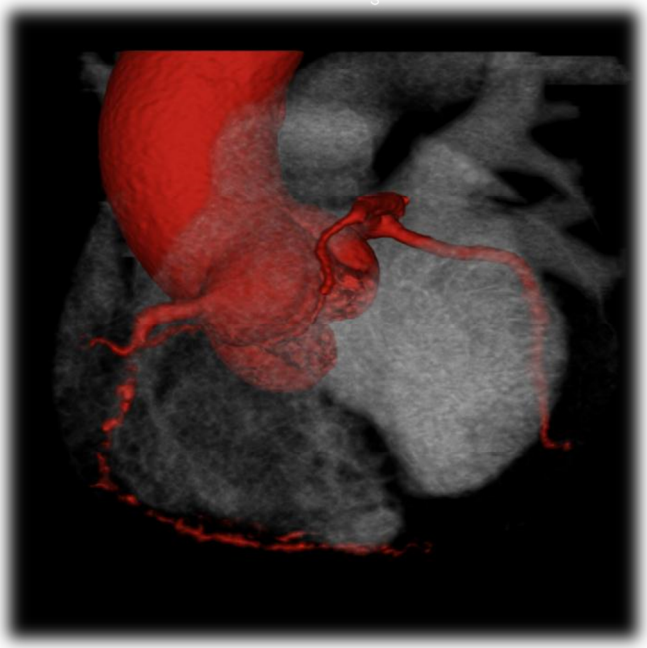
Imaging in the diagnosis and prediction of allograft vasculopathy after heart transplantation

Thesis for the degree of philosophiae doctor

Anne Günther



Division of Radiology and Nuclear Medicine

Oslo University Hospital, Rikshospitalet

Institute of Clinical Medicine

Faculty of Medicine

University of Oslo

2017

Series of dissertations submitted to the
Faculty of Medicine, University of Oslo

ISBN 978-82-8377-091-9

Cover: Hanne Baadsgaard Utigard.
Print production: Reprosentralen, University of Oslo.

To Jonas

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Acknowledgements

In 2004 the Department of Radiology at Oslo University Hospital, Rikshospitalet installed their first multidetector CT. This opened for imaging of the coronary arteries and Dr. Rune Andersen, in close collaboration with radiographer Joanna Kristiansen, started performing coronary CT-angiography at our hospital. Simultaneously, Dr. Rune Andersen initiated a project on coronary CT-angiography of heart transplanted patients in collaboration with Prof. Thor Edvardsen the Department of Cardiology, and I was invited to join the project. This was the start of my work towards a PhD-degree.

Many people have been involved and made this project possible. First of all I would like to thank Dr. Rune Andersen for having faith in me and inviting me to join the project. It is with his initiative, creativity and never failing enthusiasm that this project has been carried out. I am very grateful to Prof. Jarl Jakobsen, my main supervisor, for his valuable advice, contribution in providing funding and for his efforts to ensure my progress during the PhD work. I am also indebted to my co-supervisor, Dr. Andreas Abildgaard. His constructive criticism, endless patience and constantly optimistic encouragement enabled me to complete the papers and the thesis. Radiographer Joanna Kristiansen has been crucial to the project with her great technical knowledge in computed tomography, as well as her dedication for acquiring the best possible images.

This work could not have been done without the close collaboration with the Department of Cardiology. I am extremely grateful for the significant contributions made by Prof. Lars Gullestad, Prof. Thor Edvardsen, and Dr. Lars Aaberge in planning and carrying out this project. Dr. Satish Arora, Dr. Einar Gude, Dr. Asgrimur Ragnarsson, Prof. Svein Simonsen, radiographer Ingrid Erikstad, and the heart transplant nurses, each made important contributions that made completion of the project possible.

I wish to thank statistician Leiv Sandvik for teaching me basic knowledge in Cox regression analysis and supervising the statistical analysis of the coronary artery calcium study.

I also wish to thank my colleges, both at the Section of General Radiology and the Section of Thoracic, Vascular and ENT Radiology, as well as the CT radiographers, for their support and interest in my project. In particular, I thank Dr. Einar Hopp, Dr. Mogens Aaløkken, and Dr. Knut Brabrand for their encouragement and good advice through all these years.

I want to express my gratitude to the Head of the Division of Radiology and Nuclear Medicine and professor at the University of Oslo, Hans-Jørgen Smith, for giving me the opportunity to complete my

research at the Institute of Clinical Medicine, University of Oslo. I also want to thank my subsequent leaders at Oslo University Hospital for their support throughout the years; Dr. Tor Egge, Dr. Andreas Abildgaard, Dr. Mogens Aaløkken and Dr. Paulina Due-Tønnesen.

Family and friends have provided endless support and encouragement since I embarked upon my research project many years ago. My mother has been especially important; she has both given practical help as well as never ending encouragement. My mother and my sister have had unfailing belief in my ability to complete my thesis and I always enjoy the company when our family gets together. Thank you to all my friends for being patient with a non-existing friend for this past year, and for your continuous encouragement. In particular, I thank Hilde Haukeland and Hege Russnes for their expert support for my thesis, as well as backing and fun talks along with the rest of the «dame doktor klubb».

Most important though, is the love and affection I receive every day from Morten and Jonas. Your patience with me has been never ending, especially this past year! I could not have completed this work without Morten's full support and complete care for the family's every-day life!

Finally I wish to acknowledge the heart transplanted patients who have participated in this project and express gratitude for the grants received from John Fredriksen's Cardiac Research Fund and Professor Frimann-Dahl's Fund. The project also received financial support from the Radiology Departement's Medinnova Fund and the South-Eastern Norway Regional Health Authority.

Oslo, February 26th, 2017
Anne Günther

Abbreviations

BMI = body mass index

BNP = brain natriuretic peptide

bpm = beats per minutes

CAC = coronary artery calcium

CAD = coronary atherosclerotic disease

CAV = cardiac allograft vasculopathy

CCTA = coronary CT angiography

CI = confidence interval

CIN = contrast-material–induced nephropathy

CMV = cytomegalovirus

CNIs = Calcineurin inhibitors

CRP = C-reactive protein

CT = computed tomography

$CTDI_{vol}$ = volume CT dose index

CT-FFR = CT-derived fractional flow reserve

CTP = CT myocardial perfusion

CX = left circumflex

D/GL = death or graft loss

DAP = dose area product

DLP = dose-length product

DS = Dual-source

DSE = Dobutamine stress echocardiography

ECG = electrocardiogram

EEM = external elastic membrane

FFR = fractional flow reserve

GFR = glomerular filtration rate

GP130 = glycoprotein 130

HR = hazard ratio

HTX = Heart transplantation

HU = Hounsfield units

ICA = invasive coronary angiography

IFN-ɣ = interferon-ɣ

IL = interleukin

IMR = index of microcirculatory resistance

ISHLT = International Society for Heart and Lung Transplantation

IVUS = intravascular ultrasound

k = organ-weighting factor

LAD = left anterior descending

LM = left main

MD = multidetector

MIT = maximal intimal thickness

MMF = mycophenolate mofetil

MPI = myocardial perfusion imaging

mTOR = mammalian target-of-rapamycin

NF-MACE = non-fatal major adverse cardiac events

NPV = negative predictive value

OCT = optical coherence tomography

OPG = Osteoprotegerin

OR = odds ratio

PCI = percutaneous coronary intervention

PPV = positive predictive value

RCA = right coronary artery

SPECT = single-photon emission computed tomography

Th1= T helper type 1 cells

TNF-α = tumor necrosis factor-α

VCAM-1= receptors cell adhesion molecule-1

VH = Virtual histology

vWf = von Willebrand factor

64-MDCT = 64-slice multidetector CT

List of papers

I. Anne Günther MD, Lars Aaberge MD PhD, Asgrimur Ragnarsson MD, Jarl Jakobsen MD PhD, Lars Gullestad MD PhD, Thor Edvardsen MD PhD, Andreas Abildgaard, MD PhD, Arora Satish MD PhD, Joanna Fenn Kristiansen MS, Rune Andersen MD.
Coronary computed tomography in heart transplant patients – detection of significant stenosis and cardiac allograft vasculopathy, image quality, and radiation dose.
Submitted to European Heart Journal – Cardiovascular Imaging

II. Anne Günther MD, Rune Andersen MD, Einar Gude MD PhD, Jarl Jakobsen MD PhD, Thor Edvardsen MD PhD, Leiv Sandvik MS PhD, Andreas Abildgaard MD PhD, Lars Aaberge MD PhD, Lars Gullestad MD PhD.
The predictive value of coronary artery calcium detected by computed tomography for short- and long-term outcomes in heart transplant patients.
Submitted to Transplant International

III. Satish Arora MD PhD, Anne Gunther MD, Bertil Wennerblom MD PhD, Thor Ueland MD PhD, Arne K. Andreassen MD PhD, Einar Gude MD PhD, Knut Endresen MD PhD, Odd Geiran MD PhD, Nils Wilhelmsen, Rune Andersen MD, Pål Aukrust MD PhD, Lars Gullestad MD PhD.
Systemic markers of inflammation are associated with cardiac allograft vasculopathy and an increased intimal inflammatory component.
American Journal of Transplantation 2010;10(6):1428-36.

1 Introduction

1.1 Heart transplant patients

Heart transplantation (HTX) has been performed since 1967 when Christiaan Barnard performed the first procedure at the Groote Schuur Hospital in Cape Town, South Africa. HTX is today an established therapy for end-stage heart disease. The two leading etiological causes are heart failure secondary to ischemic heart disease and non-ischemic cardiomyopathy.[1]

More than 5000 HTX procedures are performed annually worldwide. Oslo University Hospital, Rikshospitalet, is the only center in Norway to perform HTX, and about 35 patients are transplanted each year. Approximately 550 patients are living with a heart transplant in Norway, and they are followed at our hospital annually at a minimum.

Survival after HTX has improved significantly from 18 days for the first transplanted patient to a median reported survival of 11 years at present[1], primarily because of improved immunosuppression. In the first year after HTX, infection and acute rejections are leading causes of death. Beyond the first year, malignancy, renal failure, and cardiac allograft vasculopathy (CAV) progressively become the most important causes of death.[1]

1.2 Coronary allograft vasculopathy

CAV is an accelerated form of atherosclerosis exclusive to HTX patients. It affects both epicardial and intramyocardial vessels and typically causes a gradual development of myocardial fibrosis with graft failure as the end stage. Symptoms of myocardial ischemia are mostly absent or atypical, especially in the early phase, because transplanted hearts are denervated. Clinically, CAV may present as congestive heart failure, arrhythmia, or sudden death. Early onset of CAV has been recognized as an adverse prognostic indicator.[2-4]

1.2.1 Definition

Coronary atherosclerosis is often applied as a broad term to include several types of diseases found in coronary arteries. Native coronary atherosclerotic disease (CAD) and CAV are two subgroups of coronary atherosclerosis. Both types may coexist in the transplanted heart because CAD can be donor mediated or develop de novo after HTX synchronously with CAV. Although the two types of lesions have distinctive features, they do have some overlap, which makes it difficult to differentiate definitively by morphology and distribution.[5] In a clinical setting, all coronary artery lesions in HTX patients are often referred to as CAV.

1.2.2 Prevalence

Registry data show that within the first year after transplantation, the incidence of CAV is 7.7%, increasing to 30% by 5 years and to 50% by 10 years.[1] In studies using intravascular ultrasound (IVUS) to evaluate the coronary arteries, the incidence of CAV at 1 year post transplantation has been reported to be up to 75%.[6] In a large autopsy study by Billingham, histopathologic findings of graft coronary disease were seen to some extent in all patients at 1 year or more after transplantation.[7]

1.2.3 Pathogenesis

The development of CAV is thought to be multifactorial and include both immunological and non-immunological factors causing chronic vascular inflammation.

Allograft endothelial cells are recognized as foreign bodies by the recipient's immune system, triggering activation of the cellular immune system involving the T lymphocytes and macrophages.[8] Non-immunological factors include traditional vascular risk factors (older age, male gender, obesity, hyperglycemia, and hyperlipidemia), ischemic heart disease etiology, brain death, organ preservation, ischemia–reperfusion injury, and cytomegalovirus (CMV) infection.[9]

The chronic vascular inflammation results in endothelial injury and fibroproliferative cellular responses with subsequent intimal thickening.[8,9] Intimal hyperplasia progresses to flow-limiting stenoses and occlusions of epicardial and intramyocardial arteries. Myocardial ischemia develops in the more advanced stages CAV, which progresses to myocardial fibrosis. Allograft dysfunction and graft failure are the end stage of the disease.

1.2.4 Morphology and histopathology

CAV is typically seen as diffuse and concentric intimal thickening affecting both epicardial and intramural arteries whereas native CAD typically is seen as focal, eccentric lesions in proximal epicardial arteries.[10,11] Histopathologically, both types display fibrofatty plaques consisting of smooth muscle cell proliferation and accumulation of extracellular lipids. Spontaneously occurring native CAD plaques are histopathologically indistinguishable from CAV lesions,[7,12] although disruption of the internal elastic lamina and calcium deposits are typical in native CAD.[12] A necrotic core and calcium deposits are not common in early stages of CAV but have been found to be more prevalent in late CAV.[5] Whether this phenomenon represents a transformation of existing CAV or infiltration of native atherosclerosis is not known, although an association among traditional cardiovascular risk factors and the presence of a necrotic core and calcium[5] is in favor of the latter.

In the early stages of CAV, there is little lumen obstruction despite the intimal thickening, a compensatory enlargement of the external elastic membrane (EEM). Later, there is a constrictive EEM phase that is predominantly responsible for the observed lumen loss.[13]

Time of progression is accelerated and rapid in CAV (months) whereas native CAD typically has a slower development (years).[14] The progression of intimal thickening is most rapid during the first year post HTX, followed by a slower continuous progression.[6,13]

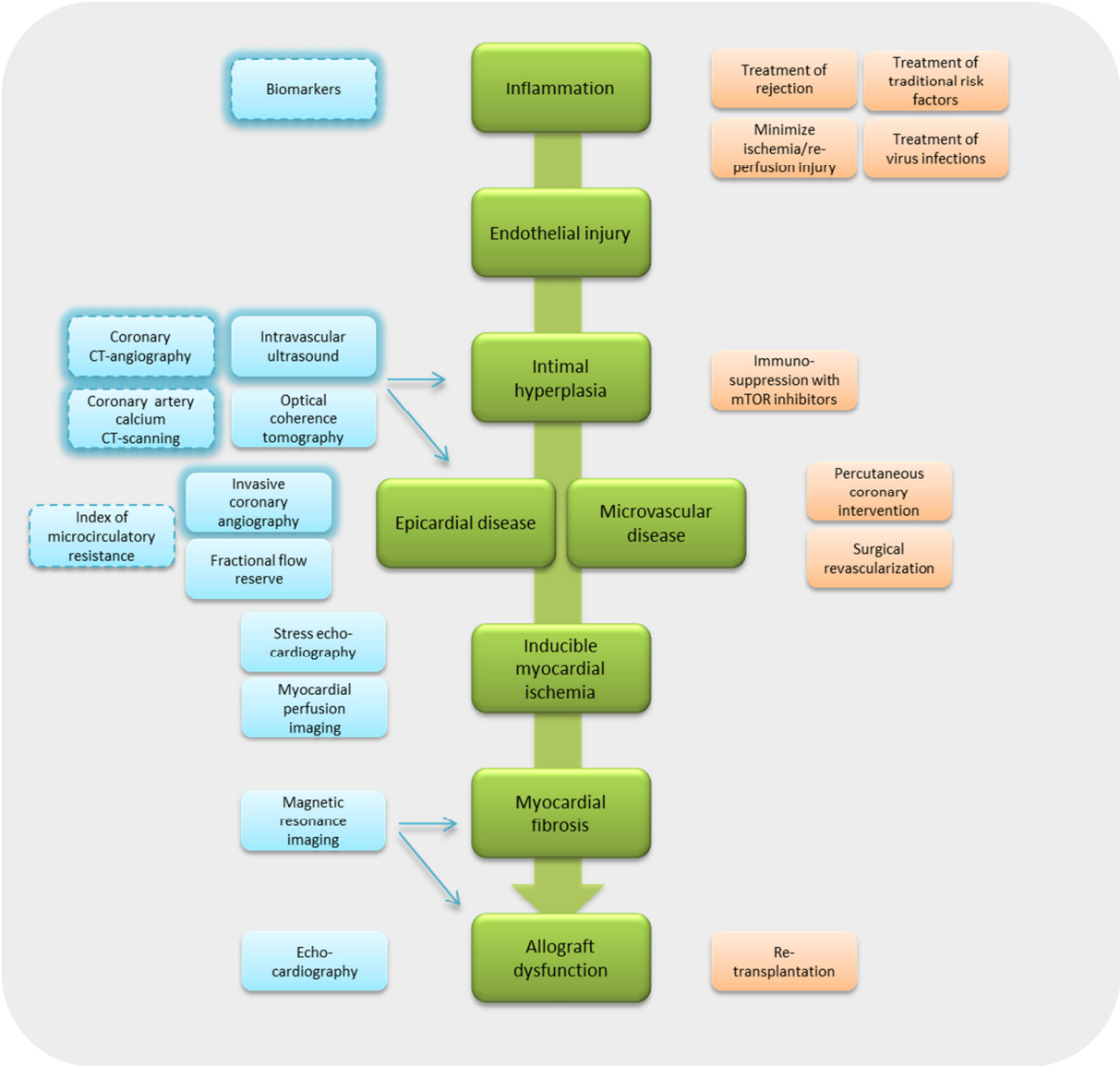

Figure 1. *Overview of cardiac allograft vasculopathy pathogenesis (green), methods of diagnosis (blue), and treatment (orange). Diagnostic methods studied or used in this thesis are marked with a shadowed frame. Dotted frames indicate experimental/investigational methods.*

1.2.5 Diagnosis

In the early era of cardiac transplantation, the diagnosis of CAV was made pathologically. Angiographic diagnosis soon emerged as survival increased and has remained the most important diagnostic tool. The development of IVUS allowed for detection of early stage CAV not identified by invasive coronary angiography (ICA).[15] In later years, circulating immunohistologic markers as well as gene-based and protein-based biomarkers have been studied to see if they can contribute to grading or detecting CAV.[16]

Routine surveillance is important because HTX patients frequently are asymptomatic, particularly in the early stages of the disease. Surveillance includes both evaluation of graft function and visualization of the coronary arteries.

Echocardiography is the first-line imaging modality to assess graft function and is part of all serial evaluations during post-transplant follow-up.[17] With echocardiography, CAV is detected in a late stage when reduced coronary blood flow has resulted in allograft dysfunction. Dysfunction first manifests as diastolic dysfunction with restrictive physiology, then as systolic dysfunction with reduced ejection fraction.

To detect the presence of CAV and identify potential significant stenosis eligible for intervention, annual or biannual screening with ICA is the current standard of care.[16] A number of other non-invasive and invasive imaging modalities are used for CAV evaluation.

1.2.5.1 Non-invasive methods

Detection of CAV is challenging with non-invasive techniques, especially in the early stages. Various non-invasive techniques such as echocardiography, technetium rest/exercise-gated wall motion assessment, thallium single-photon emission computed tomography (SPECT) perfusion imaging, exercise electrocardiogram (ECG), and ambulatory ECG monitoring are insensitive for identifying early stage CAV, most probably because they reflect ischemia rather than coronary obstruction alone.[18]

Dobutamine stress echocardiography (DSE) and SPECT myocardial perfusion imaging (MPI) both have shown prognostic value but have a moderate diagnostic accuracy, which can be related to a limited ability to detect balanced ischemia.[9,19,20] Promising results have been demonstrated for MPI with both positron emission tomography and in magnetic resonance imaging in small studies.[21,22]

The above-mentioned techniques evaluate myocardial structure, function, and/or perfusion. Coronary computed tomography (CT) is the only non-invasive technique assessing the coronary arteries. In the most recent International Society for Heart and Lung Transplantation (ISHLT) guidelines for the care of heart transplant recipients, coronary CT angiography (CCTA) is given a class IIb recommendation (usefulness/efficacy is less well established by evidence/opinion) with a C level

of evidence (recommendations are based on expert consensus and not on randomized controlled clinical trials); CCTA shows promise in the evaluation of CAV in HTX recipients, although higher resting heart rates in these patients limit the technical image quality.[23]

1.2.5.2 Invasive methods

Invasive methods for coronary evaluation include visualizing vessel lumen (coronary angiography), evaluation of vessel wall dimensions and wall components (IVUS, IVUS virtual histology, and optical coherence tomography [OCT]), and evaluation of coronary flow parameters (fractional flow reserve [FFR], and index of microcirculatory resistance (IMR)).

Although a relatively insensitive method for diagnosing CAV, ICA remains the accepted standard of care serving as a screening tool to grossly detect the presence of CAV and is typically performed at an annual or biannual routine basis.[15] The method is clinically available and has documented prognostic significance.[16] The ISHLT recommendations for CAV nomenclature is based on angiographically depicted lesions of CAV.[16] CAV is underestimated by ICA because of the typical features of CAV with a diffuse, concentric intimal thickening. With a diffuse and longitudinal distribution of CAV, there is also a lack of normal lumen diameter reference. Additionally, there is little lumen obstruction in early CAV because of the compensatory enlargement of the external elastic membrane.[13]

IVUS is superior to ICA in detecting CAV. IVUS has been documented to detect CAV in apparently normal angiograms[15] and to predict development of cardiac events even in the presence of a normal coronary angiogram.[2,24] A coronary artery intimal thickness ≥0.5 mm is defined as abnormal by ISHLT guidelines.[23] A rapid progression of maximal intimal thickness (MIT) ≥0.5 mm during the first year after transplantation is a predictor of all-cause mortality and adverse cardiac events.[3,25] On the other side, it has been demonstrated that IVUS-detected intimal hyperplasia does not correlate well with small-artery disease by histologic or immunohistochemical analysis.[26] Although IVUS is very sensitive for defining CAV, the ISHLT guidelines consider it to be an investigational tool and do not recommended IVUS for routine surveillance of CAV.[16] According to the same guidelines, IVUS is optional at baseline (5–6 weeks) and at 1 year after HTX to exclude donor CAD and detect rapidly progressive CAV, respectively, thus providing prognostic information. Being very sensitive for defining CAV, IVUS is an important research tool helping investigators to explore surrogate markers for CAV and evaluate the outcome of various therapeutic conditions.

Virtual histology (VH) is a relatively new IVUS-based technique providing information about plaque components. Four basic tissue components can be identified: fibrous, fibrofatty, calcified, and necrotic core.[27,28] Although VH and IVUS are not a part of the routine surveillance of CAV, the added information on prevalence, morphologic patterns, and distribution from studies using these methods

has led to a better understanding of the CAV process.[29] Much of what has been learned regarding the distribution and morphology of CAV has come from the use of IVUS.[30]

1.2.5.3 Biomarkers

Identification and validation of noninvasive biomarkers aiding in diagnosis and/or predicting outcomes would improve clinical care of HTX recipients. Markers generally used in cardiology such as troponins, brain natriuretic peptide (BNP), and C-reactive protein (CRP) have been studied.[31] Chronic vascular inflammation is thought to play a vital role in the development of CAV, leading to an interest in studying circulating biomarkers related to inflammation.[32] Immunological markers and molecular markers have also been studied.[31,33] No biomarker has proven to be reliable as a detector of CAV according to the ISHLT guidelines published in 2010,[16] and currently no biomarker is routinely used for the evaluation of CAV.

1.2.6 Classification

Prior to 2010, there was no uniform international classification, and definitions of CAV were diverse. In 1988, Gao et al. published a classification of angiographic anatomic abnormalities in transplant coronary vascular disease.[11] The angiographic lesions were coded as type A, B1, B2, and C (Figure 2).

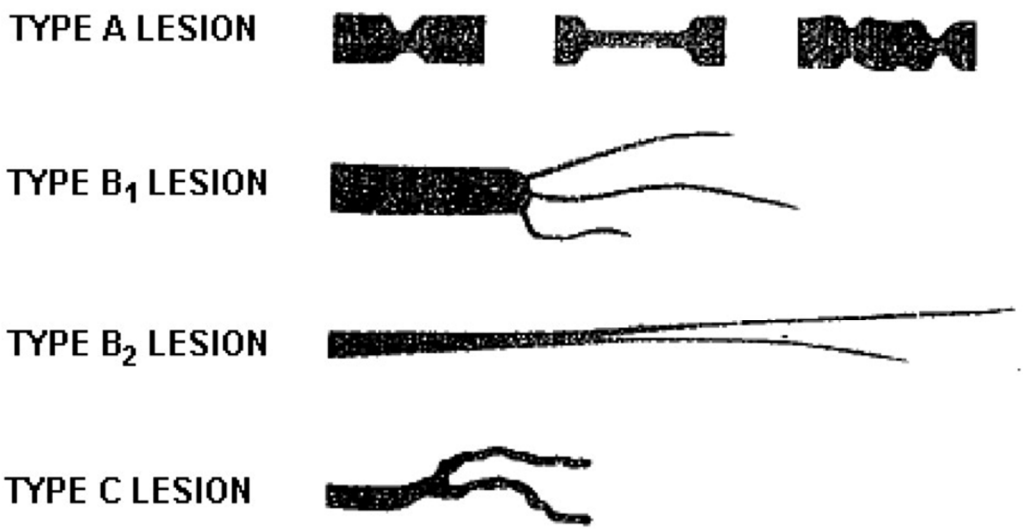

Figure 2. *Anatomic abnormalities in transplant coronary vascular disease. Type A lesion: discrete, tubular, or multiple stenoses. Type B_1 lesion: abrupt onset with distal diffuse concentric narrowing and obliterated vessels. Type B_2 lesion: gradual, concentric tapering with the distal portion having some residual lumen. Type C lesion: narrowed irregular distal branches with terminations that are often non-tapered and squared off, ending abruptly.*

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In 1992, St Goar et al. published the Stanford classification of CAV severity on IVUS.[15] Vascular disease severity was classified according to intimal thickness and degree of vessel circumference involved (Table 1). In 2001, the American College of Cardiology Clinical Expert Consensus Document on Standards for Acquisition, Measurement and Reporting of Intravascular Ultrasound Studies was published, which recommended a threshold for transplant vasculopathy as an intimal thickness of >0.5 mm measured at a target segment of a vessel.[34] This is a widely accepted definition of CAV by IVUS today.

Table 1

Stanford classification of CAV severity on IVUS

	Class I	Class II	Class III	Class IV
Severity	Minimal	Mild	Moderated	Severe
Intimal thickness	<0.3 mm	<0.3 mm	0.3–0.5 mm	>1.0 mm
Extent of plaque	<180	>180	>0.5 mm, <180	>0.5 mm, >180

CAV, cardiac allograft vasculopathy; IVUS, intravascular ultrasound.

Badano, European Association of Cardiovascular Imaging/Cardiovascular Imaging Department of the Brazilian Society of Cardiology recommendations for the use of cardiac imaging to assess and follow patients after heart transplantation, European Heart Journal – Cardiovascular Imaging (2015) 16, 919–948, by permission of Oxford University Press. Original figure from St Goar et al. Circulation, (1992) 85, 979–987, with permission from Wolters Kluwer Health, Inc.

In a large, multi-institutional study published in 1998, Costanzo et al. categorized CAV into normal, mild, moderate, or severe based on angiographic findings; they used a combination of stenosis grading, lesion location in three defined vessel levels (left main [LM] artery, primary vessel, and branch vessel), and number of systems affected (left anterior descending [LAD], left circumflex [CX], or right coronary artery[RCA]).[35]

Finally, in 2010, ISHLT published a working formulation of a standardized nomenclature for cardiac allograft vasculopathy (Figure ISHLT Cardiac Allograft Vasculopathy Nomenclature).[16] These current guidelines classify CAV into four categories, ISHLT CAV_{0-3}, with CAV_0 being “not significant” to CAV_3 being “severe” (Table 2). The nomenclature is based on angiographic findings in combination with assessment of graft function. The angiographic evaluation is a continuation of Costanzo et al.’s categories and includes grading of maximal lumen stenosis at three different vessel levels (left main

artery, primary vessels, and secondary branch vessels). In addition, the presence of allograft dysfunction or evidence of significant restrictive physiology is assessed.

Table 2

Recommended Nomenclature For Cardiac Allograft Vasculopathy

ISHLT CAV_0 (Not significant): No detectable angiographic lesion

ISHLT CAV_1 (Mild): Angiographic left main (LM) <50%, or primary vessel with maximum lesion of <70%, or any branch stenosis <70% (including diffuse narrowing) without allograft dysfunction

ISHLT CAV_2 (Moderate): Angiographic LM <50%; a single primary vessel ≥70%, or isolated branch stenosis ≥70% in branches of 2 systems, without allograft dysfunction

ISHLT CAV_3 (Severe): Angiographic LM ≥50%, or two or more primary vessels ≥70% stenosis, or isolated branch stenosis ≥70% in all 3 systems; or ISHLT CAV1 or CAV2 with allograft dysfunction (defined as LVEF ≤45% usually in the presence of regional wall motion abnormalities) or evidence of significant restrictive physiology (which is common but not specific; see text for definitions)

Definitions

a). A "Primary Vessel" denotes the proximal and Middle 33% of the left anterior descending artery, the left circumflex, the ramus and the dominant or co-dominant right coronary artery with the posterior descending and posterolateral branches.

b). A "Secondary Branch Vessel" includes the distal 33% of the primary vessels or any segment within a large septal perforator, diagonals and obtuse marginal branches or any portion of a non-dominant right coronary artery.

c). Restrictive cardiac allograft physiology is defined as symptomatic heart failure with echocardiographic E to A velocity ratio >2 (>1.5 in children), shortened isovolumetric relaxation time (<60 msec), shortened deceleration time (<150 msec), or restrictive hemodynamic values (Right Atrial Pressure >12mmHg, Pulmonary Capillary Wedge Pressure >25 mmHg, Cardiac Index <2 l/min/m2)

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1.2.7 Prognosis

In a much quoted multicenter study by Costanzo et al., the overall incidence of coronary artery disease–related death or retransplantation 5 years after HTX was 7%.[35] In those with angiographically severe coronary artery disease, two out of three patients experienced coronary artery disease–related death or retransplantation. Using the ISHLT nomenclature, Prada-Delgado et al. found that patients with severe CAV at 1 year after HTX were at a high risk of having an adverse cardiovascular event, with an adjusted hazard ratio of 9.2.[4] According to registry data, CAV is the cause of death in 11–13% of the patients beyond the first year after HTX.[36] It is argued that this number is likely higher because CAV probably is often an unreported underlying cause of graft failure,[37,38] a diagnosis that is registered as the cause of death in 17–26% beyond the first year.[36]

1.2.8 Treatment

There is no effective treatment once CAV is established. Management focus is on strategies and medication targeting triggering factors to prevent development of CAV, and on interventional procedures of eligible lesions when CAV develops. Retransplantation is the only option in end-stage CAV with graft failure.

1.2.8.1 Medication

Medications targeting nonimmunological factors have limited efficacy. Strict control of the cardiovascular risk factors hypertension, diabetes, hyperlipidemia, smoking, and obesity is recommended.[23] Statins are standard care after HTX. In addition to producing an improved lipid profile, statins reduce severe rejection, CAV, and mortality, probably through inhibition of inflammatory and immune responses.[39-41] Antiplatelet therapy with aspirin is used empirically.[9] Prophylaxis and treatment of CMV infection are recommended because CMV infection is associated with the development of CAV.[42] Patients with antibody-mediated rejection have poorer survival and are at increased risk for the development of CAV, and prompt diagnosis and treatment are essential.[43,44]

Calcineurin inhibitors (CNIs) constitute the foundation of immunosuppression after HTX and are most often used in combination with mycophenolate mofetil (MMF) or azathioprine, and with prednisolone. In recent years, much focus has been on a new group of drugs, the mTOR (mammalian target-of-rapamycin) inhibitors, as an alternative to or as a supplement that permits reduction of CNI therapy. The mTORs inhibit proliferation of B and T lymphocytes, as well as fibroblast and smooth muscle cells, and randomized controlled studies have demonstrated reduced CAV incidence or progression in de novo HTX recipients.[45-47] Similar results have not been demonstrated in maintenance HTX recipients, possibly related to different plaque composition at various stages of CAV development.[48] The use of MMF is preferred to the use of azathioprine because it has been documented that MMF reduces progression of intimal thickening compared to azathioprine.[49] The beneficial effect of MMF is thought to be anti-proliferative by suppressing lymphocyte function and controlling arterial smooth muscle cell migration and proliferation, in addition to a decreased systemic inflammatory activity via inhibition of adhesion molecule glycosylation.[8,49]

1.2.8.2 Revascularization and retransplantation

The diffuse and often peripheral distribution of CAV limits the use of both percutaneous coronary intervention (PCI) and surgical revascularization. PCI is undertaken for appropriate discrete lesions, although an increase in overall survival has not been documented.[50-52] Coronary artery bypass grafting in HTX recipients is an option only in highly selected patients who have lesions amenable to surgical revascularization according to current guidelines.[23]

Retransplantation comprises only a minimal volume (2–3%)[1] of the total number of transplantations and is associated with increased post-transplant mortality.[53] Graft failure caused by severe CAV is one of three selected indications currently recommended for retransplantation.[54]

1.3 Malignancy – association with immunosuppression and radiation exposure

Malignancy is a major cause of morbidity and mortality in HTX patients and is caused by the lifelong immunosuppression. The immunosuppressed state *per se* and various potentially oncogenic viruses play a major role.[55] In an Australian publication, HTX patients were found to have a 2.6-fold risk of cancer compared to the general population.[56] An excess risk of non-Hodgkin lymphomas, lip cancer, and lung cancer was identified, and cancers with a viral etiology dominated. A Spanish study found 50% of the post-HTX cancers to be cutaneous and 10% were lymphomas.[57] Statistics from the Cincinnati Transplant Tumor Registry (a worldwide registry) showed a 3–4-fold general increase in cancer risk, but only for certain cancers.[58] As in the Australian study, the most common cancers were cancers of the skin and lips and post-transplant lymphoproliferative disease, which are not so common in the general population. There was no increase in the incidence of lung, breast, prostate, colon, and uterine cervix cancers, malignancies that are common in the general population.

Exposure to ionizing radiation is associated with a risk of cancer. The excess risk in low-dose exposures is uncertain, but a linear no-threshold risk model is often assumed, i.e., the risk is directly proportional to dose at all dose levels.[59] The American Heart Association scientific statement on cardiac CT cites the US Food and Drug Administration as indicating that a 10-mSv CT study may be associated with an approximately 1 in 2000 increase in the possibility of fatal cancer.[60] Based on simulation models, Einstein et al. found that a CCTA with the use of 64-slice multidetector CT (64-MDCT) and ECG-modulated tube current was associated with a lifetime cancer risk of 1 in 715 for a 60-year old woman and 1 in 1911 for a 60-year old man.[61] The risks varied considerably and were markedly greater for women and younger patients. The lifetime attributable organ risk was highest for lung cancer and for breast cancer in younger women.

1.4 Renal impairment and contrast-induced nephropathy

Glomerular filtration rate (GFR) is used to measure overall kidney function. In young adults the normal GFR is 120-130 mL/min per 1.73 m^2, it declines with age and also varies according to gender and body size. Chronic kidney disease is classified into five stages based on GFR according to The National Kidney Foundation (Table 3).[62]

Table 3 Stages of chronic kidney disease according to The National Kidney foundation.

Stage	Description	GFR mL/min per 1.73 m^2
1	Kidney damage with normal or increased GFR	≥90
2	Kidney damage with mild decreased GFR	60–89
3	Moderately decreased GFR	30–59
4	Severely decreased GFR	15–29
5	Kidney failure	<15 (or dialysis)

Adapted from the National Kidney Foundation[62]
GFR, glomerular filtration rate

GFR can be accurately measured using radioactive substances, but more often equations are used to approximate GFR. Estimated GFR (eGFR) by the Modification of Diet in Renal Disease (MDRD)formula, is calculated using serum creatinine, age, ethnicity, and gender.[63] The Cockcroft-Gault formula employs serum creatinine measurements, patient's weight, and gender to predict estimated creatinine clearance rate.[64] Creatinine is a waste substance measured in the blood plasma and is frequently used alone to estimate renal function. However, as the creatinine level is not elevated until 50% of the total kidney function is lost, it is unable to detect the early stages of kidney failure.[65]

Renal impairment after HTX is common. According to statistics, a total of 45% of the patients have abnormal creatinine at 5 years post-transplantation and 12% have creatinine ≥2.5 mg/dL (220 µmol/L).[1] After HTX, there is first a rapid decline in renal function and thereafter a slower deterioration. Pooled results from seven studies indicate that development of end-stage renal failure occurs in 2–4% during 4–7 years of follow-up after HTX.[66] A 44% decline in the average GFR was reported by Lindelöw et al. at 9 years after HTX compared to preoperative values.[67] Cyclosporine-induced nephrotoxicity is thought to be an important contributor.[68,69]

Intravascular iodinated contrast material is associated with the development of subsequent acute kidney injury, often termed contrast-material–induced nephropathy (CIN). Several potential nephrotoxic mechanisms have been suggest in animal models, including vasoconstriction, the formation of reactive oxygen species, and direct tubular toxicity.[70] CIN generally refers to a condition

in which an impairment in renal function (an increase in serum creatinine by more than 25% or 44 mmol/L) occurs within 3 days following the intravascular administration of contrast media in the absence of an alternative etiology.[71] A widely cited paper by Nash et al. suggests that CIN is the third most common cause of acute kidney injury in hospitalized patients.[72] In recent years, the incidence and severity of CIN have been questioned.[73,74]

A significant implication of reduced renal function is a restricted use of iodine contrast media based on the risk of developing CIN. Impaired renal function excludes a large number of HTX patients from iodine contrast-enhanced imaging in the follow-up of the coronary arteries.

1.5 Coronary computed tomography

1.5.1 General aspects

1.5.1.1 Technical aspects

Cardiac CT imaging includes coronary artery calcium (CAC) CT scanning and CCTA. The following technical considerations are predominantly concerned with CCTA but are also relevant for understanding CAC CT scanning.

Imaging the coronary arteries with CT is technically demanding. Spatial and temporal resolution is challenged by the small, torturous vessels moving synchronously with the beating heart. Electron beam CT was the first non-invasive imaging modality with cross-sectional visualization of the heart. It has a high temporal resolution of 100 ms, but the spatial resolution is limited by a slice thickness of 3 mm. With the introduction of multidetector technology, cardiac imaging with mechanical helical CT systems became possible.[75] Starting out with cardiac imaging using 4-slice MDCT, a 64-MDCT is considered the minimum prerequisite for adequate scanning of the heart today.[76,77]

Spatial resolution

To be visualized adequately, the coronary arteries require isotropic submillimeter spatial resolution. Spatial resolution with contemporary 64-MDCT is 300–400 μm[78] and is 230–240 μm on the newest high-end scanners (vendor website information; GE Revolution CT, Siemens Somatom Force). Coarse coronary calcifications are still a challenge to reliable visualization of the lumen because of blooming artifacts and reduce the specificity of CCTA.[79-81] Likewise, assessment of coronary stent patency can be inadequate because of metal artifacts and limited spatial resolution[82,83] and is not recommended in the European guidelines.[76]

Temporal resolution

High temporal resolution is a prerequisite for imaging the coronary arteries to avoid cardiac motion artifacts.[84] The data acquisition time per image is referred to as temporal resolution. In cardiac imaging, a half gantry rotation is sufficient for reconstruction of one image; therefore, temporal resolution is half the gantry rotation time. High-end CT systems have rotation times of 250–280 ms (vendor website information; Siemens Somatom Force, Phillips Brilliance iCT, Toshiba Aquillion Genesis, GE Revolution CT), resulting in a temporal resolution of 125–140 ms. Dual-source (DS) systems with two x-ray tubes and corresponding detectors operating simultaneously provide temporal resolution close to a quarter of a rotation time, which presently is 66 ms with the fastest scanner (vendor website information; Siemens Somatom Force). Shorter rotation time enables adequate imaging of higher heart rates.[84] Medication to lower the heart rate to 60–65 beats per minutes (bpm) is currently recommended by European guidelines.[76]

Another important temporal aspect is to minimize the time needed to cover the heart in the z-axis (the long axis of the patient). The optimum is to cover the heart in only one heartbeat to avoid misalignment artifacts related to the heart being differently positioned in consecutive heartbeats, which is especially noticeable in arrhythmia. One-heartbeat coverage is achieved with wide detector technology or with DSCT high-pitch technology. The widest detectors are 16 cm wide and cover the whole heart in one rotation.[85,86] In high-pitch technology, the high pitch facilitates data acquisition of the whole length of the heart within the diastole of a heartbeat, and the dual detector system enables gapless volume coverage despite the high pitch by doing two helical acquisitions almost simultaneously.[87,88]

Scan modes

When imaging a beating heart, the images need to be reconstructed in consistency with a cardiac phase, i.e., systole or diastole. This reconstruction is facilitated with ECG-synchronized data acquisition. There are two types of ECG-synchronized scanning modes: retrospective ECG-gated helical scanning and prospective ECG-triggered axial (sequential) scanning. A variant of the prospective ECG-triggered method is used in high-pitch DS scanning where a helical data acquisition in the diastole of one heartbeat is prospectively triggered by the patient's ECG.

In retrospective ECG-gating, the data are acquired in a continuous, helical scan and a continuous movement of the table with simultaneous recording of the patient's ECG. The ECG recording guides data selection to ensure phase-consistent image reconstruction of data taken from several cardiac cycles. Sets of data can be reconstructed from any phase of the cardiac cycle, and the availability of both systolic and diastolic reconstructions makes the technique quite robust and can be essential in patients with high heart rates. In high heart rates, the optimal phase for

reconstructing the left part of the coronary tree is most often the diastole while the right part is often best reconstructed in the late systole.[89]

In prospective ECG-triggered sequential scanning, the data are acquired at a predefined phase of the cardiac cycle in an axial scan with a stationary table. Data acquisition is initiated by the patient's ECG signal using the R peak as a reference. Depending on the detector width, one or more sequential axial scans are needed to cover the entire heart volume. If more than one scan is needed, the table has to be moved to the next scan position between each data acquisition; hence, the term "step-and-shoot." The prospectively ECG-triggered scan mode effectively reduces the time of radiation exposure because only a short part of the cardiac cycle is scanned. The short exposure time does, however, restrict the possibility of multiple reconstructions throughout the cardiac cycle. Prospective triggering is limited to patients with low heart rates (<70–75 bpm in systems with gantry rotation time 250–280 ms) and with stable sinus rhythm.[59]

1.5.1.2 Radiation exposure

Dose-saving strategies

In all procedures involving ionizing radiation, the small stochastic risk of malignancy induction should be taken into consideration, and radiation exposure should always be kept as low as reasonably achievable. There has been much focus on radiation dose in cardiac imaging, and great efforts have been put into the development of dose-saving strategies by the vendors. The most important dose-saving strategies/techniques are choice of scan mode, ECG-synchronized tube current modulation, tube voltage reduction, and iterative CT data reconstruction.[90] Choice of scan mode is probably the single most important factor influencing radiation exposure. Prospectively ECG-triggered axial scanning significantly reduces the radiation dose compared to retrospective ECG-gated helical scanning; reductions of up to around 70–80% have been reported.[91,92] In the latest generation of high-end scanners, submillisievert dose levels have been demonstrated with the combined use of ECG-triggered scan mode, lower tube voltage, automated exposure control, and iterative reconstruction algorithms with both high-pitch and wide-volume scanners.[84,88,93]

Patient-related dose factors

Patient-related factors are important predictors of radiation dose. Heart rate and heart rate regularity are important determinants of radiation dose because most of the dose-reducing alternatives depend on a low and steady heart rate. Depending on gantry rotation speed, there is an upper limit for prospective ECG-triggered scanning of 60–65 bpm in earlier systems[94] and 70–75 bpm in high-end scanners.[84] Body weight is another factor with a profound effect on radiation dose.

Heavier patients require higher tube voltage and current to achieve acceptable image noise levels, which consequently increases radiation exposure.[95]

Radiation dose parameters

The radiation dose parameters used for CT are volume CT dose index ($CTDI_{vol}$), expressed in units of mGy, and dose-length product (DLP), expressed in units of mGy*cm. Simplified, $CTDI_{vol}$ is an estimate of the average radiation dose for a specific scan protocol for one tomographic image with pitch incorporated. DLP is the product of the $CTDI_{vol}$ and the scan length. The $CTDI_{vol}$ is recommended for optimizing CT protocols whereas DLP should be used for comparing radiation doses and characterizing radiation dose from CT studies.[59] To estimate an effective dose for adult patients, the DLP is multiplied by an organ-weighting factor (k). In cardiovascular imaging, the k value for chest examination is used, which currently is 0.014 mSv per mGy*cm.[96]

1.5.2 Coronary CT-angiography

CCTA is a contrast-enhanced examination requiring high spatial and temporal resolution and low noise to depict the coronary artery lumen, analogous to invasive coronary angiography. In addition to lumen visualization, CCTA depicts the vessel wall and surrounding structures.

The clinical utility of CCTA in the general population has been established.[97] The strengths of CCTA are its high sensitivity and high negative predictive value (NPV), which in a meta-analysis of 28 studies including 3674 patients were reported to be 91% and 99%, respectively.[98] CCTA identifies the majority of patients with significant disease, and most important, it is an effective non-invasive alternative to ICA for ruling out significant stenosis. A greater part of HTX patients undergo annual ICA to identify potential significant stenosis, so a non-invasive method that could serve as a gatekeeper for ICA would be of great interest in this population. It has been proposed that CCTA with its ability to visualize the coronary wall would be superior to ICA in detecting earlier stages of CAV.[99,100]

CCTA in HTX patients can be challenging because of characteristics typical for this group. Because their hearts are denervated, most HTX patients have higher heart rates than non-transplanted persons, and beta-blockers also have a limited effect. High heart rates may reduce image quality and also increase radiation dose because prospective gating requires a low heart rate. Increased body mass index (BMI) reduces image quality and precludes the use of low tube voltage to reduce radiation exposure. CAV frequently afflicts the periphery of the coronary tree with small-caliber vessels; thus, spatial resolution and image noise are challenges. The need for repeated examinations requires radiation dose awareness.

With the risk for CIN, the use of contrast agent is a limitation with CCTA in the HTX population because impaired kidney function is frequent after HTX. At present, more contrast agent

is needed for CCTA than for ICA. Barthélémy et al. reported a mean total contrast agent volume of 91 mL for CCTA versus 56 mL for ICA in a study of HTX patients.[101]

1.5.3 Coronary artery calcium scoring

CAC CT scanning is a non-contrast examination done to quantify calcified atherosclerotic plaques. Calcifications are detected without the use of contrast media, so patients can be scanned regardless of kidney function. High spatial resolution and low image noise are not as critical for detection of coronary calcium, allowing for low-dose technical settings. The estimated effective dose is usually 1–3 mSv.[59] In 1990, Agatston et al. published a method of quantifying CAC.[102] Later, volume score[103] and calcium mass score[104] were introduced as methods of quantification, but the CAC scoring Agatston et al. introduced remains the most widely used method both in clinical and research settings.[105]

CAC is a marker of coronary atherosclerosis.[106,107] The amount of coronary calcium detected by CT correlates with the total atherosclerotic plaque burden.[107,108] Large cohort studies in the general population have demonstrated that absence of CAC dependably excludes significant stenoses and predicts a low risk of future cardiac events.[109,110] As previously described, the development of CAV is thought to be a multifactorial inflammatory process including immunological and non-immunological factors and different from the native atherosclerotic process. CAC CT scanning assesses the calcified and necrotic components of a coronary plaque, which are typical in native CAD.[12] A necrotic core and calcium deposits are not common in early CAV but have been found to be more prevalent in late CAV.[5] Thus, studies of CAC in the general population with native CAD might not be valid for HTX patients with vasculopathy predominantly of a different etiology.

1.6 Invasive coronary angiography

ICA provides a planar two-dimensional silhouette of the coronary artery lumen, usually in a cine function and in multiple views. The coronary arteries are evaluated for percentage luminal diameter reduction. ICA has a spatial resolution of 150–200 μm and a temporal resolution of 10 ms.[78] ICA is universal in availability and applicable at any time after HTX.

ICA is generally regarded as a safe procedure, but because it is invasive, it carries a small, though not negligible, risk of complications. Today, most patients are accessed via the radial artery, which significantly reduces the risk of risk of major bleeding compared to femoral access.[111] The total risk for major complications of death, myocardial infarction, or stroke using radial access and femoral access is 2.5% and 3.8%, respectively.[111] In addition, the procedure depends on the use of contrast agent with the risk of inducing CIN.

Vascular access can in some cases be challenging, particularly in HTX patients who have repeated examinations as well as often having had other vascular procedures performed in the course of being transplanted. Patient discomfort and use of resources are additional negative factors.

An advantage of the invasive nature of ICA is that it facilitates the option of performing percutaneous intervention within the same procedure. ICA may also be combined with other catheter-based techniques, such as IVUS, OCT, and FFR and IMR.

The radiation dose parameter used for ICA examinations is the dose area product (DAP), expressed in units of mGy*cm^2. DAP is the product of dose in air in a given plane and the area of the irradiating beam and is independent of the distance from the x-ray source.[112] To estimate an effective dose for adult patients, the DAP is multiplied by a conversion factor of 0.2 mSv/(Gy.cm^2).[96,113]

Radiation exposure varies widely according to a review by Einstein et al.,[114] with studies reporting effective doses from 2.3–22.7 mSv, but with a typically cited value of 7 mSv. The latter is in line with an effective dose of 6.0 mSv for ICA reported in a study of CCTA in HTX patients.[101] In two studies comparing MDCT with ICA, a mean effective dose of 5.6 ± 3.6 mSv and 6.0 ± 3.5 mSv, respectively, was reported for ICA.[101,115] The latter of the two studies involved a HTX population.

1.7 Intravascular ultrasound and virtual histology

IVUS provides cross-sectional grey-scale images of the coronary lumen and arterial wall using a miniaturized ultrasound transducer mounted on the tip of a catheter. The images have an axial resolution of 50–80 μm,[116] which is highly superior to ICA and CCTA. The high resolution enables a unique visualization of the entire vessel wall, including the elastic lamina, intima, and lumen.

For measurements, contour detection of both the lumen and EEM is done, and several parameters can be recorded. Published IVUS parameters include (1) intimal thickness, (2) intimal index, (3) change in maximal intimal thickness at a reference point, (4) total atheroma volume, (5) percentage of atheroma volume, and (6) rapidly progressive CAV.[16] Serial IVUS measurements have been proved to be highly reproducible.[117] If performed in HTX patients, ISHLT recommends maximal intimal thickening evaluation based on automated pullback in one or more epicardial vessels over a 40- to 50-mm long segment.[16] Usually one of the major epicardial vessels is imaged, though detection of early stage CAV is increased with multi-vessel imaging.[118]

The IVUS catheter is approximately 1 mm in diameter.[116,119] The size of the IVUS catheter limits the method to imaging the larger epicardial vessels; consequently, small-caliber vessel evaluation and assessment of microvascular disease are not feasible.[26,119] As with all catheter-based procedures, there is a risk of complications. The most frequent adverse event is vessel spasm; other less frequent events are acute occlusion, dissection, and arrhythmia.[120,121] In a multicenter study by Hausmann et al., spasm occurred in 2.9% of the patients and complications other than spasms (occlusion, embolism, dissection, and thrombus) in 0.4%.[120] In multi-vessel imaging, Stone et al. documented a complication rate of 1.6%; complications recorded were dissections and one

perforation.[122] Other factors in disfavor of the use of IVUS on a regular basis in surveillance of HTX patients are increased procedure time, increased cost, and lack of expertise.[123]

VH is a relatively new technique using spectral analysis of backscatter radiofrequency data obtained during IVUS pullback to assess plaque components. The frequency components of the reflected ultrasound signal are analyzed, resulting in a power spectrum that represents the magnitudes of all the frequencies within the returned signal.[34]

Four basic tissue components can be identified with a predictive accuracy of 94–97% *ex vivo* and 87–97% *in vivo*: fibrous, fibrofatty, calcified, and necrotic core.[27,28] The necrotic core component consists of lipid cells and necrotic and lymphocyte remnants together with tissue microcalcification. Necrotic and dense calcified tissue has been considered to at least partly reflect inflammatory tissue components of the vessel wall being associated with a higher subsequent progression of CAV.[27,124] An increased intimal inflammatory tissue component was defined by Raichlin et al. as >30% necrotic and dense calcified tissue.[124] This cut-off is supported by the results of other *ex vivo* and *in vivo* VH-IVUS studies performed in patients with ischemic heart disease.[125-127]

1.8 Systemic markers of inflammation

The soluble inflammatory markers evaluated in paper III are derived from the cellular immune system involving the T helper type 1 cells (Th1). Antigen presentation by cells from the innate immunity induces proliferation of naïve T cells to active Th1 cells. Macrophages are activated by interferon-ɣ (IFN-ɣ) secreted by the Th1 cells. Activated macrophages produce the proinflammatory cytokines (upstream inflammatory pathway) tumor necrosis factor-α (TNF-α), interleukin (IL)-1, and IL-6, which induce and enhance inflammatory responses from target organs (downstream inflammatory pathway). Responses include the production of the prototypical acute-phase protein CRP by the liver, as well as endothelial cell activation by upregulation of the vascular cell adhesion molecule-1 (VCAM-1) and glycoprotein 130 (GP130), and production/release of von Willebrand factor (vWf).

Neopterin is a catabolic product synthesized by the macrophages and is a marker of macrophage activation. Increased neopterin concentrations are an independent marker for cardiovascular disease and a predictor of future cardiovascular events in patients with coronary artery disease.[128]

Osteoprotegerin (OPG) is a soluble glycoprotein that regulates bone resorption by inhibiting osteoclast precursors.[129] It is a decoy cytokine receptor of the TNF receptor family. Vascular cells producing OPG include endothelial cells and vascular smooth muscle cells.[130] Elevated serum OPG levels are associated with the presence and severity of CAD determined by coronary angiography.[131]

Research indicates that OPG is not only a marker but also a mediator of vascular pathology modulating osteogenic, inflammatory, and apoptotic responses.[130]

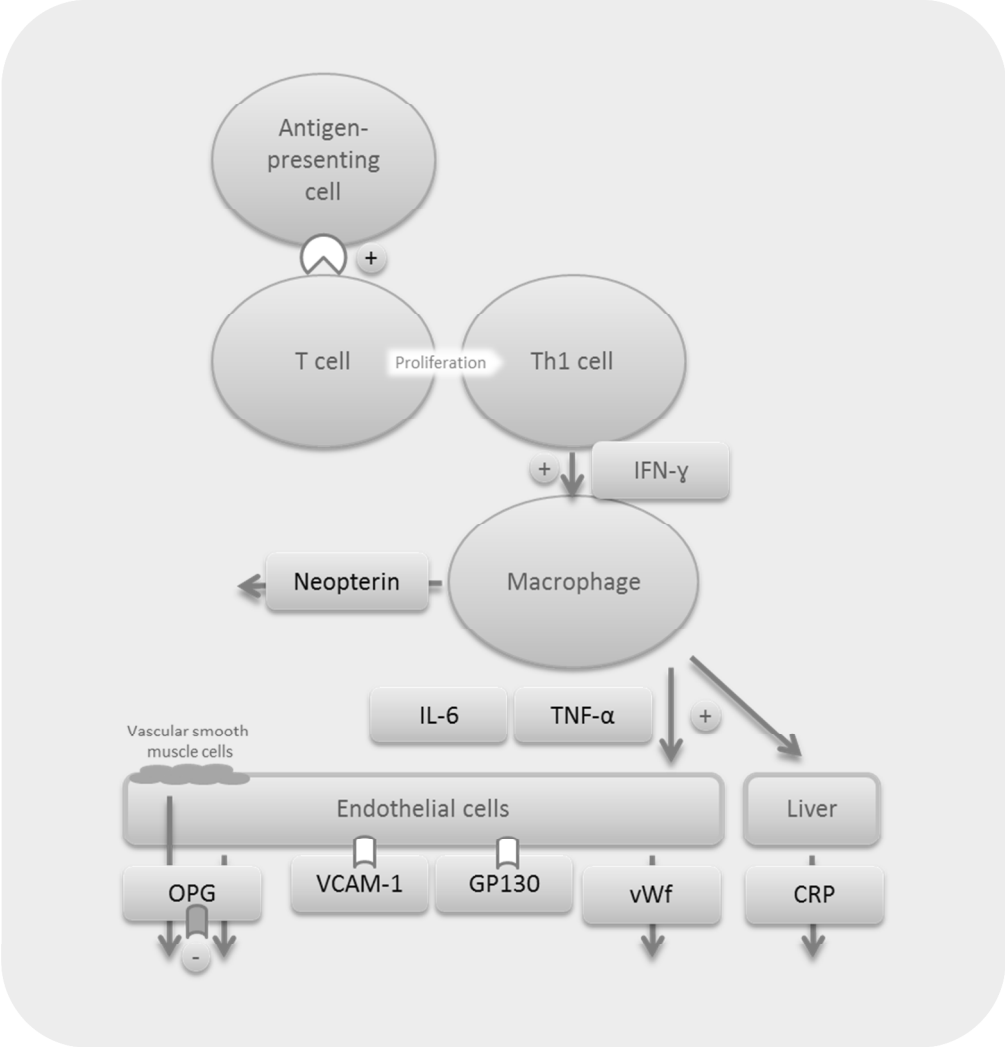

Figure 3. *Systemic markers of inflammation. The pathway of activating the cellular immune system involving the T-helper type 1 cells (Th1) inducing inflammatory responses. IFN-ɣ, interferon-ɣ; TNF-α, tumor necrosis factor-α; IL, interleukin; CRP, C-reactive protein; VCAM-1, vascular cell adhesion molecule-1; GP130, glycoprotein 130; vWf , von Willebrand factor.*

2 Aims of the study

2.1 General aims

The overall aim of the thesis was to explore the utility of different imaging modalities in the diagnosis and prediction of allograft vasculopathy after heart transplantation.

2.2 Specific aims

Paper I

To evaluate the performance of CCTA in a HTX population

CCTA was evaluated for its:

- ability to rule out significant stenosis compared to ICA
- ability to detect CAV with IVUS as reference standard
- image quality
- estimated radiation dose

Paper II

To evaluate CAC detected by CT for its predictive value for short- and long-term outcomes in a HTX population

The absence of CAC was evaluated for its ability to:

- exclude CAV of moderate to severe grade (CAV_{2-3}) using the ISHLT recommended nomenclature
- exclude significant stenosis using ICA as the reference standard

CAC was evaluated as a surrogate marker for long-term outcome using death or graft loss (D/GL), and non-fatal major adverse cardiac events (NF-MACE) as outcome variables

Paper III

With the use of quantitative and qualitative CAV measurements by IVUS, to evaluate the inflammatory milieu associated with CAV

Systemic markers of inflammation represented by an extensive profile of clinical variables and immune markers were analyzed for their association with:

- CAV quantitatively determined by IVUS measurements of MIT
- CAV qualitatively assessed by the proportion of inflammatory tissue in the thickened intimal area estimated by VH

3 Material and methods

3.1 Study design

The study had an observational, cross-sectional, prospective, and blinded design. In paper II, the included participants were also followed longitudinally for up to 10 years.

3.2 Patients

Eligible subjects were patients ≥18 years of age with ≥12 months since HTX, scheduled for annual follow-up including coronary angiography. Exclusion criteria were related to administration of iodine contrast agent, ability of breath-holding, ECG synchronizing of image reconstruction, and radiation exposure, as appropriate for the imaging methods included in each paper.

In papers II and III, estimated GFR <30 mL/min using the Modification of Diet in Renal Diseases formula[63] was an exclusion criterion because HTX recipients with severe renal impairment at our center do not undergo annual angiography unless clinically indicated. In paper I, the additional contrast agent given for the CCTA warranted stricter kidney function criteria, and patients with estimated creatinine clearance <50 mL/min/1.73 m², calculated using the Cockcroft–Gault formula,[64] were defined as not eligible for inclusion. A history of severe adverse reaction to iodinated contrast agent was also a criterion for exclusion in paper I.

Severe heart failure and severe lung disease restricting breath-hold during CT scanning and atrial fibrillation disturbing the ECG-synchronized image reconstruction were exclusion criteria in papers I and II. Pregnant women were not eligible for inclusion because of the risk associated with radiation exposure.

An overview over the study population is presented in Figure 4.

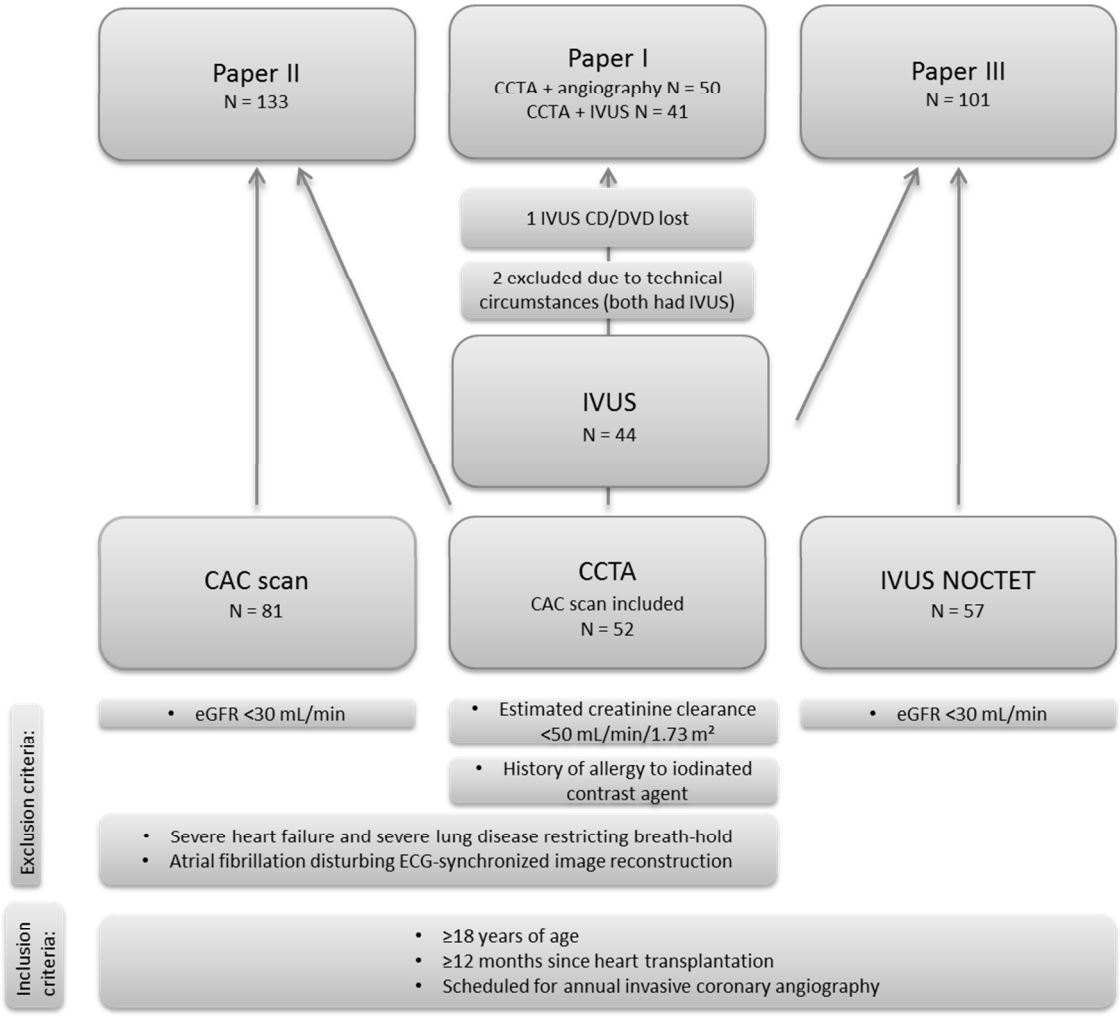

Figure 4. Overview study population with inclusion and exclusion criteria.
N, number; CCTA, coronary computed tomography angiography; IVUS, intravascular ultrasound; CAC, coronary artery calcium; eGFR, estimated glomerular filtration rate. The NOCTET (NOrdic Certican Trial in HEart and lung Transplantation) trial.[48,132]

3.3 Methods

3.3.1 Clinical data

Baseline information about the patients including demographics, medication, biochemical data, and echocardiography (for paper II) were recorded during the annual follow-up at time of inclusion. Information about medical history and donor characteristics was obtained from medical records. These variables were previously identified as being associated with the development of atherosclerosis or CAV or generally associated with an elevated risk of D/GL following HTX.

In paper II, the patients were followed to the time of D/GL or to their last clinical follow-up before study closure, and long-term outcome variables were obtained from medical records, which in turn are continuously updated with information from the Norwegian Population Register and computerized medical records. Figure 5 gives an overview of all baseline and outcome variables recorded in paper II.

Immunosuppressive therapy consisted of maintenance therapy with prednisolone, cyclosporine or tacrolimus, and azathioprine or mycophenolate mofetil. No cytotoxic induction therapy was given, and statins were introduced as standard therapy from 1997 onwards. Prednisolone was commenced immediately following HTX, with weaning off by 1.25 mg at every negative endomyocardial biopsy performed during the first 3 months post-HTX, to a maintenance dose of 0.1 mg/kg.

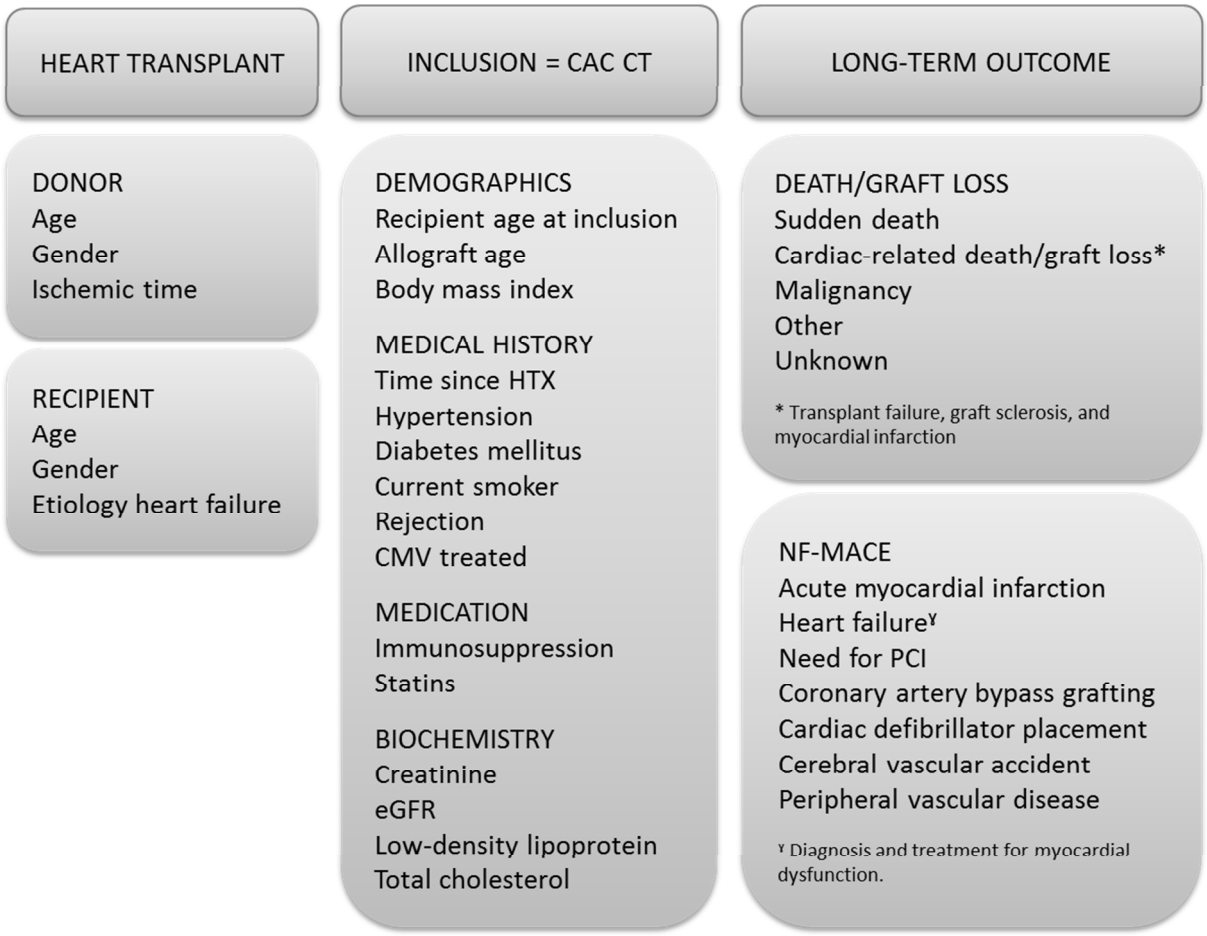

Figure 5. *Demographic, clinical characteristics, and outcome variables of the study population in paper II at time of heart transplant, inclusion, and long-term follow-up.*
CAC CT, coronary artery calcium computed tomography; HTX, heart transplant; CMV, cytomegalovirus; eGFR, estimated glomerular filtration rate; D/GL, death/graft loss; NF-MACE, non-fatal major cardiac event; PCI, percutaneous cardiac intervention.

3.3.2 Long-term outcomes

Long-term outcomes were defined using the same variables as in other studies,[3,133] which included graft survival and NF-MACE, defined as acute myocardial infarction, congestive heart failure, need for PCI, coronary artery bypass grafting, cardiac defibrillator placement, cerebral vascular accident, and peripheral vascular disease (Figure 5). The inclusion date was defined as the date of the CT scan. Censored date for the outcome graft survival was either the time of D/GL or the date of the patient's last recorded clinical follow-up. A combined outcome was defined as the date of the first event of either a NF-MACE or D/GL, or the date of the patient's last recorded clinical follow-up if no adverse event occurred.

3.3.3 Coronary artery calcium CT

In paper II, the patients were examined with either 16- or 64-MDCT (GE Light Speed Pro16 and VCT; General Electric Healthcare Technologies, Milwaukee, Wisconsin, USA) using prospective electrocardiographic triggering. Images were reconstructed with a slice thickness of 2.5 mm at an increment of 2.5 mm. All the scan and reconstruction parameters are listed in Table 4.

3.3.3.1 Analysis

An Advantage Windows 4.3 workstation (General Electric Healthcare Technologies, Milwaukee, Wisconsin, USA) was used for calculating the CAC score, which is presented as the Agatston score,[102] a semi-quantitative measurement where regions of interest in the coronary arteries with two or more adjacent voxels with density ≥130 Hounsfield units (HU) are scored. The area of each region (mm^2) is multiplied by a number related to the maximal density of that region of interest, as follows: 1 if maximal density is 130 to 199 HU; 2 if 200 to 299 HU; 3 if 300-399 HU; and 4 if 400 HU or more. Scoring was performed by two experienced readers blinded to the ICA and ISHLT CAV results. If there was a discrepancy in scoring between the readers, a consensus reading was performed.

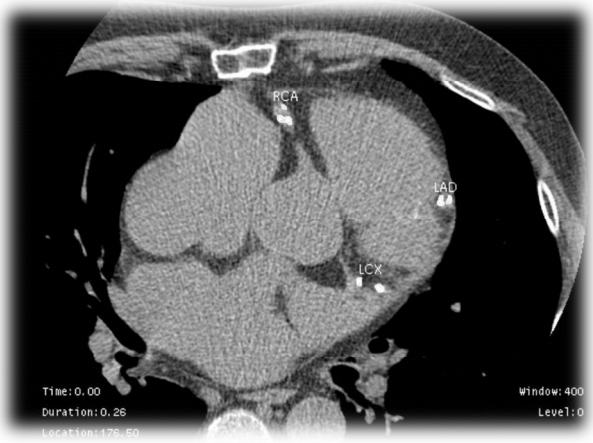

Figure 6. *Example of coronary artery calcium scoring with computed tomography. Regions of interest in the coronary arteries representing coronary calcium have been marked using a computerized program on the Advantage Windows workstation.*

3.3.4 Coronary CT-angiography

Enrolled subjects underwent CCTA 2–4 weeks prior to their annual ICA. All scans were obtained with a 64-MDCT scanner (GE Light Speed VCT; General Electric Healthcare Technologies, Milwaukee, Wisconsin, USA) using retrospective ECG-gating and ECG-synchronized tube current modulation. Preceding the CCTA, a prospectively ECG-triggered non-enhanced scan was obtained for CAC scoring, as described above. All the scan and reconstruction parameters are listed in Table 4.

An intravenous beta-blocker was administered prior to the CCTA if the heart rate was >70 bpm, and there were no patient-related contraindications.

The patients received 20 + 80 mL of the non-ionic contrast medium iodixanol (Visipaque 320; GE Healthcare, Oslo, Norway). The first 20 mL was used in a test bolus for calculation of optimal scan delay.

Datasets were reconstructed at 40, 50, 60, 70, 75, and 80% of the R-R interval. An Advantage Windows 4.3 workstation (General Electric Healthcare Technologies, Milwaukee, Wisconsin, USA) was used for image post-processing of the CCTA and for calculating the CAC score. Maximum and minimum heart rate during the scan was recorded, and heart rate variability was calculated.

Table 4. Coronary CT-angiography and coronary artery calcium CT scan and reconstruction parameters.

Parameter	Coronary CT-angiography	Coronary artery calcium CT
Tube potential (kV)	120	120
Tube current (mA)	100–800 (ECG-modulated)*	300–400**
Gantry rotation time (s)	0.35	0.35 or 0.40
Pitch	0.18–0.24 (heart rate dependent)	NA
Detector collimation (mm)	40	20
Detector configuration	64 × 0.625 mm	8 × 2.5 mm
Section width (mm)	0.625	2.5
Reconstruction increment (mm)	0.625	2.5
Reconstruction algorithm	Standard	Standard

* Maximum tube current between 40% and 80% of the R-R interval and a minimum tube current during the remainder of the cardiac cycle. The maximum tube current was chosen at the radiographer's discretion according to body habitus, and the minimum tube current was set at 20% of the maximum.
**Tube current depending on patient body weight.

3.3.4.1 Analysis

Stenosis grading and coronary allograft vasculopathy detection

Analyses were performed on a per-segment basis using the 16-segment classification of the American Heart Association.[134] Coronary artery segments with a luminal diameter ≥2 mm and a quality score better than 4 on the CCTA were included and graded on the basis of luminal diameter reduction, as follows: normal, minimal (lumen reduction <33%), non-significant stenosis (lumen reduction 33–50%), and significant stenosis (lumen reduction ≥50%).The CCTAs were independently analyzed by two investigators blinded to the outcome of the ICA. Consensus reading was performed if there was a discrepancy in grading between readers. With any discrepancy in stenosis grading compared to ICA, a validation of the anatomy was performed to ensure segment match.
Coronary segments were grouped as proximal (LM, proximal and middle LAD and RCA, and proximal and distal CX) and distal (distal LAD and RCA, and side branches).
CAV was defined as any wall irregularity on CCTA, and the most severe lesion was used to classify each segment. Analyses were done by one investigator blinded to the result of ICA and IVUS.

Image quality

Each segment was assessed by two independent readers for image quality and scored on a scale from 1 to 4, as follows: 1 = no artifacts, 2 = minor artifacts, 3 = some artifacts but interpretable, and 4 = not interpretable (Figure 7). The same grading scale has been used by others evaluating CCTA images of HTX patients.[135,136] A middle value was set if there was a discrepancy of one step between the readers. A discrepancy regarding grade 4 or a discrepancy of 2 steps prompted a consensus reading.

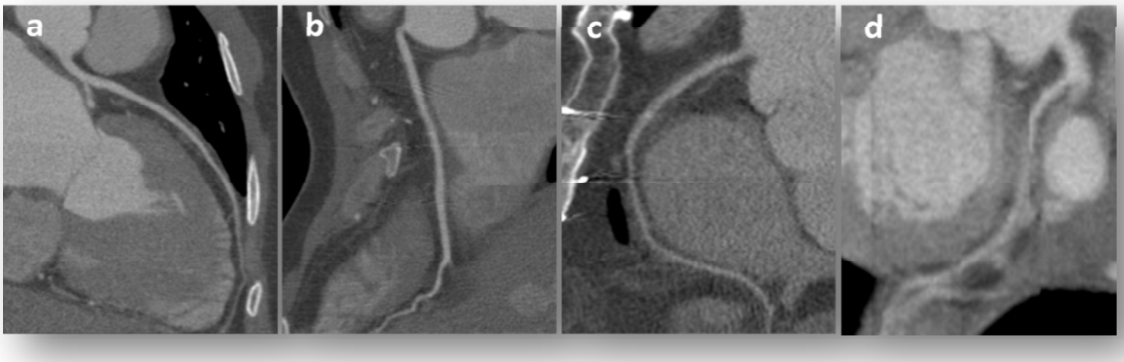

Figure 7. *Image quality. Examples of image quality grading: (a) grade 1 = no artifacts, (b) grade 2 = minor artifacts, (c) grade 3 = some artifacts but interpretable, and (d) grade 4 = not interpretable.*

Estimated radiation dose

The DLP was recorded for each examination. An effective radiation dose was estimated by multiplying the DLP by a conversion factor of 0.014 mSv/(mGy*cm) according to standard methodology.[96]

3.3.5 Invasive coronary angiography

In both papers I and II, ICA was performed by standard hospital procedure. Analyses were performed on a per-segment basis using the 16-segment classification of the American Heart Association[134] and graded based on luminal diameter reduction.

3.3.5.1 Analysis

Stenosis grading, coronary allograft vasculopathy detection, and ISHLT classification

The segments of all ICA examinations included in papers I and II were initially graded as normal or with lumen diameter reduction <33%, 33–49%, 50–70%, 75%, 90%, or 100%.

In paper I, the segment grading was recategorized to normal or a lumen diameter reduction <33%, 33–50%, or ≥50%. Significant stenosis was defined as ≥50% luminal reduction. ICA was independently analyzed by two investigators who were blinded to the result of the CCTA modality. Consensus reading was performed if there was a discrepancy in grading between readers. For discrepancies in stenosis grading compared to CCTA, a validation of the anatomy was performed to ensure segment match. In paper I, a separate analysis of ICA was performed for the evaluation of CAV. CAV was defined as any lumen irregularity, and the most severe lesion was used to classify each segment. The CAV analyses were done by one investigator blinded to the result of ICA and IVUS.

In paper II, the initial segment grading was recategorized to fit the ISHLT CAV nomenclature, as follows: coronary arteries were classified as normal or with lumen diameter reduction of <50%, 50–70%, or ≥70% and the location of lesion in either primary or secondary vessels was noted. Based on the results of the ICA and echocardiography, each patient was classified according to ISHLT CAV recommended nomenclature, as follows: not significant (CAV_0), mild (CAV_1), moderate (CAV_2), or severe (CAV_3).[16]

Estimated radiation dose

The DAP was recorded for each examination. Effective radiation dose was estimated by multiplying the DAP by a conversion factor of 0.2 mSv/(mGy.cm^2).[96,113]

3.3.6 Intravascular ultrasound and virtual histology

In papers I and III, the routine angiography of the included patients was done with a 6F introductory sheet to facilitate IVUS examinations of one major coronary artery. After intracoronary

administration of 200 µg nitroglycerin, the IVUS examination was carried out using a 20 MHz, 2.9F, monorail electronic Eagle Eye Gold IVUS imaging catheter (Volcano Therapeutics Inc., Rancho Cordova, California, USA) and a dedicated IVUS/VH scanner (Volcano Therapeutics). The catheter was placed as distally as possible, and mechanical pullback was performed from this start point to the ostium. Images were acquired at a rate of 30 frames per second during mechanical pullback at a speed of 0.5 mm per second. IVUS images were stored on CD-ROM for subsequent offline analysis. In paper III, the VH images were captured at the top of the R wave of each heartbeat.

3.3.6.1 Analysis

Intravascular ultrasonography

Semiautomated contour detection of both the lumen and EEM was performed in papers II and III, using dedicated software (QIVUS Clinical Edition, Medis Medical Imaging, Netherlands). Following automatic contour detection, borders were edited manually (Figure 8). The largest distance from the intimal leading edge to the EEM was defined as MIT. CAV was defined MIT > 0.5 mm, as suggested by the American College of Cardiology clinical expert consensus document on the standards for acquisition, measurement, and reporting of IVUS studies.[34]

In paper I, the whole imaged length of the coronary artery was evaluated on a per-segment basis using the 16-segment classification of the American Heart Association.[134] Side branches were used as anatomic landmarks to separate the segments. In the absence of a side branch, the two adjacent segments were analyzed as one segment. MIT of the most severe lesion was measured and used to classify each segment.

In paper III, the longest possible segment was analyzed for each patient, defined as the segment between the most distal and proximal side branches identified. MIT was measured at 1 mm intervals, and a mean MIT was calculated for the analyzed segment, as recommended by the ISHLT.[16]

Virtual histology

In paper III, the same segment length and portion that were used for CAV quantification underwent qualitative assessment by dedicated VH software (pcVH, v.2.2, Volcano Corporation). Following manual editing of contours, the software used stored radiofrequency data to reconstruct tissue maps with the four identifiable components—fibrous, fibrofatty, dense calcified, and necrotic core—all expressed as a percentage of total intima area (Figure 8).

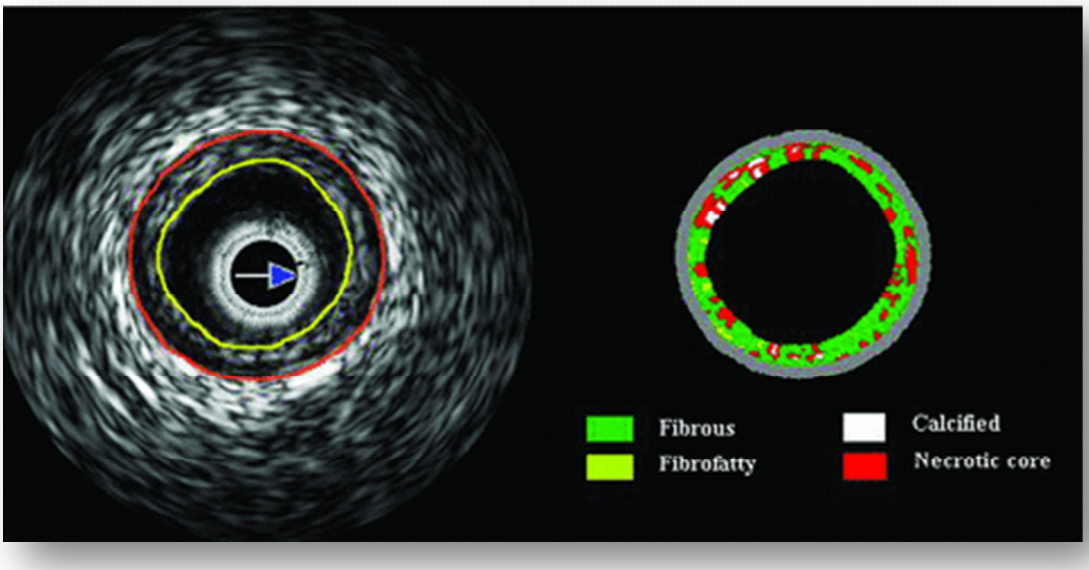

Figure 8. Example of an intravascular ultrasound and virtual histology recording.

Left panel: A transverse frame from an intravascular (IVUS) recording obtained by the Volcano scanner manually edited after semiautomated contour detection. Light green marking = lumen contour (LC); red marking = external elastic membrane (EEM). Right panel: Analysis of a virtual histology frame obtained at the time of IVUS acquisition. Analysis of radiofrequency data following contour detection provides color-coded tissue component characterization where green is fibrous, light green is fibrofatty, white is dense calcified, and red is the necrotic core component. Reprinted with permission from Satish Arora.

3.3.7 Inflammatory markers

Plasma samples were obtained by standard venipuncture immediately prior to angiography and IVUS examination. Peripheral venous blood was drawn into sterile blood collection tubes with EDTA as anticoagulant, immediately immersed in melting ice, and centrifuged within 30 min at 2000 ×g for 20 min to obtain platelet-poor plasma. Plasma specimens were stored at −80°C and thawed less than three times. The samples were collected at the time of inclusion, and consequently, the study population's range in time post-HTX also applied to plasma sampling (i.e., plasma sampling was performed at varying points in time post-HTX). The storage time before analyzing the plasma samples was mean 13.9 and range 1.8–28.4 months.

Plasma levels of soluble TNF receptor 1, IL-6, OPG, GP130, and VCAM-1, neopterin, CRP, and vWf were measured by enzyme immunoassays. All biomarker assays were performed upon study closure to minimize differences related to run variability.

3.4 Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics, version 15 or 21 (SPSS Inc. Chicago, Illinois, USA). A p value <0.05 was considered significant. Categorical variables are presented as frequency (percentage), and continuous variables are presented as mean ± standard deviation or median (interquartile range). Independent or paired samples t-tests were used for normally distributed continuous variables and the Mann–Whitney test for other continuous variables. Categorical variables were compared using the Pearson Chi-square test.

In paper I, sensitivity, specificity, positive predictive value (PPV), and NPV were calculated for detection of significant stenosis by CCTA using ICA as the standard of reference, and for detection of CAV by CCTA and by ICA with IVUS as standard of reference. Logistic regression analysis was used to evaluate the effects of BMI, heart rate, heart rate variability, CAC score, attenuation of the aortic root, and image noise on image quality.

In paper II, the association of CAC with long-term outcomes was examined using Kaplan–Meier plots and Cox regression analysis. Possible confounders were identified. These were variables significantly different in the group with and without CAC at a $p < 0.10$, as well as being significantly associated with long-term outcome, either with D/GL and/or with the combined outcome NF-MACE and D/GL.

In paper III, differences among two or more groups were compared using one-way analysis of variance (ANOVA) for normally distributed variables and the Kruskal–Wallis test for non-normally distributed variables. The association between variables of demographic and clinical characteristics of the study population and the outcomes «MIT > 0.5 mm» and «intimal inflammatory component > 30%» was evaluated by univariate analysis. Significant predictors (two-tailed $p < 0.05$) were entered into a forward stepwise logistic regression analysis with criteria for entry and exit at $p < 0.05$ and <0.10, respectively. Model performance was assessed by the Hosmer–Lemeshow goodness-of-fit test. Advanced statistical techniques were used to evaluate the validation of the multivariate models. With SAS version 9 (SAS Inc., Cary, North Carolina, USA), 1000 bootstrap samples were randomly generated, and stepwise logistic regression was employed with the same selection criteria as the original modeling. The discriminatory ability of the original multivariate model was compared with the bootstrapped model by evaluating the area under the receiver operating characteristic curve.

3.5 Ethics

The three studies included in this thesis all comply with the Declaration of Helsinki, the Regional Ethics Committee approved the research protocol, and written informed consent was obtained from all patients.

4 Summary of results

Paper I

The aim of paper I was to evaluate CCTA for its ability to rule out significant stenosis compared to ICA and its ability to detect CAV with IVUS as reference standard in the follow-up of HTX patients. Furthermore, we sought to assess image quality and radiation exposure of CCTA in HTX patients.

A total of 52 patients were prospectively included and underwent CCTA. Of these, 44 patients had IVUS completed in combination with their annual ICA. Two patients had incomplete CCTA data and were excluded from further analysis; both had IVUS completed. For one patient, IVUS data were lost.

The findings were as follows:

- In 570 included segments, ICA identified one segment (0.2%) with significant stenosis; 7 (1.2%) with 33–50% stenosis; 181 (32%) with <33% stenosis; and 381 (67%) normal segments. CCTA correctly identified the segment with significant stenosis detected on ICA and 557 segments without significant stenosis. The sensitivity, specificity, PPV, and NPV of CCTA compared with ICA for the detection of segments with significant stenosis were 100%, 98%, 7.7%, and 100% respectively.

  A total of 125 segments were excluded from the calculation of sensitivity, specificity, PPV, and NPV of the CCTA. Of 27 segments graded as not interpretable on CCTA, ICA identified four significant stenoses and two occlusions. In the 98 segments with diameter <2 mm, ICA identified an additional two significant stenoses and three occlusions.
- In 41 patients and 134 segments, CAV was detected by IVUS in 33 (81%) patients and in 74 (55%) segments. Of the 33 patients confirmed to have CAV on IVUS, CCTA and ICA detected 18 (44%) and 14 (34%), respectively. Of the 74 (55%) IVUS-proven CAV-affected segments, 50 (68%) were identified by CCTA and 49 (66%) by ICA.
- Mean image quality score per patient was 2.26 ± 0.40 for the CCTA. There was a significant difference in mean quality score between proximal segments and distal segments (2.03 ± 0.42 vs. 2.66 ± 0.50, $p < 0.001$). Multivariate logistic regression analysis found image quality to be inversely related to the amount of coronary calcium ($p = 0.006$). For the distal segment group, coronary calcium ($p = 0.009$) and image noise ($p = 0.013$) were inversely related to image quality. Maximum heart rate, heart rate variability, attenuation of the aortic root, and BMI were not associated with image quality.
- Mean DLP for the CCTA examinations was 1356 ± 246 mGy*cm, and the mean estimated radiation dose was 19.0 ± 3.4 mSv. Mean DAP for the ICA examinations was 2856 ± 1637

cGy*cm^2, and the mean estimated radiation dose was 5.7 ± 3.3 mSv. The estimated radiation dose for CCTA was significantly higher compared with ICA, p < 001.

Paper II

The aim of paper II was to evaluate CAC detected by CT to explore if absence of CAC could exclude CAV and predict outcome in HTX patients.

A total of 133 HTX recipients scheduled for coronary angiography were prospectively enrolled and underwent CAC scoring with CT at their annual follow-up. The medical records of the patients were reviewed in February 2016 to determine long-term outcomes, including graft survival and NF-MACE, defined as acute myocardial infarction, congestive heart failure, need for PCI, coronary artery bypass grafting, cardiac defibrillator placement, cerebral vascular accident, and peripheral vascular disease.

The findings were as follows:

- The median CAC Agatston score was 1 (interquartile range 0–51). Of the 133 patients examined, 60 (45%) had no CAC (NCAC group) and 73 (55%) had a CAC score >0 (CAC group). The donor age, time since HTX, allograft age, and history of rejection were significantly higher in the CAC group compared with the NCAC group. Otherwise, the groups were comparable. The CAC score displayed a continuous increase with allograft age and time interval since HTX, but not with recipient age at time of inclusion.
- The CAC score increased continuously with the severity of ISHLT CAV grades. In the NCAC group, 2 (3.3%) patients had ISHLT CAV_{2-3} while, 14 (19%) in the CAC group had ISHLT CAV_{2-3}. The CAC CT had an overall sensitivity, specificity, PPV, and NPV for ISHLT CAV_{2-3} of 88%, 50%, 19%, and 97%, respectively.
- 12% and 18% of the patients had significant coronary stenosis confirmed by ICA in the NCAC group and CAC group, respectively. The CAC CT with ICA as a reference had an overall sensitivity, specificity, PPV, and NPV for significant coronary stenosis of 65%, 47%, 18%, and 88%, respectively.
- All 133 participants were followed to the time of D/GL (n = 52) or to their last clinical follow-up (81), with an overall mean follow-up time of 7.5 ± 2.6 (range 0.3–10) years after CAC CT. A total of 127 participants were included in a combined outcome analysis (five patients were excluded because outcome variables had occurred before inclusion), and there were 57 (45%) NF-MACE and 23 (18%) D/GL registered as first events. During follow-up, 49 (71%) patients in the CAC group experienced NF-MACE or D/GL vs 31 (53%) in the NCAC group (p = 0.041) while D/GL occurred in 34 (47%) and 18 (30%) patients in the two groups, respectively (p = 0.051).
- Unadjusted Cox regression analysis showed a significant association between the presence of CAC and a worse outcome both for D/GL alone (hazard ratio [HR] 1.9, 95% CI 1.1–3.3; p =

0.032) and for the combined outcome of either NF-MACE or D/GL (HR 1.8, 95% CI 1.1–2.8; p = 0.016). In an adjusted Cox regression analysis, CAC was significantly associated with the combined outcome NF-MACE or D/GL (HR 1.8, 95% CI 1.1–3.0; p = 0.023), but not with D/GL alone (HR 1.7, 95% CI 0.93–3.2; p = 0.081).

Paper III

The aim of paper III was to study the role of inflammation in the development of CAV, the latter quantified by IVUS and qualitatively evaluated by VH. Systemic markers of inflammation were analyzed for their association with IVUS-defined CAV and with the proportion of inflammatory tissue in the thickened intimal area estimated by VH.

A total of 101 HTX recipients were included and underwent IVUS/VH examination and measurement of plasma CRP, soluble TNF receptor-1, IL-6, OPG, soluble GP130, vWF, VCAM-1, and neopterin.

The findings were as follows:

- Mean MIT was 0.61 ± 0.19 mm, and 47 (47%) patients were found to have advanced CAV defined as MIT > 0.5 mm. When comparing demographic and clinical characteristics, patients with advanced CAV were older, had a higher serum creatinine, displayed higher N-terminal proBNP levels, and were more likely to be *Toxoplasma gondii* seropositive.
- Fibrous tissue was the predominant identifiable component of the intimal area (55 ± 15%), followed by very similar proportions of fibrofatty (14 ± 10%), dense calcified (15 ± 13%), and necrotic core (17 ± 9%) tissue. Our data indicated increased fibrotic and less inflammatory tissue (dense calcified and necrotic core) with increasing time since HTX, but this association did not reach statistical significance ($p = 0.07$). An increased intimal inflammatory component was associated with increased serum creatinine ($p < 0.01$) and lower high-density lipoprotein levels ($p < 0.01$).
- In multivariate analysis, CRP > 1.5 mg/L (odds ratio [OR](95% CI 4.7, CI 1.7–12.2; $p < 0.01$), VCAM-1 > 391 ng/mL (adjusted OR 3.2, 95% CI 1.1–9.7; $p = 0.04$), and neopterin > 7.7 nmol/L (OR 3.8, 95% CI 1.2–11.6; $p = 0.02$) were independently associated with MIT > 0.5 mm.
- Similarly, CRP > 1.5 mg/L (OR 3.7, 95% CI 1.4–9.5; $p < 0.01$) and VCAM-1 > 391 (OR 2.7, 95% CI 1.1–6.9; $p = 0.04$) were independently associated with an increased intimal inflammatory component (dense calcified/necrotic core component > 30%).

5 Discussion

5.1 Methodological considerations

5.1.1 Study design

The strength of this thesis is the prospective design of all three papers. A prospective design enables standardized conditions for how methods are employed and information is recorded to minimize confounding factors and biases.

In a cross-sectional study, the aim is to provide data on the entire population under study. The cross-sectional design in all three papers is both an advantage and a disadvantage. It resulted in a heterogeneous study population; time since HTX varied considerably (range 1–20 years), and all stages of CAV were included. For studying diagnostic methods that identify the disease, either directly as in paper I or indirectly via markers as in papers II and III, it is valuable that all stages of the disease are represented.

The heterogeneity of the study population made it difficult, however, to draw conclusions regarding the practical clinical use of the CAC score in paper II. A cohort study, for example, 1 year after HTX could have given more solid information about the possible use of CAC CT at that particular time after HTX. Such an approach would, though, have limited the number of available subjects substantially because the number of heart transplants is limited to 30–40 a year. A cohort study would have been difficult to combine with a study of the short-term outcomes for ISHLT CAV_{2-3} and significant stenosis, preferably including all stages of the disease, as was done in paper II.

Paper III, with its cross-sectional design, demonstrated an important association between inflammatory markers and IVUS-detected CAV, but could not establish causality. A study design that included a baseline IVUS could have discriminated donor-transmitted disease and CAV, but would markedly have limited the number of available patients.

A major strength of paper II is the long follow-up time of up to 10 years with complete data for all but one patient.

5.1.2 Patients

Oslo University Hospital, Rikshospitalet, is the only center in Norway to perform heart transplantations, and the recipients are followed at our center annually, at a minimum. Hence, the study recruited patients from a complete HTX population. The continuous follow-up at one center allows for almost complete clinical data recording of long-term outcome variables. Only one person was lost to follow-up on NF-MACE; this person moved out of the country.

Impaired kidney function is a bias in the recruitment to this study. This bias is likely most prominent for paper I, for which better kidney function was required for the patients than for the other two studies. For the other two papers, recruitment depended on the patient's being scheduled for ICA, and in our center, HTX recipients with severe renal impairment do not undergo annual ICA unless clinically indicated. According to registry data, 33% of the HTX patients have creatinine ≤2.5 mg/dL (220 µmol/L) at 5 years post-transplant.[1] Because both impairment of kidney function and development of CAV increase with time after HTX, a bias towards patients with normal and less severe coronary artery pathology must be expected. In paper I, this bias probably was a major contributor to the low prevalence of significantly diseased segments.

5.1.3 Methods of detection and quantification

5.1.3.1 Gold standard

Evaluating the performance of a diagnostic method or a surrogate marker requires a gold standard against which to measure. A gold standard should ideally give the perfect measurement or the true outcome.

In medical imaging, a gold standard can be another imaging method, another test, or a clinical outcome. In medical imaging as well as in medicine in general, there is often not a perfect gold standard. A method is sometimes chosen as the reference standard because it is the only available method or because it is the clinically established method. Outcomes can be hard endpoints, which typically are dichotomous, i.e., death, or a set of events or variables can be used to create a composite endpoint if one outcome or measurement is not obtainable.

The objective of a study will also influence which standard reference to use; different gold standards would be chosen if the study objective were to examine if a diagnostic method could detect the presence of a condition, to grade the presence of the condition, or to detect/measure an outcome related to the condition. For CAV, what comes closest to being a gold standard, both for its presence (intimal hyperplasia) and for its final outcome (myocardial fibrosis causing heart failure), is histology of the coronary artery wall and myocardium in an autopsy. Histology is, however, not the perfect gold standard because native atherosclerosis plaques are indistinguishable from CAV lesions.[7,12] Histology of the coronary wall is attainable only at autopsies, and other methods have to be taken into use to define a gold standard for CAV. In this thesis, ICA, IVUS, and the combination of ICA with echocardiography have been used as substitute methods of reference, as well as the outcome variables D/GL and the composite outcome NF-MACE for end-stage clinical outcome of CAV. Their strengths and shortcomings are discussed below.

Another aspect of defining a gold standard for CAV is the transformation of a continuous variable into a categorical variable. The development of CAV is a continuous process from the first insignificant intimal increase to obliteration of the vessel lumen. An artificial cut-off point of a continuous measurement has been set to dichotomize CAV into a categorical value, defining it as present or not present.

Finally, there is the aspect of which type of information our measurements provide about the condition we are investigating. The diameter measurement of CAV, either intimal thickness or vessel lumen reduction, is anatomic information. When it comes to significant coronary stenosis, the anatomic visual descriptors are substitutes for the information that is clinically important: the restriction of blood flow to the myocardium. Variables measuring blood flow, myocardial perfusion, and heart function would give functional information related to the presence of CAV.

5.1.3.2 Coronary CT-angiography

In the evaluation of significant stenosis detection with ICA as gold standard, a lumen-based definition of significant stenosis was used for both ICA and CCTA. In the evaluation of CAV detected by IVUS, CAV was defined differently for all three modalities included in the study: with IVUS, the definition was wall diameter >0.5 mm; for CCTA, “any wall irregularity,” and for ICA, “any lumen irregularity.” Thus, the definition encompassed two dimensions: the structure evaluated (lumen or vessel wall) and the type of evaluation (quantitative or descriptive).

CAV defined by IVUS includes more subtle coronary pathology because of the high resolution of IVUS, which presents a challenge for the spatial resolution of both CCTA and ICA. It has been proposed that CCTA with its ability to visualize the coronary wall would be superior to ICA in detecting CAV.[99,100] Several publications on CCTA of HTX patients have included the vessel wall in their definition of CAV; however, the spatial resolution of CCTA is markedly inferior to IVUS. The latest generation high-end CT scanners have spatial resolution of 230–240 µm and slice thickness of 0.5–0.6 mm; thus, valid measurements of MIT in the range of 0.5 mm are not feasible. In an effort to use the vessel wall to define CAV, descriptive variables have been applied as substitutes for the measurement of the vessel wall, such as “proliferative changes in the coronary arterial wall,”[137] and “any coronary plaque.”[138]

5.1.3.3 Invasive coronary angiography

ICA provides contours of the coronary artery lumen and is based on detection of luminal narrowing. CAV is, however, not a disease of the vessel lumen but a disease of the vessel wall. In the early stage of CAV, there is an outward remodeling of the external elastic membrane, and first in the late stage of CAV, there is a restrictive remodeling causing luminal narrowing, which is angiographically

detectable.[13] To detect luminal narrowing, a normal lumen diameter is needed as reference. CAV often has a diffuse distribution, so a normal reference may be difficult to find/define. All of these factors contribute to the underestimation of CAV by ICA.

Historically, most studies of revascularization have defined a "significant" stenosis as ≥70% diameter reduction with angiography.[139] In daily practice, lesions with a diameter stenosis of ≥50% are generally considered for revascularization but are often inaccurate in predicting ischemia. In one study comparing angiography with FFR, only 35% of 50–70% stenoses were functionally significant (FFR ≤ 0.80), and 80% of 71–90% stenoses were functionally significant.[140] In all publications on CCTA of HTX patients, angiographic ≥50% lumen reduction has been defined as the gold standard for significant stenosis, most likely to avoid overlooking any possible significant stenosis.[99,101,136,137,141-147]

There is more variation in the definition of ICA as a gold standard for CAV: "any lumen irregularity," "lumen reduction <50%," or both.[99,136,141,145,148] In these publications, another variable is whether or not segments with >50% stenosis were included in the analysis.

In spite of its shortcomings, ICA is usually used as the gold standard for studies on CCTA of HTX. The availability and clinical acceptance, together with the recommendation of annually or biannually coronary angiography[16,23], makes it a convenient method to use as reference in terms of logistics and economy. The two methods ICA and CCTA are also alike in that contrast fills the vessel lumen and that nearly the same parts of the coronary vasculature are depicted. The fact that CCTA is investigated with the aim of replacing ICA or being a gatekeeper for ICA, makes it reasonable to use ICA as gold standard.

5.1.3.4 Intravascular ultrasound

Most often, one-vessel morphology is used as a surrogate for the entire coronary tree when HTX patients are investigated with IVUS. Detection of early stage CAV is increased with multi-vessel imaging.[118] The distal parts of the epicardial vessels with smaller lumen diameter as well as the myocardial vasculature are not available for IVUS examination at all. It has also been demonstrated that IVUS-detected intimal hyperplasia does not correlate well with small artery disease by histologic or immunohistochemical analysis.[26]

The strength of IVUS in detection of CAV is the direct visualization of the vessel wall where the disease is located. The excellent resolution and reproducibility of this technique enable detection of early stages of CAV as well as detection of subtle disease progression.[117]

The VH provides a unique dimension of CAV compared to the other modalities: qualitative information.

5.1.3.5 ISHLT coronary allograft vasculopathy nomenclature

The ISHLT recommended nomenclature for CAV is a classification based on a combination of anatomic and functional measurements. Still, the limitations of the two modalities included in this consensus statement are not overcome by combining the two. ICA is insensitive in detecting early stages of CAV as described previously and does not evaluate small-caliber vessels. Allograft dysfunction caused by both epicardial and myocardial vessel pathology would be picked up by echocardiography. However, dysfunction evaluated with echocardiography reflects a late stage of CAV when the coronary pathology has caused irreversible damage of the myocardium. None of the two included variables are sensitive in detecting early stages of CAV, which clearly is underestimated by both methods when compared to IVUS. However, a classification including both anatomy and function should, at least in theory, be superior to either variable alone in diagnosing and classifying CAV. Another important asset is that this classification provides a standardization of language used in research on CAV, which hopefully will make interpretation of and comparison amongst studies more reliable.

5.1.3.6 Long-term outcome

For long-term outcome, two variables were used: death or graft loss and the combination of D/GL and a composite endpoint of NF-MACE. A number of variables can be included in the term "MACE."[149] The set of composite endpoints included in paper II represented the same variables used in previous publications by renowned researchers in the field of HTX.[3,133]

Ordinary Cox regression was used for analysis of D/GL and the combined outcome NF-MACE. We also considered performing a Cox analysis focusing on a more CAV-specific long-term outcome like CAV_{2-3} or significant stenosis. However, these outcomes require the use of a more complex Cox regression model, e.g., a model where death is treated as censoring. To be appropriate, this model requires that CAC is not associated with mortality, which most likely is not true. Furthermore, in a statistical perspective, the sample size in our study was relatively small, making it almost impossible to evaluate whether the assumptions of a more complex Cox model are fulfilled. Thus, we set aside the idea of analyzing CAV_{2-3} or significant stenosis as a long-term outcome.

5.1.4 Statistical limitations

The number of patients transplanted each year and the total number of living HTX patients are limited both in Norway and worldwide. Single-center studies on HTX patients are even more limited by the number of available patients. The high frequency of chronic renal impairment in HTX patients further reduces the number of available patients and leads to a selection bias.

From a statistical perspective, the sample sizes in all three papers are relatively small, which limits conclusions. Particularly, the low prevalence of significantly diseased segments in paper I is an

obvious limitation. From a HTX research perspective, however, the numbers included are not particularly small and in line with most other publications.

5.2 Discussion of main findings

5.2.1 Coronary CT-angiography

5.2.1.1 Detection of stenosis and coronary allograft vasculopathy by coronary CT-angiography

In paper I, we found that CCTA with interpretable image quality had a high NPV for ruling out significant stenosis suitable for PCI, supporting its role as a gatekeeper. This result is consistent with other studies of CCTA in HTX patients, as well as with a meta-analysis.[100,101,136-138,141-144,146,147] Even with the challenging clinical characteristics of this population, the sensitivity and NPV found for HTX patients are comparable with results in non-transplanted populations.[98] CCTA is recommended in symptomatic patients with a low to intermediate pretest probability of CAD in native hearts,[76,97] but not for patients with transplanted hearts.[16,23] The number of studies of CCTA in HTX patients is very modest compared to the non-transplanted population, the sample sizes are small, and no multicenter studies have been carried out. The main concerns raised are lack of adequate branch vessel assessment, excess radiation, and a lack of data providing prognostic outcomes.[16]

The peripheral lesions are a challenge because of the small vessel caliber. The typical tapering nature of these lesions (type B2 in particular, but also type B1; Figure 2) results in small vessel diameters, which are a challenge to the spatial resolution of the CCTA. In the 98 (14%) segments with vessel diameter <2 mm not evaluated in our study, ICA identified two significant stenoses and three occlusions. Significant stenoses in distal coronary arteries and branches are, however, usually not suited for PCI and represent a lower clinical risk compared with the more proximal stenoses and therefore are of lesser clinical relevance.

We found CCTA to have a modest detection rate for CAV as assessed by IVUS, and at the same level as ICA. CAV detected by IVUS includes disease with lumen reduction <50% diameter on ICA, i.e., more subtle CAV. There are conflicting results among studies published on CAV of lesser degree than <50% lumen reduction. The two publications using IVUS MIT > 0.5 mm as the gold standard both found CCTA to be inferior in detecting CAV.[138,143] Only one of these two studies evaluated both CCTA and ICA with IVUS-detected CAV. In 20 patients, Gregory et al. found that CCTA (64-MDCT) detected 39 (70%) of 56 segments with CAV whereas ICA detected only 6 (11%) segments.[138] Although the ability of CCTA to detect CAV was comparable in our study, the ability of ICA to detect CAV was markedly greater. Schepis et al. evaluated the detection of CAV by DSCT using IVUS as the gold

standard in 30 patients and found a slightly higher detection rate (35/41 segments, 85%),[143] a result that may reflect the improved performance of high-end equipment.

5.2.1.2 Image quality by coronary CT-angiography

In our study, 95.5% of the segments were graded as interpretable. Previous publications on CCTA of HTX recipients have reported interpretable segments within the range of 81.4–99.6%.[101,136-138,141-144,146,147] In the 27 segments graded as uninterpretable in our study, ICA identified four significant stenoses and two occlusions. Of these, only one lesion was located in a proximal segment (LAD middle segment); this was also the only significant stenosis in the study eligible for treatment. In general, we found that image quality was significantly lower in distal than in proximal segments. Gregory et al. reported that 87% of the non-evaluable segments were in distal coronary arteries and branches.[138] Significant stenoses in these parts of the coronary tree are, as mentioned above, usually of lesser clinical relevance. The lower quality of the distal segments may therefore not be of paramount significance.

The only significant determinant of image quality in our study was CAC. Mittal et al. found a tendency, but not a significant relationship, towards worsening image quality with increasing CAC score.[136] In several publications on CCTA in non-transplanted populations, CAC significantly reduces image quality.[79,81,150]

Increased heart rate was not associated with worsening image quality, which was slightly unexpected because this variable is considered important for image quality of CCTA in non-transplanted patients.[150] A heart rate < 65 bpm is recommended in the European guidelines.[76] All but four (8%) of the patients in our study had heart rate R > 65 bpm. Gregory et al. examined visualized vessel length without motion artifacts and found substantial deterioration of image quality for heart rate > 85 bpm.[138] The very low heart rate variability in HTX patients owing to the denervation of the heart is advantageous in CCTA. Only three (6%) patients in our material had heart rate variability > 5 bpm, and Mittal et al. found a tendency toward worsening image quality with heart rate variability > 5 bpm.[136]

5.2.1.3 Radiation exposure by coronary CT-angiography

The mean estimated radiation dose of 19.0 ± 3.4 mSv in our study using a 64-MDCT was high compared to the dose of 5.7 ± 3.3 mSv for ICA, and to a mean dose of 2.5 ± 1.7 mSv in 102 consecutive non-HTX patients examined at our department in 2014 with a 16-cm wide detector CT (A. Günther and J. Kristiansen, unpublished data). Recent publications by Mittal et al. and Barthélémy et al. report equivalent radiation doses for 64-MDCT of 17.5 ± 6.9 mSv and 17.8 ± 5.5 mSv, respectively.[101,136]

The high heart rate in HTX patients with limited response to betablockers often prevents the use of important dose-lowering techniques or renders them less effective.

ECG-synchronized tube current modulation in helical scanning is less effective in high heart rates.[151] Prospective ECG-triggered sequential scanning is one of the most effective dose-saving strategies[91,151-153] but requires heart rates below 60–65 bpm in scanners with a rotation time higher than 30 ms and in high-pitch mode on second-generation DSCT.[84,154]

One feasibility study addressing DSCT with ECG triggering in HTX patients reported diagnostic image quality and an estimated radiation dose of 4.5 mSv.[135] The newest high-end scanners have gantry rotation times as low as 25–28 ms, enabling the use of prospective ECG-triggered scan techniques in heart rates up to 75 bpm.[86,88] Thus, more of the HTX population could benefit from this dose-reducing approach. In our study, 29 (58%) of the patients had a maximum heart rate ≤ 75 bpm during the CCTA and could potentially have been scanned with prospective ECG-triggering using a high-end scanner. A stable heart rate is an absolute requirement when scanning with ECG triggering, and the very low heart rate variability in HTX patients is especially advantageous.

The use of tube voltages below 120 kV reduces radiation exposure.[155,156] A 100 kV tube voltage has recently become the standard for small- to medium-sized patients. One publication has reported the use of 100 kV in a low-weight (<80 kg) HTX population and found a significantly lower radiation dose when compared to 120 kV.[146] In newer CT systems with x-ray tubes capable of providing high power at lower tube voltages, 80 and 70 kV have been introduced.[157] The mean effective dose of CCTA in non-obese patients has been reported to be 0.44 mSv at 70 kV. In obese patients, the high power x-ray tubes could enable reduction of tube voltage from today's standard of 120 kV to 100 kV, which could make a significant contribution to reducing radiation exposure to an acceptable level in the HTX population.

Novel iterative reconstruction algorithms have demonstrated significant dose reduction with preserved image quality using lower tube current in CCTA studies in the general population.[158,159]

When considering the risk of CCTA radiation exposure, two aspects are special to HTX patients: their increased risk for cancer and the repeated examinations they undergo.

HTX patients have an increased risk for cancer. The overall malignancy risk associated with radiation exposure is small, but whether it is increased or potentiated by the immunosuppressive-associated cancer risk in HTX patients is not known. The organs associated with an increased risk for cancers being exposed to ionizing radiation by a CCTA are the lungs and breasts. The incidences of these cancers were not increased in the Cincinnati Transplant Tumor Registry study[58] whereas the incidence of lung cancer was increased in an Australian study.[56]

The high radiation dose with the use of 64-MDCT documented in our study and in the two other resent publications[101,136], is of concern undertaking repetitive CCTAs in HTX patients. Recent technical development with faster scanners, x-ray tubes with higher power, and iterative reconstruction algorithms will almost certainly reduce the dose also in the challenging HTX population. The degree of dose reduction is not yet documented.

5.2.2 Coronary artery calcium CT

A modest number of publications have studied CAC CT in a HTX population, and CAC have been evaluated with various standards of reference.[136,160-164]

5.2.2.1 Coronary artery calcium and prediction of ISHLT coronary allograft vasculopathy and significant stenosis

To our knowledge, this study is the first to assess the prognostic value of CAC CT using the ISHLT CAV nomenclature. This nomenclature has been the recommended classification for CAV since 2010[16] and is based on a combination of coronary visualization and allograft function. We found that the absence of CAC on CT predicts a low prevalence of ISHLT CAV_{2-3}. This prediction is of prognostic value because as Prada-Delgado et al. demonstrated, CAV_2 and CAV_3 detected at 1 year after HTX are associated with poor prognosis.[4]

Others have used various definitions of CAV. Ratliff et al. applied a coronary index for extent of disease on ICA,[160] Barbir et al. used ICA stenosis ≥25%,[161] von Ziegler et al. used any angiographic lesions,[162] and Knollman et al. used degree of intimal proliferation with IVUS.[163] The various definitions of CAV make it difficult to compare the predictive value of CAC CT in these studies. One explanation for the discrepancy in the predictive values could be that the varying definitions of CAV reflect different stages of the disease as calcified and necrotic components increase with time after HTX: Early, subtle CAV, i.e., IVUS-detected low-grade CAV and any angiographic lesions, have less CAC and therefore these studies report a modest predictive value of CAC CT. More advanced CAV, i.e., IVUS-detected high-grade CAV and ISHLT CAV_{2-3}, have more CAC and the predictive value of CAC CT increases. This hypothesis is supported by Knollmann et al.'s finding that total CAC score is associated with the degree of intimal proliferation on IVUS.[163]

We also evaluated CAC and its association with significant stenosis on ICA and found that the absence of CAC on CT predicts a low prevalence of significant coronary artery stenosis on a concurrent ICA with a NPV of 88%. Our findings are consistent with those of others; Barbir et al. and Mittal et al. also found high NPVs of 95% and 94%, respectively, for a negative CAC CT.[136,161] Knollmann and colleagues reported a NPV of 99% for a CAC score >55.[163]

Even with a NPV coming close to 90%, the absence of CAC in HTX patients does not entirely exclude the presence of significant stenosis, which is of paramount importance if CAC CT is to be used as a gatekeeper for ICA. This importance is underscored by the finding that two of three patients treated with PCI had no CAC. Similar findings have also been reported by Mittal et al.,[136] and the manifestation of significant stenosis in patients with no CAC is also documented in a non-transplanted population.[165] CAC CT could be used in a highly selected group of patients with relative contraindications for ICA, i.e., difficult access for invasive procedures, severe kidney impairment, or allergy to iodine contrast agent. In such patients, a negative CAC CT could support more limited use of ICA.

5.2.2.2 The association of coronary artery calcium with long-term outcomes

Our CAC long-term outcome results are in line with our finding that CAC reflects the severity of CAV. Our study showed a relation between the presence of CAC and a worse long-term outcome. This finding is in line with those of Prada-Delgado et al., who demonstrated that ISHLT CAV severity had a prognostic significance for long-term outcome.[4] We found CAC associated with the combined outcome NF-MACE or D/GL, but not with D/GL alone. The latter could be explained by the high number of non-cardiac deaths that do not reflect CAV; 31 (60%) patients had malignancy or "other" registered as the cause of death.

To our knowledge, only one other study is available on CAC and long-term outcome in the HTX population. Lazem et al. studied CAC as a predictor of cardiac events in 91 subjects with a mean follow-up of 2.12 years and found that absence of CAC was a significant predictor of event-free survival.[164] Knollmann et al. also found ICA stenosis ≥25% to significantly predict event-free survival and that CAC CT did not add incremental information to angiography; these authors suggested that the two methods reflect the same pathological process.[163]

5.2.3 The association of systemic markers of inflammation with coronary allograft vasculopathy

Previous publications have demonstrated that elevated CRP levels predict development of angiographically evident CAV among HTX recipients.[166,167] These findings have led to a proposal that CRP reflects a general inflammatory state associated with CAV development and adverse outcome.[168] With IVUS to determine advanced CAV (MIT > 0.5 mm) and characterization of tissue composition by VH analysis, our study demonstrated that elevated CRP is strongly associated with advanced CAV and inflammatory tissue content in the affected intima. We found that advanced CAV was associated with increased plasma levels of VCAM-1 and neopterin, as well as CRP, suggesting a pathogenic role of inflammation, including endothelial cell and monocyte/macrophage activation. The association of an increased proportion of inflammatory tissue in the thickened intimal area evaluated by VH

analysis, with elevated levels of CRP and VCAM-1, suggests a link between systemic and local inflammation within the coronary arteries.

Our study has demonstrated an association between inflammatory markers and CAV but does not establish causality. The design and the exploratory nature as well as lack of baseline IVUS to exclude donor-transmitted disease are important limitations.

5.3 Future aspects

Presently, no effective treatment exists for established CAV. This lack may well be an important reason for ISHLT to recommend surveillance with ICA in HTX patients, even though it is a modality that is described to only "grossly detect CAV,"[16] indicating that accurate detection and quantification have limited clinical implications. Future development of drugs that can treat or efficiently stop progression of CAV will define the detection and staging accuracy needed for image methods and surrogate markers of CAV. Parallel to the clinical diagnostic needs is a continuous ongoing effort to increase our understanding of the disease, which also stimulates the search for better diagnostic methods that are both more accurate and offer new types of information.

5.3.1 Coronary CT-angiography

Enormous technical progress has been made over the past decade since the start of cardiac imaging with 4-MDCT. Advances in spatial and temporal resolution have significantly improved the quality of CT depiction of the coronary arteries.[84,85,157] Technical developments have markedly reduced radiation exposure.[88,92,93] New techniques have provided information other than visualization of anatomic structures; plaque characterization and functional information.[169,170]

5.3.1.1 Stenosis detection

If technological development continues with the same progression as in the past decade, we can anticipate an increase in the accuracy of stenosis detection in large epicardial coronary arteries and valid evaluation of the smaller caliber branch vessels. Improvement in image quality of patients with higher heart rates and large body size is also to be expected, as well as a continuous decrease in radiation exposure in patients with such characteristics.

Currently, research evaluating high-end equipment in HTX patients is warranted, including third-generation DSCT and wide detectors with the lowest rotation times. In particular, research is needed in evaluation and validation of the performance of prospective ECG-triggered scan modes in combination with iterative reconstruction and lower tube voltage to ascertain if a majority of HTX patients can be examined with sufficient image quality at an acceptable level of radiation exposure. Evaluation of CCTA in larger samples of multi-center studies is also needed.

Outcome analysis using CCTA in the follow-up in HTX patients has been done in only one study,[171] and such research is encouraged by ISHLT.[16]

5.3.1.2 Plaque characterization

CT visualizes not only the vessel lumen but also the surrounding structures, raising interest in studying CT attenuation to characterize the components of coronary atherosclerotic plaques. Differentiation between calcified and non-calcified plaques is relatively simple because of the high density of calcium compared to soft tissue. More challenging is the differentiation between the two non-calcified plaques components lipid-rich or fibrous. Studies of plaque density have found the mean attenuation to differ between the two plaque components, both in studies using IVUS VH analysis and those using OCT as reference standard.[169] A substantial overlap in attenuation prevents reliable differentiation between the two lesion types. Limited contrast and spatial resolutions, motion artifacts, and heterogeneous plaque composition hamper correct analysis of plaque components with CT.[172] Most of these limitations are technical issues that will improve with technical advances, resulting in better spatial and temporal resolution.

Dual energy CT is a technology in which two CT datasets are acquired with different x-ray spectra. These spectra are generated using different tube potentials.[173] In a study by Barreto et al. on characterizing coronary plaques with dual energy CT, improved differentiation between calcified and non-calcified coronary plaques was demonstrated, but further classification of plaque types was not possible.[174]

5.3.1.3 Functional image analysis

In recent years, feasibility studies on noninvasive functional imaging analysis based on CTA have been published; CT-derived fractional flow reserve (CT-FFR),[175] CT myocardial perfusion (CTP) imaging,[176] and transluminal attenuation gradient.[177]

FFR has become the gold standard for guiding intervention of coronary artery stenosis.[178] It is defined as the coronary pressure distal to a stenotic lesion (P_d) divided by the aortic pressure (P_a) obtained during maximal coronary vasodilation.[178] This ratio expresses the maximum blood flow down a vessel with a stenotic lesion compared to the hypothetical normal maximum flow in the same vessel. Studies on CT-FFR have demonstrated increased diagnostic accuracy in detecting FFR significant stenosis (FFR ≤ 0.80) compared with CCTA alone.[179] Similar results have been demonstrated with CTP in combination with CCTA.[180] CT-FFR and CTP have not yet been studied in HTX patients but could hold potential for improving diagnostic accuracy as in non-HTX patients.

In HTX patients with no angiographic evidence of significant CAV, Hirohata et al. demonstrated a significant inverse correlation between epicardial physiology and anatomy; FFR decreased significantly and percentage plaque volume derived by IVUS increased significantly during

the first year after HTX.[181] One could, at least in theory, anticipate that CT-FFR of HTX patients holds potential for assisting CCTA in the detection of subtle CAV.

5.3.2 Coronary artery calcium CT

Serial examinations of CAC CT in a cohort of de novo HTX patients have not yet been done and would provide valuable knowledge both for evaluating CAC CT as a gatekeeper for ICA and as a marker predicting long-term outcome.

5.3.3 Invasive techniques

IVUS has been an important contributor to understanding the distribution and morphology of CAV, as well as facilitating research on CAV-associated parameters such as markers of systemic inflammation.

In recent years, OCT has emerged as a new imaging modality, providing insight on CAV pathogenesis. With OCT, images are generated by measuring the echo time delay and intensity of light that is reflected or back-scattered from internal structures in the tissue.[182] OCT provides images of the vessel wall with even higher resolution than IVUS, in the range of 10–20 μm. Compared to IVUS, OCT can provide more accurate information on plaque characteristics[183] and a higher sensitivity of OCT than IVUS for early detection of CAV.[182] An example of new insight into CAV pathogenesis provided by OCT is a study by Ichibori et al. on the impact of neovascularization represented by OCT-identified microchannels on intimal proliferation evaluated by IVUS.[184]

IMR is a new, flow-based technique quantifying microvascular circulation. The detection of microvasculopathy has previously been done only with myocardial biopsy specimens. IMR is defined as the product of the distal pressure and mean transit time of a saline bolus during maximum hyperemia using a dual temperature and pressure wire.[185] Haddad et al. demonstrated that microvascular dysfunction assessed using IMR was correlated with worse graft function and possibly worse clinical outcomes.[186] Solberg et al. used IMR to evaluate the effect of immunosuppression conversion from calcineurin inhibitor to everolimus (mTOR inhibitor) on microvascular function during the first year after HTX.[187]

OCT and IMR hold promise for further increasing our understanding of CAV, as well as providing new types of reference standards in CAV research.

6 Conclusions

Thesis

In this thesis, different imaging methods have been employed in the diagnosis and prediction of CAV after HTX; CCTA was evaluated for its performance in a HTX population, CAC CT was evaluated as a surrogate marker for short- and long-term outcomes, and IVUS/VH facilitated the investigation of possible biomarkers for CAV.

Paper I

CCTA with interpretable image quality had a high NPV for ruling out significant stenosis appropriate for PCI, giving support to using the method as a gatekeeper for ICA. However, the high estimated radiation dose found in this study is a matter of concern because HTX patients are subject to repetitive examinations. In detecting CAV, CCTA was inferior to IVUS and at the same level as ICA, implying that CCTA has limited usefulness in detection of early CAV.

Paper II

An absence of CAC on CT predicts a low prevalence of ISHLT CAV_{2-3} and of significant coronary artery stenosis on a concurrent ICA with NPVs of 97% and 88%, respectively. In a follow-up period of up to 10 years, we found that the presence of CAC was significantly associated with a worse combined long-term outcome of NF-MACE and D/GL.

Paper III

Advanced CAV, as determined by IVUS, was associated with increased plasma levels of CRP as well as of VCAM-1 and neopterin, suggesting a pathogenic role in inflammation, including endothelial cell and monocyte/macrophage activation. An increased proportion of inflammatory tissue in the thickened intimal area, as evaluated by VH analysis, was associated with elevated levels of CRP and VCAM-1, suggesting a link between systemic and local inflammation within the coronary artery of the transplanted heart.

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