in predicting outcome (event or no event) for each observation time (1–8 years) using the random and independent test sets ($N = 571$). In *Figure 1*, AUCs and their 95% confidence intervals (CIs) are visualized for the two sets of Xboost models: (i) with all input variables and (ii) without PET variables.

The results show that the predictive power of the data is reducing over time from the best AUC of 0.82 (95% CI 0.804–0.827) at Year 1 of the observation time to AUC of 0.75 (95% CI 0.745–0.757) at the maximum observation time of 8 years. The AUCs with the data including PET variables were significantly higher than when using only clinical and CCTA variables ($P < 0.05$). However, the significant difference was detected only during the first 4 years of the observation time.

As revascularization is used to treat ischaemia and is strongly linked with the ischaemic imaging findings,[11] we performed a separate analysis

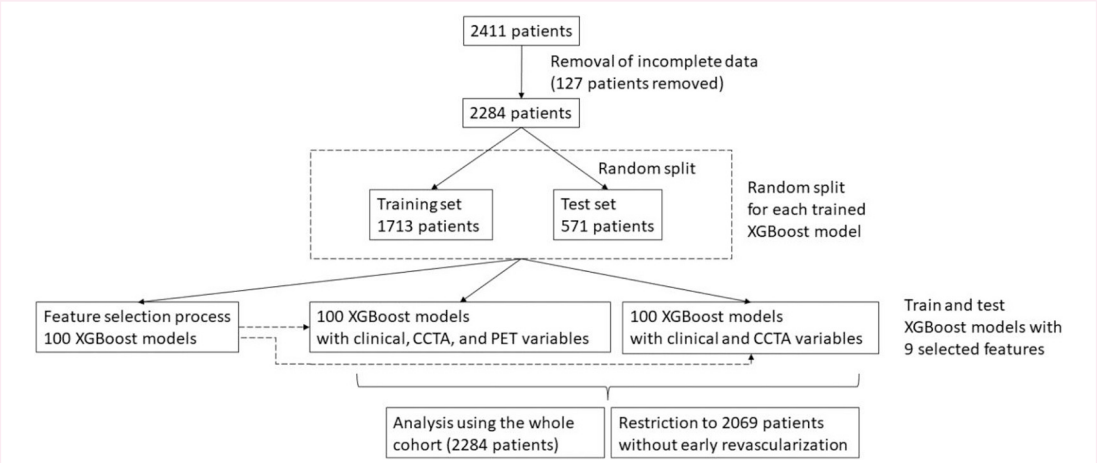


Figure 1 Flow diagram of the presented work. After removal of incomplete data entries, data from 2284 patients were retained for further analysis. In the feature selection process, 100 XGBoost models were trained using all the considered input variables, and based on the testing of these models, 9 most important features were selected. These nine variables were considered in training and testing of two sets of 100 XGBoost models (using clinical, CCTA, and PET variables vs. using only clinical and CCTA variables). Finally, we considered the subsets of the test data, restricted to patients who did not undergo early revascularization.

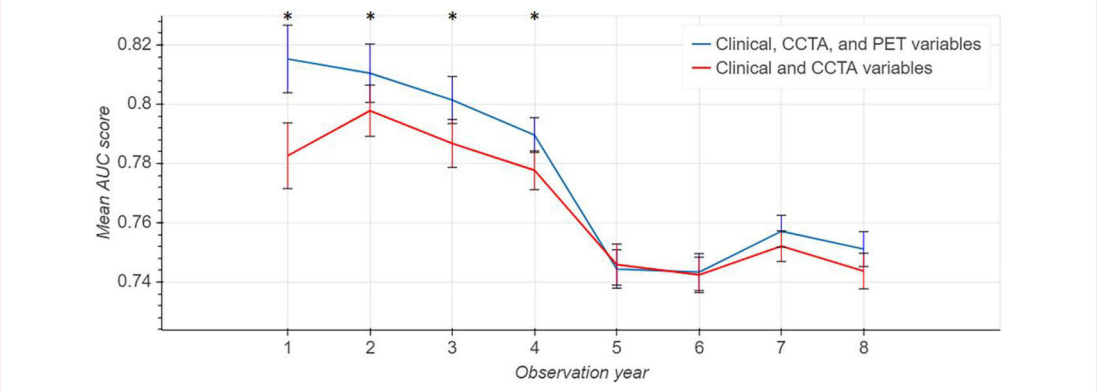


Figure 2 Mean AUCs and 95% CIs for the XGBoost models trained with all variables, and without PET variables in the test sets randomly selected from the whole cohort. Per each observation year, 100 XGBoost models were trained and tested for each input variable set; an independent and random training and test data split was performed for each of these models. The AUCs are calculated using the test sets ($N = 571$). The difference between these curves is statistically significant for the first four observation years; this is highlighted by the black asterisks.

restricted to patients without early revascularization, as illustrated in *Figure 3*. In this analysis, there was no statistically significant difference between the mean AUCs.

Figure 4 illustrates the mean AUC difference curves for XGBoost models trained with all the variables and without PET variables, separately drawn for all patients and patients without early revascularization. The difference between the curves is larger during the first 4 years of observation time and becomes diminished with time.

Discussion

Predicting the outcome of patients with suspected CAD is one of the main goals of diagnostic testing. The imaging methods used for the diagnosis of CAD are well known to also provide prognostic information. Furthermore, combining data from anatomical imaging and functional imaging may further improve the predictive power.[10] We use ML in predicting outcome, as it enables the automated fusion of large number

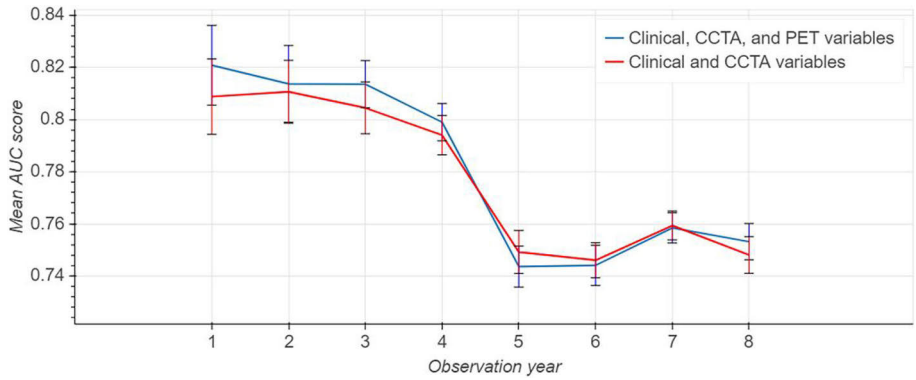


Figure 3 Mean AUCs and 95% CIs for the restricted set of patients who did not undergo early revascularization. The XGBoost models trained with all variables, and without PET variables using the whole cohort data. The AUCs are calculated using the same test sets as in the whole cohort case, restricted to patients who did not undergo early revascularization.

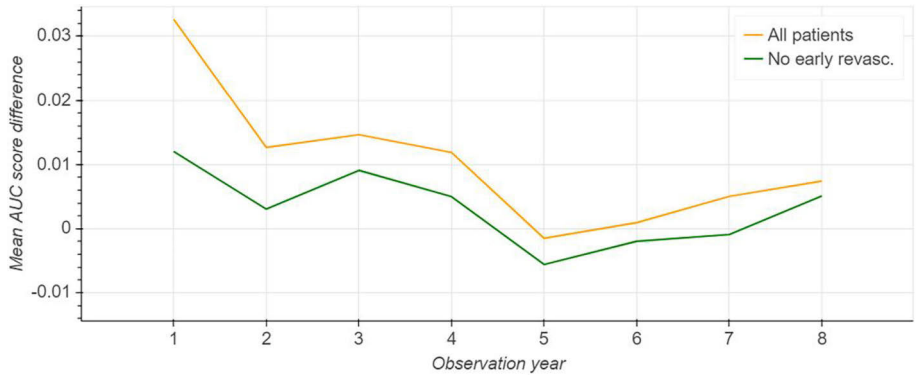


Figure 4 Mean AUC differences (all variables vs. no PET variables) for XGBoost models trained and tested with data corresponding to all patients, and with data corresponding to patients without early revascularization.

of input variables—our analysis began with 53 different input variables. In particular, the chosen ML framework, XGBoost, has been applied previously with state-of-the-art results to various ML and data mining challenges.[23] The highest AUC in the presented study for predicting event was 0.82 which is comparable to the highest score reported in an earlier study combining atherosclerotic plaque microcalcification and anatomical features of CAD (0.85, 95% CI 0.79–0.91).[8]

Our findings support the hypothesis that the presence of ischaemia as a sign of more severe CAD could be stronger in predicting short-term outcome while anatomical findings, reflecting also earlier phases of the development of CAD, might be better in predicting events in the long term. The predictive power increased by adding myocardial perfusion parameters on top of the clinical and CCTA variables, but this impact was only seen during the first 4 years.

In contrast to our study, in a recent study utilizing SPECT/CCTA, hybrid imaging provided independent prognostic information for short- and long-term risk prediction.[24,25] Unlike in our work, the prognostic benefit of hybrid imaging lasted for 10 years (median 6.8) follow-up.[24] However, in that study, the patient population was rather small ($n =$ 357) and included only 46 patients with matched anatomical stenosis and perfusion deficit.[24]

The incremental prognostic value of perfusion imaging was not detected when the patients with early revascularization were excluded (note that early revascularization was not used as an endpoint). We have earlier shown, in partly overlapping patient population, that the adherence of early revascularization to myocardial ischaemia detected by PET perfusion imaging is very high.[11] As the ischaemic finding triggers revascularization, a large proportion of the high-risk patients based on the perfusion findings did enter the revascularization procedure and were, therefore, excluded from the secondary analysis. These findings further suggest that the increased event risk is associated with abnormal perfusion, which commonly triggers revascularization procedures to eliminate ischaemia. This is consistent with previous studies showing an interplay between the severity of ischaemia, myocardial

revascularization, and outcomes.[26–29] However, our study emphasizes that the residual risk of an event is still significant despite revascularization. This risk is likely associated with generally more severe CAD in the patients with detected ischaemia in the diagnostic phase.

In the clinical routine protocol applied in our hospital, the downstream PET perfusion imaging is triggered by abnormal CCTA finding, as suggested by the current European guidelines.[30] This approach is based on the high negative predictive value of CCTA in excluding obstructive CAD and excellent outcome of patients without obstructive CAD, obviating the need for further diagnostic testing in over half of symptomatic patients with suspected CAD.[10,31] Due to this, the patients who entered perfusion imaging were selected based on CCTA findings, and therefore, our results can be only transferred to similar situations than in the present study.

As limitations of the current work, we note that it is a single-centre study, and the numbers of events in test data sets are unavoidably quite small (in the whole data set, there are 17–33 events per year). Furthermore, the treatment of patients is not blinded and can affect the results. It should be noted that our ML model included all patients referred to CCTA regardless of the performance of downstream PET perfusion imaging. For patients with obstructive CAD excluded by CCTA alone, and therefore not undergoing further PET imaging, were classified as having non-obstructive CAD. We think that this method resembles the clinical setting in which a patient has obstructive CAD excluded by CCTA and no further testing is performed, although we acknowledge that, for example, coronary microvascular dysfunction can impair myocardial blood flow in the absence of obstructive epicardial CAD. Another potential limitation is that we did not measure detailed CCTA characteristics for prognostic information, such as quantitative plaque volumes, but, on the other hand, the CCTA variables in our study are those that are widely used in clinical practice.[32] We used 64-row CT scanners that are fulfilling the current requirements for CCTA but believe that the results should be generalizable also to CCTA scanners with a higher number of rows because the scans were analysed by experienced human readers and these features were subsequently provided as an input for the ML algorithm rather than directly feeding the CCTA images for the model. Furthermore, we could not include PET rest myocardial blood flow values into this study, as the considered study used stress-only protocol.[16] However, according to a previous study from our institution,[16] absolute stress MBF was superior to perfusion reserve (i.e. stress MBF divided by rest MBF) in the detection of haemodynamically significant CAD.

Interestingly, ML determined some specific regional PET perfusion results as selected features, mostly located to the inferior wall of the left ventricle and area supplied by RCA. This selection by the XGBoost model is difficult to understand as classically anterior ischaemia has been considered prognostically the most important.

Conclusion

Based on the ML approach, downstream MPI after CCTA improves the prediction of adverse events beyond CCTA alone for the 4 years of follow-up, but no longer-term benefit was seen. The results illustrate the differences and complementary nature of anatomic and functional information in predicting the outcome of patients with chest pain and suspected CAD.

Supplementary data

Supplementary data are available at *European Heart Journal - Cardiovascular Imaging* online.

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Conflict of interest: J.K. received consultancy fees from GE Healthcare and AstraZeneca and speaker fees from GE Healthcare, Bayer, Lundbeck, Boehringer-Ingelheim, Pfizer, and Merck, outside of the submitted work. A.S. received consultancy fees from Amgen and Astra Zeneca, Boehringer-Ingelheim, and Pfizer, and speaker fees from Abbott, Astra Zeneca, and Bayer. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Data availability

The data underlying this article cannot be shared publicly due to the privacy of individuals that participated in the study. The data will be shared on reasonable request to the corresponding author.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed mainly by E.L., I.K., T.M., and J.T. The first draft of the manuscript was written by E. L., I.K., T.M., J.T., R.K., and J.K., and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. The first two authors contributed equally: E.L. oversaw the algorithm development and mathematical part of this work, while I.K. was in charge of the medical part of this work, and both of the first authors equally contributed to the writing of the manuscript.

Ethics approval

The study complies with the Declaration of Helsinki. The Ethics Committee of the Hospital District of Southwest Finland approved the study protocol and waived the need for written informed consent by patients due to observational nature.

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Polygenic Risk Scores in Predicting Coronary Artery Disease in Symptomatic Patients. A Validation Study

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Aim: Clinical risk scores for coronary artery disease (CAD) are used in clinical practice to select patients for diagnostic testing and therapy. Several studies have proposed that polygenic risk scores (PRSs) can improve the prediction of CAD, but the scores need to be validated in clinical populations with accurately characterized phenotypes. We assessed the predictive power of the three most promising PRSs for the prediction of coronary atherosclerosis and obstructive CAD.

Methods: This study was conducted on 943 symptomatic patients with suspected CAD for whom the phenotype was accurately characterized using anatomic and functional imaging. Previously published genome-wide polygenic scores were generated to compare a genetic model based on PRSs with a model based on clinical data. The test and PRS cohorts were predominantly Caucasian of northern European ancestry.

Results: All three PRSs predicted coronary atherosclerosis and obstructive CAD statistically significantly. The predictive accuracy of the models combining clinical data and different PRSs varied between 0.778 and 0.805 in terms of the area under the receiver operating characteristic (AUROC), being close to the model including only clinical variables (AUROC 0.769). The difference between the clinical model and combined clinical + PRS model was not significant for PRS1 ($p=0.627$) and PRS3 ($p=0.061$). Only PRS2 slightly improved the predictive power of the model ($p=0.04$). The likelihood ratios showed the very weak diagnostic power of all PRSs.

Conclusion: The addition of PRSs to conventional risk factors did not clinically significantly improve the predictive accuracy for either coronary atherosclerosis or obstructive CAD, showing that current PRSs are not justified for routine clinical use in CAD.

Key words: Coronary artery disease, Coronary atherosclerosis, Risk factors, Polygenic risk score

Introduction

Coronary artery disease (CAD) remains a leading cause of mortality and morbidity worldwide[1]. Strategies to identify patients with a higher likelihood of CAD are needed to appropriately target diagnostic testing and tailor therapy. Currently, clinical risk scores incorporating clinical risk factors—smoking, hypertension, diabetes, dyslipidemia, age, sex, and family history—are widely used in clinical practice. In addition to these clinical risk factors, genetic risk scores, often referred to as polygenic risk scores (PRSs), have been shown to independently predict the development of CAD[2-6]. The potential use of genetic data in clinics is based on robust evidence and the importance of family history, with an estimated 40%–60% CAD heritability[6, 7].

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For common multifactorial diseases, including CAD, polygenic inheritance plays a greater role than rare monogenic mutations. Genome-wide association studies (GWAS) have previously shown that the genetic load for CAD is due to common genetic variants with small effect sizes, in addition to rare variants with stronger effects[7, 8]. Common low-risk to rare high-risk genetic variants most likely act cumulatively to drive the overall risk in an individual[7, 9].

Derived from GWAS, multiple different PRSs have been previously created. PRSs are calculated by summing risk alleles, which are preferably weighted by effect sizes derived from GWAS results[3]. Recent meta-analyses have shown that PRS can be used to evaluate a patient's genetic risk and is an incremental predictor of CAD along with clinical cardiovascular risk factors. In many studies, PRSs have been proposed for clinical work to support the diagnostic process[3]. However, in large population studies, the diagnoses of CAD are based only on general clinical reports of having or not having CAD. The real cardiac phenotype is typically uncertain; thus, the current PRSs have not yet been validated in cohorts with accurate phenotyping of CAD.

Aim

This study aimed to assess the predictive power of the previously published three most promising PRSs in the prediction of CAD in symptomatic patients with suspected CAD in whom the coronary phenotype has been carefully characterized using both anatomic and functional imaging. The applied imaging methods (coronary computed tomography angiography, CTA, and positron emission tomography, PET perfusion imaging) allow accurate detection of anatomic atherosclerotic changes in coronary arteries as well as myocardial ischemia as a sign of functionally obstructive CAD[10, 11]. We hypothesized that the recently documented PRSs would improve the prediction of the development of coronary atherosclerosis and obstructive CAD. Consequently, we assessed the predictive value of three established PRSs in predicting both incident coronary atherosclerosis and obstructive CAD as a stand-alone measure as well as when added to the currently known clinical risk factors.

Materials and Methods

This study complies with the Declaration of Helsinki. The Ethics Committee of the Hospital District of Southwest Finland approved the study protocol, and written informed consent was obtained from all patients. Genetic data were used only for research purposes. Patients or referring physicians did not receive any information regarding the genetic results.

Cohort

The study cohort consisted of 998 symptomatic patients with stable suspected obstructive CAD who had undergone coronary CTA with selective PET perfusion imaging as a diagnostic test at Turku University Hospital from 2006 to 2019. These patients are typically those with an intermediate probability of obstructive CAD and stable symptoms as an indication to undergo diagnostic procedures by clinicians. Patients provided voluntary consent for the collection of blood samples for genetic analyses.

As previously described[12, 13], in our routine practice, patients with suspected CAD first undergo coronary CTA, and immediately thereafter, PET perfusion imaging is performed if coronary CTA alone cannot rule out obstructive CAD. Typically, in all patients in whom CTA shows a suspicious coronary plaque (e.g., stenosis diameter ≥ 50%), the hemodynamic significance of the plaque was evaluated using ^{15}O-water PET perfusion imaging during adenosine stress. The detailed imaging protocol has been described previously[12, 14]. After excluding patients who did not complete the imaging protocol, 943 patients were included in the final analyses.

On the basis of the imaging findings, we classified the patients into three clinical groups. Group 1 had no coronary atherosclerosis. Group 2 had nonobstructive CAD (i.e., nonobstructive atherosclerosis based on CTA alone or atherosclerosis on CTA combined with normal PET perfusion). Group 3 had obstructive CAD (i.e., atherosclerosis on CTA combined with abnormal PET perfusion).

Clinical Data Collection

The patients' clinical characteristics, conventional cardiovascular risk factors, and symptoms were extracted from electronic medical records and saved to a specific cardiac registry database. Age, sex, body mass index, current smoking status, diabetes, hypertension, dyslipidemia, and a family history of premature CAD were considered conventional risk factors. A family history of premature CAD was defined as CAD in a first-degree relative, who is either <55 years (male) or <65 years (female). Patients without a family history of premature CAD were pooled together with patients with an unknown family history. For symptoms, the patients were classified into three categories 1) typical angina, 2) atypical/nonanginal pain/dyspnea, or 3) no chest pain

or dyspnea. The existence and severity of symptoms were derived from electronic medical records. Chest pain was classified according to the Canadian Cardiovascular Society Angina Grade.

Selection of PRSs and Genetic Data Analysis

We selected the following three PRS profiles to be tested: PGS000329 [5], PGS000018 [6], and PGS000013 [2]. These PRSs are among the largest and most recognized in populations genetically close to our study cohort. For clarity, we call them PRS1, PRS2, and PRS3 in this study.

A total of 950 samples were genotyped using Illumina's GSAMD-24v2_0 bead chip. All genotypes were identified using GenomeStudio v. 2.0.3 software. Results were checked using Plink software for sex, identity-by-descent, and Hardy–Weinberg equilibrium. One duplicate sample was found and excluded, and four first-degree relatives were also excluded. Genotyping success rates for the samples were 98.4%–99.6% (GenomeStudio software) after removing the low-quality single-nucleotide polymorphisms (SNPs) and discarded samples. In the quality control, 28904/759993 (3.8%) SNPs were discarded. The plus/forward strand was based on information on "WRayner" (https://www.well.ox.ac.uk/~wrayner/strand/), and map positions were based on the genome build GRCh38.

For imputation, SNPs with a 99% genotyping rate were included. SNPs in linkage disequilibrium were excluded using a window size of 50 kb, a step size of 5, and an r2 threshold of 0.7. Imputation was performed using Minimac4 based on the MIT server (https://imputationserver.sph.umich.edu/) using HRC (HRC-r1.1) as a reference panel 15. Pre-imputation quality control was performed as recommended in the server documentation using the Will Rayner toolbox version 4.2 (https://www.well.ox.ac.uk/~wrayner/tools/index.html#Checking) and using plink. Additionally, the MIT server also employs an extensive pre-imputation quality check on the uploaded datasets and is described at (https://imputationserver.readthedocs.io/en/latest/pipeline/).

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics, version 27. All PRSs were standardized to obtain the odds ratio (OR) per SD unit. To evaluate the association between PRS and CAD, a logistic regression model was used $p<5\times10-8$ was considered statistically significant and $P<0.05$ was nominally significant. Models based on the clinical risk factors, PRS alone, and PRS with the clinical risk factors were generated to test whether the inclusion of PRSs improves CAD prediction. The discrimination of the predictive models was evaluated using the area under the receiver operating characteristic (AUROC) values. The Z-test was used to compare AUROC values of the two models. PRS was also stratified into deciles to illustrate its distribution in cases versus controls. To study the diagnostic performance of PRSs, we also calculated the sensitivity, specificity, and positive and negative predictive values, as well as the positive and negative clinical likelihood ratios (LRs) for all decile cutoffs of each PRS. Net reclassification improvement (NRI) values were also calculated for each decile of the PRS cutoff.

Results

Patient Characteristics

The study cohort included 943 patients (59.7% women). The mean age of the patients was 64 years (SD: 8.6 years). A family history of premature CAD was reported by 49.5% (n=467) of participants. Based on the imaging tests, 273 (29.0%) individuals had no coronary atherosclerosis, 465 (49.3%) had nonobstructive CAD, and 205 (21.6%) had obstructive CAD. The characteristics of the study participants are shown in **Table 1**.

To analyze the predictive power of PRS for CAD, we performed two comparisons. The first analysis compared patients with any coronary atherosclerosis (i.e., either nonobstructive or obstructive) (n=670) to those without atherosclerosis (n=273). The second analysis compared patients with obstructive CAD (n=205) to those without (n=738).

The Predictive Power of PRS

All three different PRSs predicted coronary atherosclerosis **(Table 2)** and obstructive CAD **(Table 3)**, and the ORs varied between 1.25 (95% confidence interval (CI) 1.07–1.46) and 1.70 (95% CI 1.44–2.01). The highest OR was found in PRS3 in predicting obstructive CAD. The ORs of clinical data alone were 3.13 (95% CI 2.63–3.72) and 2.53 (95% CI 2.15–2.98) for predicting coronary atherosclerosis or obstructive CAD, respectively. The ORs of models combining clinical data and different PRS varied between 2.59 (95% CI 2.20–3.05) and 3.01 (95% CI 2.53–3.57) in predicting obstructive CAD.

The PRS appeared to perform better in predicting obstructive CAD than coronary atherosclerosis. Therefore, the results for the prediction of obstructive CAD are presented in the main paper, whereas the results for predicting coronary atherosclerosis are presented in the **Supplemental Fig. 1**. **Fig. 1** depicts the receiver operating characteristic

Table 1. Clinical characteristics and imaging findings for the cohort

	(*n* = 943)
Age (years) [a]	64 ± 8.6
Body mass index (kg/m²) [a]	27.5 ± 4.9
Male sex	380 (40.3)
Current smoking	95 (10.0)
Diabetes	130 (13.8)
Hypertension	542 (57.5)
Dyslipidemia	611 (64.8)
Family history of premature CAD	
Symptoms	467 (49.5)
Typical angina	250 (26.5)
Atypical/non-anginal pain/dyspnea	594 (63.0)
No chest pain nor dyspnea	99 (10.5)
Imaging findings	
No coronary atherosclerosis	273 (29.0)
Non-obstructive CAD	465 (49.3)
Obstructive CAD	205 (21.7)

[a] mean ± standard deviation (range), CAD=Coronary artery disease

Table 2. The prediction of coronary atherosclerosis by clinical data and three PRS

Model	OR	95% CI	*p*-value
PRS1 (PGS000329)	1.40	1.21-1.62	<0.001
PRS2 (PGS000018)	1.62	1.39-1.89	<0.001
PRS3 (PGS000013)	1.62	1.39-1.88	<0.001
Clinical data [a]	3.13	2.63-3.72	<0.001
Clinical data [a] + PRS1	3.35	2.81-4.00	<0.001
Clinical data [a] + PRS2	3.59	3.00-4.30	<0.001
Clinical data [a] + PRS3	3.59	3.00-4.30	<0.001

Patients with coronary atherosclerosis (*n*=670) are compared against patients without coronary atherosclerosis (*n*=273).
[a] The clinical model includes age, sex, hypertension, diabetes, smoking, dyslipidemia, family history of premature CAD and symptoms. OR=odds ratio, CI=confidence interval.

Table 3. The prediction of obstructive CAD by clinical data and three PRS

Model	OR	95% CI	*p*-value
PRS1 (PGS000329)	1.25	1.07-1.46	0.006
PRS2 (PGS000018)	1.68	1.42-1.97	<0.001
PRS3 (PGS000013)	1.70	1.44-2.01	<0.001
Clinical data [a]	2.53	2.15-2.98	<0.001
Clinical data [a] + PRS1	2.59	2.20-3.05	<0.001
Clinical data [a] + PRS2	2.97	2.50-3.53	<0.001
Clinical data [a] + PRS3	3.01	2.53-3.57	<0.001

Patients with obstructive CAD (*n*=205) are compared against patients without obstructive CAD (*n*=738).
[a] The clinical model includes age, sex, hypertension, diabetes, smoking, dyslipidemia, family history of premature CAD and symptoms. OR=odds ratio, CI=confidence interval.

(ROC) curves of each PRS in predicting obstructive CAD. The areas under the curve of the PRS models vary between 0.561 and 0.640, indicating a rather low predictive power.

ROC curves for models combining clinical data and PRSs for predicting obstructive CAD are shown in **Fig. 2.** The predictive accuracy of the models combining clinical data and different PRS varied

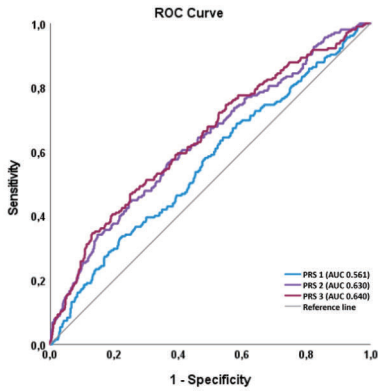

Fig. 1. ROC curves of PRSs

Receiver operating characteristic (ROC) curves of three PRSs in predicting obstructive CAD.
AUC = Area under the curve, PRS = Polygenic risk score.

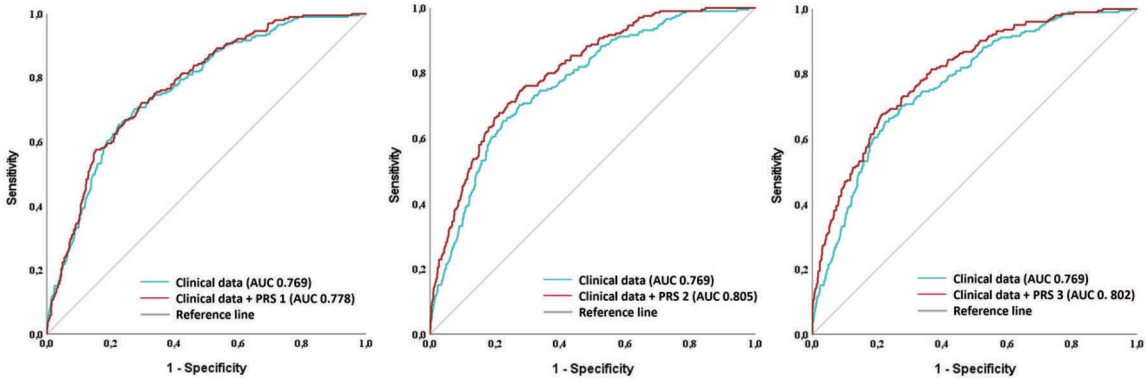

Fig. 2. ROC curves of PRSs with clinical risk factors

Receiver operating characteristic (ROC) curves of clinical data and PRS in predicting obstructive CAD.
AUC = Area under the curve, PRS = Polygenic risk score.

between 0.778 and 0.805 (in terms of AUROC), being close to the model including only clinical variables (AUROC: 0.769) and yielding an improvement of only 0.9–3.6 percentage points over clinical data alone. The difference between the clinical model and the combined clinical + PRS model was not significant for PRS1 (p=0.627) and PRS3 (p=0.061). Only PRS2 slightly (AUC change: 0.036) but nominally significantly (without Bonferroni correction for four independent tests) improved the predictive power of the model (p=0.04).

Distribution of the PRS Values

Fig. 3 shows the distribution of the PRS values in patients with and without obstructive CAD. The distribution of the PRS in the two groups completely overlapped with a minor shift toward higher PRS values in patients with obstructive CAD compared with those without obstructive CAD. In **Fig. 4,** the PRS values are categorized into deciles to visualize the relative risk in each PRS category. The prevalence of obstructive CAD was higher in higher PRS deciles; however, a large proportion of the patients classified as having high risk by PRS did not have obstructive CAD based on imaging tests. Conversely, most patients with obstructive CAD had a PRS lower than the highest deciles. **Supplemental Fig. 2 and Supplemental Fig. 3** shows similar distributions for

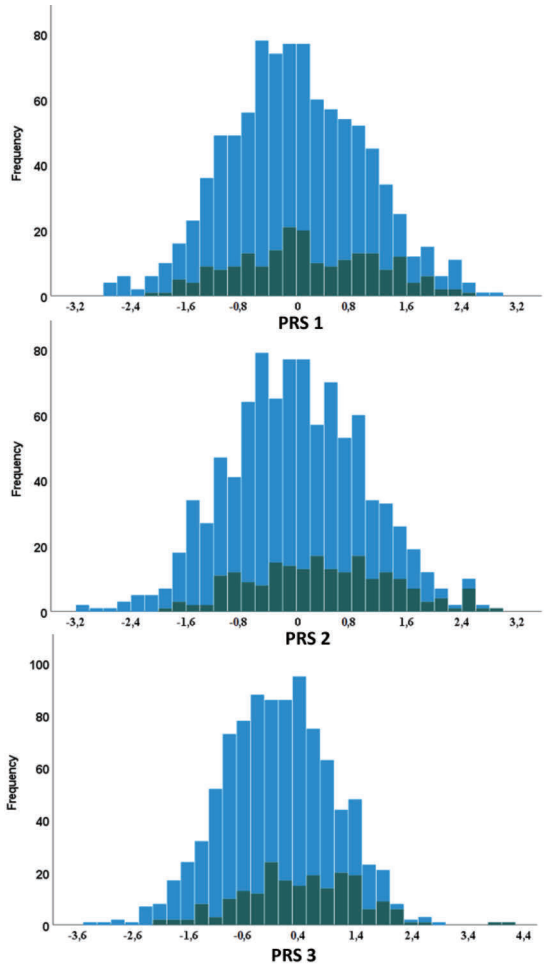

Fig. 3. Distribution charts of PRSs per standard deviation (SD)

Distribution charts demonstrating the distribution of PRSs per SD for each of the three PRSs in patients with (in green) and without (in blue) obstructive CAD.
PRS = Polygenic risk score

the prediction of atherosclerosis.

The calculated sensitivity, specificity, positive and negative predictive values, and the positive and negative LRs for all decile cutoffs of each PRS are shown in the **Supplemental Table 1**. None of the PRS cutoffs provided reasonable sensitivity and specificity at the same time. The positive LR ranged from 1.01 to 1.10 and the negative LR from 0.29 to 0.92. NRI values are shown in the **Supplemental Table 2**.

Discussion

Many studies have reported the use of PRSs to assess the risk of developing diseases, including CAD. Different cohort studies have shown that PRS are independent and incremental predictors of CAD along with clinical cardiovascular risk factors[3-6, 15]. We hypothesized that the previously documented PRSs are useful in predicting the development of coronary atherosclerosis and obstructive CAD along with the traditional risk factors and could be used in clinical workups, as shown by a recent meta-analysis[3]. However, its clinical utility has not been previously

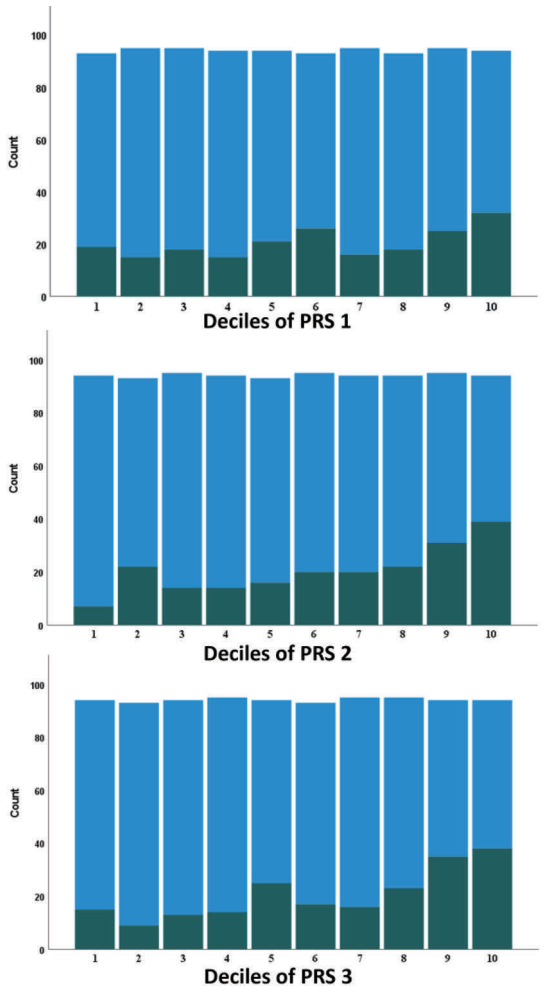

Fig. 4. Distribution of PRSs in deciles

The distribution of PRSs (in deciles) in participants with (in green) and without (in blue) obstructive CAD.
PRS = Polygenic risk score

evaluated at the individual level, and a validation of various PRSs in an independent population with an accurate phenotype is not yet available[3].

In this study, we conducted a validation study in a cohort with a very accurate cardiac imaging phenotype. The results rejected the hypothesis of the high utility of PRSs. Although PRS has some predictive power, it is small and does not appear to provide clinically useful information regarding routine clinical risk factors in the evaluation of incident CAD. Adding PRS, the predictive accuracy improved only by 0.9–3.6 percentage points over the clinical data. Our findings in the symptomatic population with suspected CAD are in agreement with those of a recent review conducted in primary prevention populations[16]. That study used five different PRSs, two of which were the same as those used in our study. In that review, PRSs were also significantly associated with the risk of CAD, but the improvement ranged from negligible to modest when the PRSs were added to traditional risk scores[16].

The strength of the current study is that we included a reasonably large number of patients with very accurate phenotyping of CAD by using anatomical (coronary atherosclerosis and plaques) and functional (ischemia) state-of-the-art imaging.

Imaging methods have been validated in large populations and are the best for this purpose[17]. As we did not aim to develop new PRSs but to validate existing ones, the cohort we studied was large enough. This is also supported by the expected performance of classic risk factors in the same population.

Another strength is that the patients were symptomatic with suspected CAD. This is the exact group in which PRSs are shown to be used if applied in the diagnostic workup. In addition, we tested three recent, most recognized, and promising PRSs, which were constructed from large sample sizes and huge number of SNPs and complex weighting modalities. The 95% CIs for ORs and AUC values obtained in our study were comparable to those reported in the original studies **(Supplemental Table 3)**. Therefore, we consider the chosen population, PRSs, and the methods as representative. The results demonstrate that despite its clinical use, the genetic characteristics obtained at birth in the form of PRSs have limited clinical value beyond the current risk factors in patients with chest pain and suspected CAD.

Another strength of our analysis is that we used two different phenotype endpoints. The genetic risk for developing atherosclerosis or obstructive CAD may be different because their pathophysiology is different. We found that all PRSs were associated with the development of both atherosclerosis and obstructive CAD[2, 5, 6]. However, it appeared that compared with developing coronary atherosclerosis, obstructive CAD was generally predicted better by PRSs (ORs for atherosclerosis were 0.68 (95% CI 0.59–0.79)–1.62 (95% CI 1.39–1.89) and for obstructive CAD 0.71 (95% CI 0.61–0.83)–1.70 (95% CI 1.44–2.01), although the differences were small.

Typically, PRSs are derived from, and their predictive power is evaluated in large-scale cohorts including hundreds of thousands of healthy individuals and individuals with CAD and a huge number of SNPs. However, in these large studies, the phenotype is typically weakly characterized because the diagnoses of CAD are based only on general clinical reports of having or not having CAD, and no details about the coronary anatomy or myocardial ischemia are available. Therefore, validation of the performance of the PRSs is needed in real-world clinical patients with an accurately characterized phenotype. Our unique setting provided valuable information on the usability of PRS in risk stratification at the individual level and enabled comparison against conventional risk factors.

Our results illustrate the dilemma of the proposed use of PRSs in risk stratification or as a screening test. ORs indicating moderate risk can have significance in population studies in determining the causes of the disease, but they are not applicable in risk prediction on the individual level[18, 19]. ORs or hazard ratios do not directly indicate the discriminatory value of a screening test[18, 19]. Despite these facts, there are big expectations for PRSs in the hope of using them in risk stratification in the future, especially in the publications that introduced PRS.

The challenge of using PRSs in the clinical workup is illustrated in **Fig. 3 and 4**. The range of risk scores by different PRSs overlap in patients who are healthy, atherosclerotic, and have obstructive CAD. This demonstrates that these PRSs do not help to separate patients with and without obstructive CAD in clinical practice. Furthermore, looking at one or two top deciles, it becomes evident that even in the highest deciles, showing a relatively higher likelihood of having obstructive CAD, most patients did not have CAD, rendering low specificity. In addition, most patients with CAD had low PRS, making the sensitivity very low, regardless of the PRS cutoff applied. This is also supported by our LR analysis with all PRS decile cutoffs **(Supplemental Table 2)**. To be clinically useful, any diagnostic test must significantly change the pre-test probability. The power of any test to change probability can be estimated from LRs. In general, LRs above 10 and below 0.1 are considered to provide strong evidence to rule in or rule out diagnoses, respectively, in most circumstances. Positive LRs between 5 and 10 are valuable in individuals with intermediate pre-test probability. In the current analyses, the positive LR ranged from 1.01 to 1.10 and the negative LR ranged from 0.29 to 0.92, suggesting that none of the PRSs in any of their cutoffs provided useful diagnostic information. The findings with NRI also showed the poor incremental diagnostic power of PRSs.

Study Limitations

One of the limitations of this study is that we did not have follow-up data regarding the progression of CAD or the medications used. Second, this study focused on three selected PRSs. We acknowledge that many other PRSs could be considered, but we believe that our selection offers a representative sample that demonstrates and evaluates the topic well. Third, the ethnicity of the study participants may significantly change the discrimination power of the PRS. However, the population tested in our study included participants of Caucasian northern European ancestry from Finland. The tested PRSs were also generated from similar European ancestry (the population in one of the tested PRSs by Mars *et al.*[5] was also Finnish),

so they should be suitable for our population. Fourth, in our study, one of the clinical risk factors was the family history of premature CAD. This risk factor may include some genetic information, as shown in the study by Mars *et al.*[5] in which PRS did not provide incremental information on the reported family history. In any case, asking for family history is common in clinical work, completely free of cost and with minimal effort, which is not the case with PRSs. In the current analysis, we did not have access to patient clinical outcomes, and further studies are warranted on this topic.

In addition, as PRSs can be constructed in different ways[20], different PRSs can give quite varying risk estimates for an individual with minimal concordance[20, 21]. This needs to be further tested in our cohort and can further hamper the usability of PRSs in a clinical real-world setting in predicting CAD at the individual level.

Conclusions

The current PRSs appear to be statistically significant predictors of coronary atherosclerosis and obstructive CAD. However, the predictive power of scores is limited and does not provide clinically useful information beyond conventional cardiovascular risk factors. The results of this study show that current PRSs are not justified for the clinical routine evaluation of symptomatic patients referred for suspected CAD.

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Disclosures

Dr. Knuuti received consultancy fees from GE Healthcare and Synektik Pharma and speaker fees from GE Healthcare, Bayer, Lundbeck, Boehringer-Ingelheim, Pfizer and Merck, outside of the submitted work. Dr. Saraste received consultancy fees from Amgen and Astra Zeneca, Boehringer Ingelheim and Pfizer, and speaker fees from Abbott, Astra Zeneca, and Bayer All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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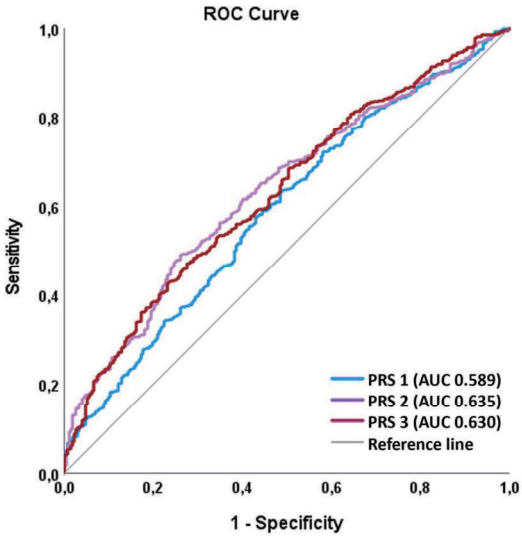
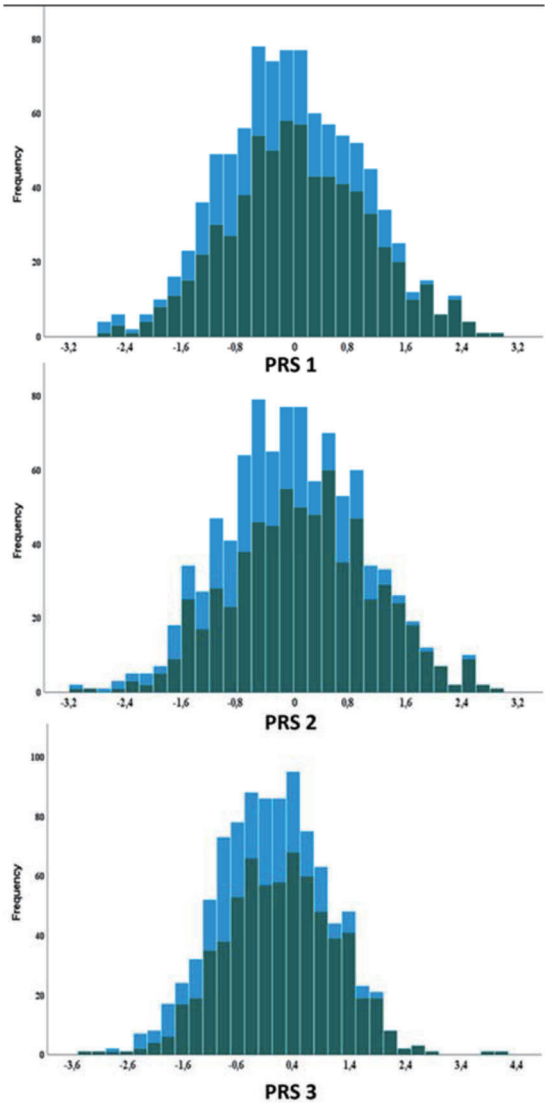


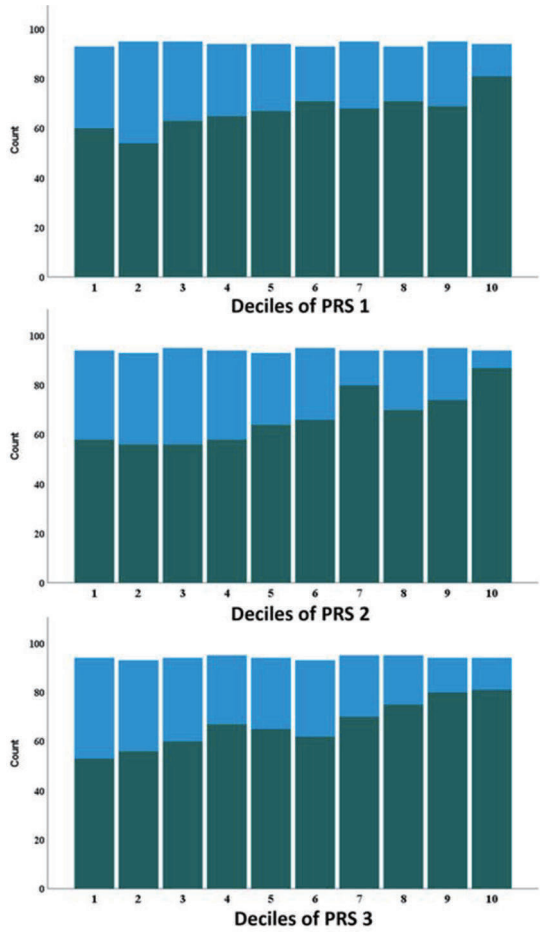
Supplemental Fig. 1. ROC curves

Receiver operating characteristic (ROC) curves of four PRS in predicting atherosclerosis.



Supplemental Fig. 2. Distribution in deciles

Distribution charts per SD. Distribution charts demonstrating distribution of PRS per SD for each four PRS separately in patients with (in green) and without (in blue) atherosclerosis.



Supplemental Fig. 3. Distribution in deciles.

Distribution of PRS (in deciles) in participants with (in green) and without (in blue) atherosclerosis.

Supplemental Table 1. Sensitivities, Specificities, LR+, LR-, PPV, NPV, accuracy

	10 %	20 %	30 %	40 %	50 %	60 %	70 %	80 %	90 %
PRS1									
Sensitivity	90.7	83.4	74.6	67.3	57.1	44.4	36.6	27.8	15.6
Specificity	10.1	20.9	31.4	42.1	52.0	61.1	71.9	82.1	91.6
Positive LR	1.01	1.05	1.09	1.16	1.19	1.14	1.30	1.55	1.86
Negative LR	0.92	0.79	0.81	0.78	0.83	0.91	0.88	0.88	0.92
NPV	79.6	81.9	81.6	82.2	81.3	79.8	80.3	80.3	79.6
PPV	21.9	22.7	23.3	24.5	24.9	24.1	26.6	30.2	34.0
Accuracy	27.6	34.5	40.8	47.6	53.1	57.5	64.2	70.2	75.0
PRS2									
Sensitivity	96.6	85.9	79.0	72.2	64.4	54.6	44.9	34.1	19.0
Specificity	11.8	21.5	32.5	43.3	53.8	64.0	74.0	83.8	92.5
Positive LR	1.10	1.09	1.17	1.27	1.39	1.52	1.73	2.10	2.53
Negative LR	0.29	0.66	0.65	0.64	0.66	0.71	0.74	0.79	0.88
NPV	92.6	84.5	84.8	84.8	84.4	83.5	82.8	82.0	80.4
PPV	23.4	23.3	24.6	26.2	28.0	29.7	32.5	37.0	41.5
Accuracy	30.3	35.5	42.6	49.6	56.1	62.0	67.7	73.0	76.5
PRS3									
Sensitivity	92.7	88.3	82.0	75.1	62.9	54.6	46.8	35.6	18.5
Specificity	10.7	22.1	33.2	44.2	53.5	63.9	74.6	84.4	92.4
Positive LR	1.04	1.13	1.23	1.35	1.35	1.51	1.84	2.28	2.43
Negative LR	0.68	0.53	0.54	0.56	0.69	0.71	0.71	0.76	0.88
NPV	84.0	87.2	86.8	86.4	83.8	83.5	83.4	82.5	80.3
PPV	22.4	24.0	25.5	27.3	27.4	29.6	33.9	38.8	40.4
Accuracy	28.6	36.6	43.8	50.9	55.6	61.8	68.5	73.8	76.3

Sensitivities, specificities, positive and negative likelihood ratio, negative predictive value, positive predictive value and accuracy for PRS 1-4, each decile separately. LR = likelihood ratio, NPV=negative predictive value, PPV=positive predictive value, PRS=polygenic risk score.

Supplemental Table 2. NRI values

Deciles	PRS1	PRS2	PRS3
10	-0,0125	-0,0623	-0,0873
9	-0,0298	-0,0111	0,0201
8	0,0534	0,0271	0,0347
7	-0,0222	-0,0146	-0,0098
6	-0,0014	0,0111	0,0000
5	0,0049	0,0173	0,0035
4	-0,0125	-0,0173	-0,0062
3	-0,0014	0,0222	0,0298
2	0,0125	0,0125	0,0125
1	0,0062	0,0125	0,0000

NRI for comparison of clinical moden and clinical model + PRS.
NRI=net reclassification index, PRS=polygenic risk score

Supplemental Table 3. PRS as predictors by original studies

	Patients' origin	Number of patients	AUC	C-index (95% CI)	NRI (95% CI)	Reference
PRS1, PGS000329	FinnGen	20,165	-	0.820 (0.816–0.824)	1.1 (-0.1,2.2)	Mars N, Koskela JT, Ripatti P, *et al.* Polygenic and clinical risk scores and their impact on age at onset and prediction of cardiometabolic diseases and common cancers. Nat. Med., 2020; 26: 549-557. Available at: http://dx.doi.org/10.1038/s41591-020-0800-0
PRS2, PGS000018	UK biobank	482,629	0.79	0.623 (0.615-0.631)	-	Inouye M, Abraham G, Nelson CP, *et al.* Genomic risk prediction of coronary artery disease in 480,000 adults: Implications for primary prevention. J. Am. Coll. Cardiol., 2018; 72: 1883-1893. Available at: http://dx.doi.org/10.1016/j.jacc.2018.07.079
PRS3, PGS000013	UK biobank	184,305	0.81 (0.81–0.81)	-	-	Khera AV, Chaffin M, Aragam KG, *et al.* Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. Nat. Genet., 2018; 50: 1219-1224. Available at: http://dx.doi.org/10.1038/s41588-018-0183

Supplemental table, PRS1-3 patients´ origin, number of patients, AUC, c-index, NRI and reference based on original publifications. PRS=polygenic risk score, AUC=area under the curve, NRI= net reclassification index, CI=confidence interval

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