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Danish study of Non-Invasive testing in Coronary Artery Disease 2 (Dan-NICAD 2)

study design for a controlled study of diagnostic accuracy

Rasmussen, Laust Dupont; Winther, Simon; Westra, Jelmer; Isaksen, Christin; Ejlersen, June Anita; Brix, Lau; Kirk, Jane; Urbonaviciene, Grazina; Søndergaard, Hanne Maare; Hammid, Osama; Schmidt, Samuel Emil; Knudsen, Lars Lyhne; Madsen, Lene Helleskov; Frost, Lars; Petersen, Steffen E.; Gormsen, Lars Christian; Christiansen, Evald Høj; Eftekhari, Ashkan; Holm, Niels Ramsing; Nyegaard, Mette; Chiribiri, Amedeo; Bøtker, Hans Erik; Böttcher, Morten

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Danish study of Non-Invasive testing in Coronary Artery Disease 2 (Dan-NICAD 2): study design for a controlled study of diagnostic accuracy

RCT# NCT03481712

Laust Dupont Rasmussen[1], Simon Winther[2], Jelmer Westra[2], Christin Isaksen[3], June Anita Ejlersen[4], Lau Brix[3], Jane Kirk[5], Grazina Urbonaviciene[5], Hanne Maare Søndergaard[6], Osama Hammid[7], Samuel Emil Schmidt[8], Lars Lyhne Knudsen[1], Lene Helleskov Madsen[1], Lars Frost[5], Steffen E. Petersen[9, 10], Lars Christian Gormsen[11], Evald Høj Christiansen[2], Ashkan Eftekhari[2], Niels Ramsing Holm[2], Mette Nyegaard[12], Amedeo Chiribiri[13], Hans Erik Bøtker[2] and Morten Böttcher[1].

[1]Department of Cardiology, Hospital Unit West, Gl. Landevej 61, DK-7400 Herning. DENMARK.

[2]Department of Cardiology, Aarhus University Hospital, Palle Juul-Jensens Boulevard 99, DK-8200 Aarhus. DENMARK.

[3] Department of Radiology, Regional Hospital Central Jutland, Falkevej 1A, DK-8600 Silkeborg, DENMARK.

[4]Department of Nuclear Medicine, Hospital Unit West, Gl. Landevej 61, DK-7400 Herning, DENMARK.

[5]Department of Cardiology, Regional Hospital Central Jutland, Falkevej 1A, DK-8600 Silkeborg, DENMARK.

[6]Department of Cardiology, Regional Hospital Central Jutland, Heibergs Allé 4, DK-8800 Viborg, DENMARK.

[7]Department of Cardiology, Regional Hospital East Jutland, Skovlyvej 15, DK-8930 Randers, DENMARK.

[8]Department of Health Science and Technology, Aalborg University, Aalborg, Denmark

[9]Barts Heart Centre, St Bartholomew's Hospital, Barts Health NHS Trust, West Smithfield, London, UNITED KINGDOM.

[10] William Harvey Research Institute, NIHR Barts Biomedical Research Centre, Queen Mary University of London, Charterhouse Square, EC1M 6BQ, London, UNITED KINGDOM

[11]Department of Nuclear Medicine, Aarhus University Hospital, Palle Juul-Jensens Boulevard 99, DK-8200 Aarhus, DENMARK.

[12]Department of Biomedicine, Aarhus University, DK-8000 Aarhus, DENMARK.

[13]Department of Cardiovascular Imaging, School of Biomedical Engineering and Imaging Sciences, King's College London, United Kingdom, UNITED KINGDOM.

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Author degrees

LDR: BSc, PhD-fellow

SW: MD, PhD

JW: BSc, PhD-fellow

CI: MD

JAE: MD, PhD

LB: MSc, PhD

JK: MD

GU: MD, PhD

HMS: MD, PhD

OH: MD, PhD

SES: MSc, PhD

LLK: MD

LHM: MD, PhD

LF; MD, PhD, DMSc

SEP: MD, PhD, MPH

LG: MD, PhD

EHC: MD, PhD

AE: MD, PhD

NRH: MD

MN: MSc, PhD

AC: MD, PhD

HEB: MD, PhD, DMSc

MB: MD, PhD

Keywords

Coronary artery disease

Heart sounds

Cardiac computed tomography angiography

Rubidium positron emission tomography

3 Tesla cardiac magnetic resonance

Invasive coronary angiography fractional flow reserve, coronary flow reserve and quantitative flow ratio.

Abbreviations

CAD: coronary artery disease

CT: computed tomography

CTA: computed tomography angiography

ICA: invasive coronary angiography

FFR: fractional flow reserve

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PCI: percutaneous coronary intervention

CABG: coronary artery bypass grafting

SPECT: single photon emission computed tomography

3T CMRI: 3 tesla cardiac magnetic resonance imaging

^{82}Rb-PET: ^{82}Rubidium positron emission tomography

FFR_{CT}: computed tomography angiography derived fractional flow reserve

ECG: electrocardiogram

QCA: quantitative coronary angiography

CFR: coronary flow reserve

QFR: quantitative flow ratio

BPM: beats per minute

LGE: late gadolinium enhancement

SSS: summed stress score

SRS: summed resting score

SDS: summed difference score

MBF: myocardial blood flow

MFR: myocardial blood flow reserve

2D: 2-dimensional

3D: 3-dimensional

LVED: left ventricular end-diastolic volume

LVESV: left ventricular end-systolic volume

LVSV: left ventricular stroke volume

LVEF: left ventricular ejection fraction

IMR: Index of microvascular resistance

ROC-AUC: Accuracy is estimated by receiver operating characteristic curve

CRS: Civil Registration

NPR: National Patient Registry

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SW received research support from Acarix A/S. JW received consultant fees and research support from Medis Medical Imaging. SEP provides consultancy for Circle Cardiovascular Imaging Inc., Calgary, Canada. All other authors declare no conflicts of interest.

Authors´ contributions

LDR, SW and MB participated in the conception and design of the study. SES is responsible for the CADScor®System algorithm. CI obtains the CMRI scans. LB aids with the CMRI scans. CI, AC and SEP analyze the CMRI scans. JAE obtains the 82Rb-PET scans. JAE and LG analyze the 82Rb-PET scans. LHM and LLK enroll participants and analyze the CTA scans at the Hospital Unit West, Herning. LF coordinates the study at Silkeborg Regional Hospital, JK and GU enroll patients and analyze the CTA scans at the Silkeborg Regional Hospital. HMS enroll patients and analyze the CTA scans at the Viborg Regional Hospital. OH enrolls patients and analyze the CTA scans at the Randers Regional Hospital. NRH and JW carry out the study regarding QFR. AE, EHC and HEB conduct the ICA and FFR measurements at the Department of Cardiology, Aarhus University Hospital. MN is responsible for the biobank including sample registration, storage, DNA extraction and genetic studies. All authors have critically reviewed, read, and approved the final version of the manuscript.

Study Sponsor

Morten Böttcher
Department of Cardiology, Hospital Unit West
Gl. Landevej 61, DK-7400 Herning. DENMARK.
Phone: +45 2577 5134, E-mail: morboett@rm.dk

Corresponding author

Laust Dupont Rasmussen
Department of Cardiology, Hospital Unit West
Gl. Landevej 61, DK-7400 Herning. DENMARK.
Phone: +45 3042 0558, E-mail: lausra@rm.dk

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Abstract

Background: Coronary computed tomography angiography (CTA) is the preferred primary diagnostic modality when examining patients with low to intermediate pre-test probability of coronary artery disease (CAD). Only 20-30% of these have potentially obstructive CAD. Because of the relatively poor positive predictive value of coronary CTA, unnecessary invasive coronary angiographies (ICA) are conducted with the costs and risks associated with the procedure. Hence, an optimized diagnostic CAD algorithm may reduce the numbers of ICAs not followed by revascularization.

The Dan-NICAD 2 study has three equivalent main aims: 1) to examine the diagnostic precision of a sound based diagnostic algorithm, The CADScor®System (Acarix A/S, Denmark), in patients with a low to intermediate pre-test risk of CAD referred to a primary examination by coronary CTA. We hypothesize that the CADScor®System provides better stratification prior to coronary CTA than clinical risk stratification scores alone. 2) to compare the diagnostic accuracy of 3 Tesla cardiac magnetic resonance imaging (3T CMRI), ^{82}Rubidium positron emission tomography (^{82}Rb-PET) and CT-derived fractional flow reserve (FFR$_{CT}$) in patients where obstructive CAD cannot be ruled out by coronary CTA using ICA fractional flow reserve (FFR) as reference standard. 3) to compare the diagnostic performance of quantitative flow ratio (QFR) and ICA-FFR in patients with low to intermediate pre-test probability of CAD using ^{82}Rb-PET as reference standard.

Methods/design: Dan-NICAD 2 is a prospective, multicenter, cross-sectional study including approximately 2,000 patients with low to intermediate pre-test probability of CAD and without previous history of CAD. Patients are referred to CTA because of symptoms suggestive of CAD, as evaluated by a cardiologist. Patient interviews, sound recordings, and blood samples are obtained in connection with the coronary CTA. If coronary CTA does not rule-out obstructive CAD, patients will be examined by both 3T CMRI, ^{82}Rb-PET, FFR$_{CT}$, ICA and FFR. Reference standard is ICA-FFR. Obstructive CAD is defined as an FFR $\leq$0.80 or as high-grade stenosis (>90 % diameter stenosis) by visual assessment.

Diagnostic performance will be evaluated as sensitivity, specificity, predictive values, likelihood ratios, calibration, and discrimination. Enrolment started January 2018 and is expected to be completed by June 2020. Patients are followed for 10 years after inclusion.

Discussion: The results of the Dan-NICAD 2 study are expected to contribute to the improvement of diagnostic strategies for patients suspected of CAD in three different steps; risk-stratification prior to coronary CTA, diagnostic strategy after coronary CTA and invasive wireless QFR analysis as an alternative to ICA-FFR.

Study registration: Clinicaltrials.gov identifier, NCT03481712. Registered on January 25th 2018.

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1. Background

An increasing number of patients are referred for evaluation of suspected obstructive coronary artery disease (CAD). Coronary computed tomographic angiographies (CTA) is currently recommended by the National Institute for Health and Care Excellence (NICE) as the initial diagnostic test for patients with stable CAD [1]. Of the patients examined, results from large databases show that coronary CTA excludes cardiovascular disease in 70-80% with an excellent negative predictive value of more than 95%.[2] However, coronary CTA alone has consistently proven to have a low positive predictive value, thus often overestimating the severity of CAD, especially in patients with moderate to severe coronary calcification.[3] Following coronary CTA, patients are frequently investigated with invasive coronary angiography (ICA) and fractional flow reserve (FFR). In this group, ICA often shows no obstructive CAD [4-6] and revascularization is not required. ICA still adds major costs and imply minor but not negligible risks of adverse events. The outlined issues raise the question of whether it is possible (1) to make a more precise stratification based on probability and consequently better patient selection prior to coronary CTA and (2) to reduce the number of patients referred for unnecessary ICAs and intracoronary flow assessments following CTA.
Acoustic detections of coronary stenosis from automatically recorded and analyzed heart sounds is a newly developed technology potentially useful for pre-test probability stratification before e.g. coronary CTA.[7] One of these devices, the CADScor®System (Acarix A/S, Denmark), extracts 8 acoustic features related to turbulent blood flow emerging from stenosed coronary segments as well as other cardiac sounds characteristic to CAD.[8, 9] In the Dan-NICAD 2 study, we will investigate the diagnostic performance of the most recently developed algorithm in the CADScor®System.

In patients with suspicion of coronary stenosis detected by coronary CTA, current guidelines recommend performing functional tests to verify the presence of inducible myocardial ischemia to reduce unnecessary ICAs.[10] Diagnostic accuracy of secondary non-invasive imaging with single photon emission computed tomography (SPECT) and 1.5 Tesla cardiac magnetic resonance imaging (CMRI) were investigated in the Dan-NICAD 1 trial.[11, 12] In Dan-NICAD 2, we intend to investigate the diagnostic accuracy of more advanced non-invasive myocardial perfusion imaging tests such as ^{82}Rubidium positron emission tomography (^{82}Rb-PET) and 3 Tesla (3T) CMRI. For both modalities, a high diagnostic accuracy was demonstrated in symptomatic patients with high probability of obstructive CAD.[13] However, the diagnostic accuracy has yet not been examined when these tests are used as second-line investigation after coronary CTA.

An alternative way to increase the diagnostic accuracy of coronary CTA and thus avoid unnecessary downstream testing is to utilize the ability to extract physiological information from the anatomical coronary CTA images. This technique - known as CT-derived fractional flow reserve (FFR_{CT}) - has become

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increasingly popular and has in preliminary studies shown promising results.[14, 15] Several companies are currently developing software for these analyses but the utilization of this technique has so far predominantly been tested in selected populations. In these selected cohorts, FFR_{CT} shows good diagnostic accuracy compared to the gold standard pressure-based ICA-FFR. Nonetheless, head to head comparison studies against non-invasive myocardial perfusion imaging tests are sparse.

Quantitative flow ratio (QFR) is a novel pressure wire-free approach for fast computation of FFR with potential to increase the global use of physiological lesion assessment.[16, 17] QFR is superior to traditional assessment of intermediate coronary lesions (QCA) and can be computed in-procedure within the same time as conventional wire-based approaches.[18] However, disagreement between FFR and QFR is identified in up to 20% of all measurements.[18] In Dan-NICAD II, the QFR-FFR disagreement is characterized by comparing paired QFR and FFR to ^{82}Rb-PET scan as a third test of reference.

This study has 3 main objectives. 1) to examine the diagnostic precision of a sound based diagnostic algorithm, the CADScor®System, in patients with a low to intermediate pre-test risk of CAD referred to a primary examination by coronary CTA. We hypothesize that the CADScor®System provides better stratification prior to coronary CTA than clinical risk stratification scores alone. 2) to determine the diagnostic accuracy of FFR_{CT}, 3T CMRI and ^{82}Rb-PET in patients where obstructive CAD cannot be ruled out by coronary CTA. 3) to compare the diagnostic precision of QFR and FFR in patients with low to intermediate pre-test probability of CAD using ^{82}Rb-PET as reference standard.

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2. Method

2.1 Study design

This study is an investigator-initiated, prospective, multicenter study. The study examines subjects without known CAD, who are referred for cardiac evaluation following symptoms suggestive of CAD. Study subjects are recruited at hospitals in the Central Denmark Region. This study will include approximately 2,000 patients with low to intermediate pre-test probability of CAD. All patients will provide their clinical history including detailed information about chest discomfort, will have a CAD-score examination, blood sampled for a biobank and finally undergo a non-enhanced and contrast-enhanced coronary CTA. It is expected that 20-23% of patients will have coronary stenosis suspected at the coronary CTA. These patients will be further examined with FFR_{CT}, ^{82}Rb-PET, 3T CMRI, and ICA with FFR, coronary flow reserve (CFR), Index of Microvascular Resistance (IMR) and QFR assessment (Figure 1). The numbers presented are based on the newly completed Dan-NICAD 1-trial.[11] Based on previous experience, the inclusion rate is expected to be 70%, and the patient inclusion is expected to be completed within 28 months.

All perfusion scans will be conducted at dedicated regional hospitals, CMRI at Regional Hospital Central Jutland and ^{82}Rb-PET at Hospital Unit West. FFR_{CT} will be analyzed by an independent corlab following an abnormal coronary CTA. ICA and invasive measurements will be conducted at Aarhus University Hospital.

2.2 Randomization and blinding procedure

If the patient has obstructive CAD at coronary CTA, a random allocation sequence stratifying for sex and inclusion site is created using a standard computerized random-number generator in regards to randomizing patients to undergo either ^{82}Rb-PET or CMRI examination first. The physicians performing coronary CTA, ^{82}Rb-PET, CMRI or ICA-FFR are initially blinded to the results of all other diagnostic test. The physicians performing FFR_{CT} are not blinded to coronary CTA results. In a second analysis, when all coronary CTA, ^{82}Rb-PET, CMRI or ICA-FFR analyses have been made, the physicians performing ^{82}Rb-PET and CMRI analyses are unblinded to coronary CTA, but not ICA, results. Results are re-calculated with information on coronary CTA findings and symptoms in order to mimic the clinical situation. The unblinded procedures are secondary investigations.

2.3 Study population

Based on a clinical assessment in an outpatient cardiology setting, the study cohort consists of patients with low or intermediate pre-test probability of CAD referred for cardiac evaluation by coronary CTA. Determination of low and intermediate pre-test probability is clinically supported of the updated Diamond-Forrester score.[19] Inclusion and exclusion criteria are listed in Table 1. All enrolled patients are systematically interviewed and undergo an acoustic CAD-score examination alongside blood sampling stored in a biobank and coronary CTA.

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2.4 Biobank

From all participants consenting to the study, five blood samples are drawn prior to coronary CTA contrast administration. Within two hours, three of the samples are centrifuged and processed into EDTA plasma, Heparin plasma, and serum, which are aliquoted into individual matrix tubes and placed at minus 20 degree. Two 4ml EDTA blood samples are placed directly in the freezer for later extraction of genomic DNA. All biospecimens are transported on dry ice to the Dan-NICAD biobank at Department of Biomedicine, Aarhus University, where all samples are stored at minus 80 degree. After extraction of genomic DNA, the samples will undergo array genotyping and whole genome sequencing for investigation of common and rare variations associated with CAD and sub-phenotypes recorded in the trial.

2.5 CADScor®System

The CADScor®System (Acarix A/S, Denmark) is a device using acoustic analysis performed just prior to coronary CTA examination and blood sampling. Heart sounds characteristic to CAD including turbulent blood flow caused by coronary stenoses are recorded using a microphone mounted at the 4th intercostal space just left to the sternum using a dedicated patch. The CAD-score examination is performed in accordance with the manufactures´ guidelines (figure 2). Using a fully automated algorithm version 3.1, a CAD-score is immediately calculated by the device following the acoustic examination. The algorithm has been adjusted compared to previously published results [9] to increase the sensitivity. A CAD-score > 20 is considered abnormal (table 2).

2.6 Imaging

2.6.1 Cardiac computed tomography angiography

Patient preparation – coronary CTA

According to the clinical routine of the radiology department, patients are instructed to abstain from all substances and drugs containing caffeine for at least 24 hours prior to the coronary CTA examination. Patients receive 50-100 mg metoprolol, 50-100mg atenolol or 7.5-15 mg ivabradin the night before and two hours prior to coronary CTA to reduce the heart rate <65 beats per minute (BPM). If not contraindicated, patients with persistent HR>65 BMP receive 2.5-20 mg metoprolol tartrate intravenously. Just prior to the coronary CTA, all patients receive 0.8 mg of sublingual nitroglycerin. The procedure is in accordance with normal clinical routine.

Imaging protocol - CTA

CTA examination is performed using a 320 multi-slice volume CT scanner (Aquilion One, Toshiba Medical Systems, Japan and Siemens Flash, Siemens Healthcare, Germany) using prospective electrocardiogram (ECG) triggering. The CTA protocol is schematically shown in figure 2. The coronary CTA includes 2 different acquisition protocols: 1) A non-enhanced examination and 2) A contrast-enhanced cardiac

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examination. Following the enhanced examination, data are reconstructed in the cardiac diastolic phase at 70-99% of the RR interval. If the patient has severe tachycardia, the diastolic scan can be combined with the systolic phases, typically phase 40%. The best phase at slice thickness 0.5 mm is transferred to a dedicated workstation (Vitrea Advanced Workstation, Vital Images, Minnetonka, MN, USA or Syngo.Via, Siemens Healthcare, Erlangen, Germany).

Imaging analyses - CTA

All coronary CTA analyses are performed by an experienced cardiologist. An Agatson calcium score is initially calculated using dedicated workstations. Using the 18-segment model described by the Society of Cardiovascular Computed Tomography, the luminal diameter stenosis is evaluated in each segment of the coronary tree.[20] Visually assessing and quantifying coronary lesions, the severity of coronary stenosis are classified as: no stenosis—0 % diameter reduction (≈ 0 % area reduction); mild stenosis—1 to 29 % diameter reduction (≈ 1 to 50 % area reduction); moderate stenosis—30 to 49 % diameter reduction (≈ 50 to 69 % area reduction); and severe stenosis—50 to 100 % diameter reduction (≈ 70 to 100 % area reduction). The criteria for an abnormal coronary CTA are shown in table 2.

FFR_{CT} are performed using dedicated software. FFR_{CT} values in the major epicardial arteries (left main, left anterior descending, left circumflex, and right coronary; including side branches). The criteria for an abnormal FFR_{CT} are outlined in table 3.

2.6.2 Positron emission tomography

Patient preparation - PET

According to the clinical routine of the nuclear department, patients are instructed to abstain from all substances and drugs containing caffeine for at least 24 hours prior to the ^{82}Rb-PET examination.

Imaging protocol and image reconstruction – PET

The ^{82}Rb-PET protocol is schematically shown in figure 2. ^{82}Rb-PET data is obtained in list mode with a Siemens Biograph mCT/64 PET-scanner (Siemens Healthcare, Knoxville, Tennessee, USA). The participants undergo two image acquisitions, 5 minutes each; the first at rest and the subsequent during hyperemia induced by adenosine. Criteria for and insurance of sufficient adenosine stress are listed in table 3.

Imaging analysis – PET

Imaging analyses are performed by an independent core lab at Aarhus University Hospital, Denmark blinded for additional patient information and results. The transaxial summed, gated and dynamic ^{82}Rb-PET perfusion images are automatically reoriented into short-axis, vertical and horizontal long-axis slices using a commercially available software (QPET 2015), Cedars-Sinai Medical Center, Los Angeles, California).[21]

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The quality of the stress and rest images is evaluated semi-quantitatively on a visual scale from 1 to 3 (1: good image quality with no artifacts; 2: moderate image quality, acceptable for clinical or research diagnosis; 3: poor image quality, and diagnosing is impossible due to severe artifacts).

For regional analysis, the recommended 17-segment American Heart Association model will be used.[22] Firstly, the summed perfusion images produced 150-300 seconds after ^{82}Rb infusion are analyzed. Segmental perfusion scores based on the average defect severity in a given segment is produced by the software and adjusted by the expert reader (0 = normal; 1= mildly abnormal; 2 = moderately abnormal; 3 = severely abnormal; 4 = absent).[23] From the segmental scores, Summed Stress Score (SSS), Summed Resting Score (SRS) and Summed Difference Score (SDS) are calculated and reported for vascular territories, regions and the entire (global) left ventricular myocardium. An abnormal ^{82}Rb-PET scan is defined as (1) an SDS ≥ 4 involving ≥ 2 contiguous segments (reversible ischemia) and ≥ 1 segment with a stress severity score ≥ 2; (2) an SRS ≥ 4 involving ≥ 2 contiguous segments and ≥ 1 segment with a stress severity score ≥ 2 (irreversible ischemia); (3) an SSS ≥ 4 involving ≥ 2 contiguous segments and ≥ 1 segment with a stress severity score ≥ 2 (combination of reversible and irreversible ischemia, mixed ischemia); 4) poor image quality (score 3, non-diagnostic). From the gated images obtained 150-300 seconds after ^{82}Rb infusion, left ventricle ejection fraction during rest and hyperemia and transient ischemic dilation (mean volume hyperemia / mean volume rest) is calculated.

Secondly, PET-derived myocardial blood flow (MBF) is calculated by the QPET software from images acquired 0-120 seconds after the ^{82}Rb infusion using the model proposed by Lortie et. al.[24] MBF and MBF reserve (MFR) defined as MBF during maximal hyperemia divided by MBF at rest will be reported for the three coronary territories. Moreover, segmental coronary flow during stress and rest will be registered.

After the blinded analysis described above has been performed, the ^{82}Rb-PET images are re-evaluated together with knowledge of patient characteristics and information from CTA (anatomy of coronary vessels and possible stenosis).

The criteria for an abnormal ^{82}Rb-PET are outlined in table 3.

2.6.3 Cardiac Magnetic Resonance Imaging

Patient preparation - CMRI

According to the clinical routine of the radiology department, patients are instructed to abstain from all substances and drugs containing caffeine for at least 24 hours prior to the CMRI examination.

Imaging protocol and image reconstruction – CMRI

The CMRI scans are conducted using a 3.0T MRI system (Siemens Skyra, Software release E11A, Siemens Healthcare GmbH, Germany) using the Body 18 and Spine 32 receive coils. Blood pressure, distal oxygen saturation and a vector-ECG will be monitored continuously during the examinations using a MedRad®

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Veris® monitoring system (Bayer Healthcare LCC, USA). All sequences are ECG gated, while motion artifacts are minimized by breath-holding. Series of MRI scans are included to assess information regarding cardiac morphology, function, perfusion and viability state of the myocardium using late gadolinium enhancement (LGE).

The CMRI protocol is schematically shown in figure 2. Cardiac morphology will be evaluated from axial and sagittal 2D image series covering the entire heart using a navigator gated single-shot echo-planar fast spin echo sequence (HASTE: Half-Fourier Acquisition Single-shot Turbo Spin-Echo).
Hyperemia will be induced using a continuous venous infusion of adenosine. Criteria for and initiatives to sufficient adenosine stress are listed in table 1. Rest perfusion scans will be performed after pharmacological stress washout (at least 10 minutes) using the same sequence parameters as for the stress perfusion. Gadolinium contrast agent (Gadovist®, Bayer Schering Pharma AG, Germany) will be injected during stress and rest using a dual-bolus method in order to minimize saturation effects during first-pass in the arterial input function.[25] The dual-bolus method consists of the injection of a 10% diluted pre-bolus, followed by a main bolus of neat contrast agent.

Imaging analyses - CMRI

CMRI analyses are carried out in an independent core lab blinded for additional patient information and results. Image quality regarding artefacts and image homogeneity are scored qualitatively using a scale from 1-3 (1 = good image quality, no significant artifacts; 2 = moderate image quality, with significant artifacts but overall diagnostic image quality; 3 = severe artifacts with poor/non-diagnostic image quality).
Several volume measurements are carried out including left ventricular end-diastolic volume (LVED), left ventricular end-systolic volume (LVESV), left ventricular stroke volume (LVSV), left ventricular ejection fraction (LVEF) and left ventricular mass.
Regional wall motion abnormalities will be scored using a scale from 1-5: 1 = normal, 2 = mild hypokinesia, 3 = severe hypokinesia, 4 = akinesia, and 5 = dyskinesia.
Myocardial perfusion will be evaluated both qualitatively and quantitatively. Qualitative (visual) perfusion analysis will be are carried out using the standard American Heart Association model for left ventricular assessments.[22] Stress-induced perfusion defects will be defined on visual assessment as a delayed wash-in of contrast persisting for at least five dynamic cardiac cycles in ≥2 contiguous segments compared with normal remote myocardium. Each AHA segment will be sub-divided into an endocardial and epicardial half, resulting in a total of 32 segments to allow an accurate calculation of ischemic burden. Moreover, the presence of LGE will be reported. The criteria for an abnormal CMRI are shown in table 3.

Quantitative perfusion analyses will be performed in collaboration with King's College London, UK using

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previously validated software.[26-29] The analyses will be performed by Fermi-constrained deconvolution according to the previously described methods, in which time-signal intensity curves for the tissue impulse response function, $h(t)$, were fitted to the Fermi function using a Marquardt-Levenberg nonlinear least-squares algorithm according to the following analytical expression:

$$h(t) = R\left[\frac{1}{e^{(t-\tau_0-\tau_d)k}+1}\right]u(t-\tau_d)$$

by letting k, R, and τ_0 vary and keeping τ_d fixed. In the preceding equation, $u(t - \tau_d)$ is the unit step function. The τ_d accounts for the delay time between the appearance of the signal in the left ventricular blood pool and myocardial region of interest (ROI); τ_0 characterizes the width of the shoulder of the Fermi function during which little or no contrast agent had left the ROI. R is the index of contrast agent influx parameter, and k represents the decay rate of $h(t)$ due to contrast agent washout. Using the preceding equation, myocardial blood flow (MBF) estimates are calculated as $h(t)$ at $t = 0$. Myocardial perfusion reserve (MPR) will be calculated as the ratio between stress and resting MBF estimates. The presence of ischemia evaluated with quantitative perfusion analyses will be defined as regions with MPR <1.5, according to previously validated criteria. In a separate analysis, CMRI analyses are compared to the coronary vessels´ anatomy and possible stenoses exposed during CTA and patient characteristics.

2.6.4 Invasive coronary angiography and invasive physiological examination – ICA, FFR, CFR, QFR

Patient preparation

According to the clinical routine of the cardiac department, patients are instructed to abstain from all substances and drugs containing caffeine for at least 24 hours prior to the ICA examination.

Cardiac catheterization protocol

Invasive coronary angiography

All diagnostic ICAs are performed at Aarhus University Hospital according to present clinical guidelines through a femoral or radial access. Before acquisition of the ICA, the operator administrates 250 µg of intra-coronary nitroglycerine. The ICA protocol is schematically shown in figure 2. All lesions with a diameter stenosis of 30-90% by visual estimate and a reference diameter of > 2mm are considered for physiological assessment. Angiographic acquisitions are performed at 15 frames per second in at least two projections more than 25 degrees apart allowing for 2D quantitative coronary angiography (QCA) and QFR analysis. Coronary artery overlap, foreshortening, zooming and planning is avoided if possible. All vessels are visualized in their full length if possible.

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Patient preparation for physiological examination

Anticoagulation (5,000 IU heparin) is administrated prior the pressure measurement. The pressure-wire (PressureWire X Guidewire, Abbott Chicago, USA) and CoroFlow systems are used according to manufacture instructions for use. The pressure wire is advanced to the tip of the guiding catheter to equalize the pressure readings.

Resting Pd/Pa and average mean resting transit time

The wire is advanced distal to all lesions in the vessel of interest and the wire-position is documented. Resting Pd/Pa is recorded as a minimum of 10 seconds with a stabilized Pa/Pa value after checking the pressure-curves. Next, 3 ml of room-temperature saline is injected rapidly by hand 3 times to record mean transit time at baseline while the coronary system is not affected by adenosine.

Fractional flow reserve, coronary flow reserve and index of microvascular resistance

Hyperemia is induced using a 1 mg/mL concentration of adenosine at 140 ug/min/kg and the infusion rate is increased to 200 µg/L/min if a stable FFR value is not achieved. When maximum hyperemia is achieved, three boluses of saline are injected to obtain hyperemic thermodilution curves for hyperemic mean transit time calculation. Coronary flow reserve (CFR) and the index of microvascular resistance (IMR) are instantly presented during the procedure. CFR is defined as the mean resting transit time by the mean hyperemic transit time. IMR is defined as the mean distal pressure multiplied by the mean hyperemic transit time. Routine checks are made to ensure that 'drift' does not occur after the recordings. Absolute drift value of ≤ ±0.02 is accepted.

Post-procedural physiological examination

Resting Pd/Pa, FFR, IMR and CFR are measured following PCI treatment of diseased vessels. QCA-projections are repeated for core-lab QFR computation of vessels treated with PCI.

Image analysis - ICA

All physiologic core-lab analyses are performed blinded to the patient´s CAD score, CTA, CMRI, and ^{82}Rb-PET examination. Invasive physiology analysis (Coroventis Research AB, Uppsala, Sweden) is performed in a suited corelab (Institute of Clinical Medicine, Aarhus University, Denmark). 2D-QCA is performed in an independent core lab (ClinFact, Leiden, The Netherlands).
The criteria for an abnormal ICA are shown in table 2.

Quantitative flow ratio analysis

QFR and 3D-QCA core-lab analyses are performed in a core-lab setting (Aarhus University Hospital, Skejby, Denmark) using the latest version of the software (QAngio XA 3D, Medis medical imaging system,

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Leiden, The Netherlands). The methodology and technical specifications were recently published.[16] Analysis is performed according to standard operating procedures.[30] QFR ≤0.80 is used as diagnostic cut-off value.

2.7 Follow-up

The cohort is followed for a period of 10 years after the coronary CTA examination. Data are extracted from the Civil Registration System (CRS) and the National Patient Registry (NPR). Data are adjudicated by look-up in the electronic patient file and verified by an adjudication committee. The purpose is to investigate the prognostic values of the CADScor®System and second second-line investigations following coronary CTA. Data recorded are: mortality, cardiovascular events, cardiac disease, revascularization treatment and medical treatments and comorbidity. Cases are electronically recorded using the patient specific electronical record with additional information on biochemistry, medication and other examination results.

2.8 Data collection and recordings

All study data are recorded in a secure web-based electronic case record form ((eCRF) – Research Electronic Data Capture (REDcap®)[31]) that enables logging of all data entries. The CAD-score measurement, patient interview, and blood samples are obtained by trained and skilled study nurses. All investigators have access to the eCRF. However, physicians performing corelab analyses have limited access in regards of blinding procedures. Data collected and registered in the dedicated eCRF are listed in Addendum. The study is monitored according to ICH-GCP (ICH Harmonized Tripartite Guideline for Good Clinical Practice).

2.9 Endpoints and statistical analysis

Data analysis and reporting will follow the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) and Standard for Reporting Diagnostic Accuracy Studies (STARD) guidelines. All demographic and baseline characteristics will be presented and analyzed using appropriate statistical methods.

2.9.1 Analyses of CADScor®

Our first main objective is to show superiority for the CADScor®System compared to the updated Diamond-Forrester score[19] for the area under the receiver-operator characteristics curve for detection of CAD. The reference standard used is anatomically significant coronary stenosis assessed by ICA-2D-QCA (≥50% diameter stenosis). Patients are categorized as having I) obstructive CAD: ≥50% diameter stenosis by ICA-2D-QCA, or II) no obstructive CAD: <50% diameter stenosis by ICA-2D-QCA or negative coronary CTA. The diagnostic performance is evaluated on a patient level as ROC-AUC with the CAD-score as a continuous variable. Similarly, calculations are made using hemodynamically significant obstructive CAD with ICA-FFR as the reference standard. Secondary, the diagnostic performance is reported with sensitivity, specificity, positive predicative value, negative predicative value, where the CAD-scores are dichotomized

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with a positive value above 20.

All analyses will be performed with CAD-scores calculated with algorithm version 3.1. Furthermore, analyses may be performed with further developed algorithms using sound files collected in the study.

2.9.2 Analysis of value of non-invasive imaging

The second main objective of this study is to investigate and compare the diagnostic precision of ^{82}Rb-PET and 3T CMRI as secondary examinations following coronary CTA where obstructive CAD cannot be excluded using ICA-FFR as reference standard.

The diagnostic precision of ^{82}Rb-PET and CMRI is evaluated by sensitivity, specificity, positive and negative predictive value, diagnostic accuracy and likelihood ratios. Comparison of ^{82}Rb-PET and CMRI sensitivity, specificity, positive and negative predictive value is tested using McNemar´s test and a weighted generalized score statistic for comparison of predictive values of diagnostic tests.[32] The reference standard used is invasive FFR≤0.80, and tests are made on both patient and coronary vessel level.

Further, we will evaluate 1) the diagnostic accuracy of FFR_{CT} compared to ^{82}Rb-PET and CMRI 2) the impact of using additional CFR and IMR on the FFR_{CT}, ^{82}Rb-PET and CMRI related diagnostic accuracy, 3) the diagnostic precision of CMRI absolute perfusion 4) the diagnostic precision of CMRI dyssynchrony analysis of coronary stenoses.

2.9.3 Analysis of QFR performance

The third main objective is to compare the diagnostic performance of QFR with FFR using ^{82}Rb-PET as reference for ischemia. Diagnostic performance of QFR is measured and compared to FFR for accuracy, ROC-AUC, sensitivity, specificity, negative predictive value, positive predictive value, positive likelihood ratio and negative likelihood ratio with ^{82}Rb-PET as reference. The same comparisons are made using CFR with a cut-point of 2.0 as reference standard. Time-frame count derived estimated contrast flow velocity is compared to mean transit time estimated by thermodilution technique during resting and hyperemic conditions. Subgroup analyses are performed for patients grouped by diagnostic match or mismatch between FFR and QFR; on 1) numerical differences in CFR and IMR between cases with matched vs mismatched FFR and QFR, 2) agreement of the two groups with ^{82}Rb-PET and separately with 3T CMRI, 3) stenosis morphology as assessed with 2D and 3D-QCA compared for the two groups. Diagnostic mismatch is defined according to the predefined cut-point of 0.80 and in a separate analysis by an absolute QFR-FFR difference >0.05.

For all statistical analyses, a two-sided p value < 0.05 is considered statistically significant, and 95 % confidence intervals are reported when appropriate. Statistical analysis is performed using dedicated statistical software (STATA, StataCorp, College Station, TX, USA).

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2.10 Sample size

Based on the DAN-NICAD 1 trial, we expect that approximately 2,000 patients are to be included and undergo coronary CTA. Following coronary CTA, we expect that 460 (23%) patients in whom coronary stenosis cannot be excluded are eligible for continuing to the perfusion examinations and ICA part of the trial. We expect 80% to complete both CMRI, ^{82}Rb-PET and undergo ICA examination. By including 2,000 patients, we are able to evaluate the predictive validity parameters (sensitivity, specificity, positive and negative predictive values) with a minimum of 6% absolute precision on both sides for the expected sensitivity (80%) and specificity (80%) for both CMRI and ^{82}Rb-PET at a disease prevalence of 50% at CMRI and ^{82}Rb-PET.

To show statistically significant superiority for the AUC for the CADScor®System compared to the AUC for the updated Diamond-Forrester score [19]for detection of CAD it is assumed that I) the AUC for CAD-score is 0.72 and the AUC for the Diamond-Forrester score is 0.66, II) the covariance between the CAD-score and the Diamond-Forrester score is 0.46, III) the CAD prevalence is 10%. Based on these assumptions a minimum of 157 CAD patients and 1413 patients without CAD are to be included in the analysis for the primary CAD-score objective.

QFR has not previously been compared to non-invasive perfusion scans. Using recent data that compared FFR to ^{82}Rb-PET, we use a sensitivity and specificity for ^{82}Rb-PET derived relative flow reserve of 0.83 and 0.84, respectively.[33] We accept a lower confidence interval limit of 0.80, and a two-sided confidence interval is used. With an expected dropout rate of 0.20, alpha=0.05 and a rate of true positives of 30 % in the population with successful FFR assessment (flow limiting stenosis prevalence), we estimate a need of 341 patients with ^{82}Rb-PET scans for sensitivity analysis and 139 patients with ^{82}Rb-PET scans for specificity analysis.

2.11 Ethical considerations

The study follows the principles outlined in the Declaration of Helsinki and ISO 14155:2011. The study´s additional radiation exposure in regards of the ^{82}Rb-PET examination increases the cumulated risk over a life-time of dying from cancer from approx. 25% to 25.1%. Patients participate in the study only after providing informed written consent. There is a small risk of incidental findings in this study. According to the Danish research ethical guidelines for genome research, an expert panel will be formed in the case of an incidental finding and clinical guidance will be provided by trained clinical geneticists within the field of that particular disease. The study was registered at ClinicalTrials.gov (Identifier; NCT03481712).

2.12 Disclosure

The study was supported by Acarix A/S. Otherwise, the authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the paper and its final contents.

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2.12 Funding

The study was supported by Aarhus University, Health Research Fund of Central Denmark Region, and Acarix A/S.

3. Discussion

Dan-NICAD 2 is a multi-purpose study assessing 1) the diagnostic performance of the CADScor®System, 2) the diagnostic benefit of performing perfusion tests and FFR_{CT} after positive coronary CTA, and 3) comparing QFR with invasive FFR using ^{82}Rb-PET as reference.

Coronary CTA has proven to be a valid tool for ruling out CAD in patients with a low to intermediate pre-test probability of CAD.[34-36] However, the low positive predictive value of coronary CTA alone causes 'unnecessary' downstream testing with e.g. ICA where patients are not revascularized. The need for more specific strategies for non-invasive diagnostic work-up of patients with suspected CAD was demonstrated in a large study comprising 398,978 patients in whom elective cardiac catheterization showed obstructive CAD in only 38%.[37] The diagnostic accuracy of SPECT and 1.5 CMRI as secondary non-invasive CAD examinations was investigated in the Dan-NICAD 1 trial and showed in general a low sensitivity for diagnosing significant stenosis as defined by $FFR \leq 0.80$.[11, 12]

The Dan-NICAD 2 study aim to test whether unnecessary ICA examination can be avoided by performing updated novel secondary perfusion imaging tests such as ^{82}Rb-PET and 3T CMRI or FFR_{CT} in patients, where coronary CTA examination does not exclude CAD. In these patients, the Dan-NICAD 2 study also applies advanced invasive measures such as CFR, IMR and QFR to allow for a more precise invasive assessment of the lesions.

To date, several acoustic detection systems are under development for CAD investigation.[7] We previously found that sound-based stratification of patients with the CADScor®System is comparable to clinical risk scores alone.[38] Further, in the DAN-NICAD 1 study we validated an automatic CADScor®System software algorithm including both sound-based features and clinical risk factors, which increases the diagnostic accuracy compared to clinical risk stratification alone. The higher specificity of the CAD-score compared to the updated Diamond-Forrester score also provides a relevant reclassification potential. With a negative predictive value of 96%, this new acoustic rule-out system could potentially supplement clinical assessment to guide decisions on the need for further diagnostic investigation.[9] In the DAN-NICAD 2 trial, we will evaluate the latest version of the software and potentially further developed algorithms. Populations change over time, and the current trends point towards a lower prevalence in populations undergoing non-invasive testing. This might change the weights of the different components, such as gender and age in the optimal algorithm. In developing an optimal algorithm, the false negative patients at coronary CTA is a limitation in the current study. However, the impact of this potential work-up bias is considered low due to the high sensitivity of the coronary CTA.

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Myocardial perfusion imaging with PET-technology enables non-invasive information of myocardial perfusion during rest and pharmacologically induced hyperemia by using a radioactive isotope taken up by vital cardiomyocytes proportionally to blood supply. Compared to SPECT, PET technique provides a higher image resolution, quantification of perfusion and causes less radiation exposure.[39] Previous studies have investigated the diagnostic accuracy of ^{82}Rb-PET for myocardial perfusion. Some of these studies are retrospective and influenced by referral biases, and some are limited by applying ICA luminal diameter stenosis as reference standard. Not using ICA-FFR as reference standard, previous studies compare functional and anatomical tests, which can be challenging.[5, 40] However, compared to SPECT, studies indicate that ^{82}Rb-PET has higher sensitivity with similar specificity in predicting CAD. A recent study using ICA-FFR as reference standard found ^{82}Rb-PET with a sensitivity of 87% and a specificity of 95%.[41]

3T CMRI is a non-invasive, non-radioactive, high resolution examination. In addition to coronary perfusion, 3T CMRI enables investigation of the general cardiac function including possible valve diseases and scar formation. CMRI has shown high sensitivity and specificity when assessing coronary stenoses.[42-44] As with ^{82}Rb-PET, investigations of CMRI accuracy has primarily been conducted with ICA diameter stenosis as reference standard.[5, 45-48] However, using ICA-FFR as reference standard, a recent metaanalysis found CMRI having an average sensitivity of 90% and a specificity of 87%.[49] This metaanalysis, however, only included 3 out of 14 studies using 3T CMRI.

Experimental software is currently being developed at St. Thomas Hospital, King´s College, London. The software enables investigation of quantified myocardial perfusion and perfusion dyssynchrony from CMRI images.[28, 50] During Dan-NICAD 1, our research group has initiated a collaboration with St. Thomas Hospital, and the software will be tested in Dan-NICAD 2 by further evaluating the diagnostic accuracy compared to ^{82}Rb-PET and invasive FFR.

The use of image-based modeling and computational fluid dynamics enables assessment of coronary blood flow and pressure from already existing coronary CTA images.[15, 51-53] The technique is known as FFR_{CT} and has in blinded trials shown high diagnostic performance compared to ICA-FFR.[15, 52, 53] Although results are promising, FFR_{CT}´s clinical utility is to our knowledge unknown.

Conventional coronary CTA enables anatomical CAD assessment, and as demonstrated in the PROMISE and SCOT-HEART trails, coronary CTA as frontline examination increases the diagnostic accuracy compared to non-invasive functional tests in patients with stable CAD.[54, 55] As outlined, conventional coronary CTA, however, tends to overestimate stenoses' severity and their functional implication.[3, 56] The discrepancy between the anatomical and functional tests might be tackled using combined information of anatomy and physiology in terms of FFR_{CT}.

Studies upon head-to-head comparison of FFR_{CT} and advanced myocardial perfusion examinations lack. In a

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single-center, non-randomized, observational study, Nørgaard et al. [57] found the introduction of FFR_{CT} to decrease ICA utilization and increase ICA diagnostic yield compared to ^{82}Rb-PET. The ReASSESS study found FFR_{CT} and SPECT with similar diagnostic accuracy, but FFR_{CT} yielded higher sensitivity.[58] Currently, the results from the CREDENCE trial are awaited.[59]

NICE currently recommends HeartFLOW FFR_{CT} analysis for patients with stable chest pain.[1] However, although FFR_{CT} is a very promising technique, it has not been evaluated for long term results. Furthermore, not all CT scans has sufficient quality to enable FFR_{CT} evaluation, and other techniques must be used for these patients.

The severity of coronary calcification has an impact on the agreement between coronary CTA and ICA when assessing obstructive lesions at the individual coronary segment level, and many patients with non-diagnostic coronary CTA have intermediate coronary stenoses.[3, 60] Hence, a population based on non-diagnostic coronary CTA have relatively few patients at the extremes (no stenosis; very severe stenosis), and a possibility is that the Dan-NICAD 2 study will find no difference in the diagnostic accuracy between ^{82}Rb-PET, 3T CMRI and FFR_{CT}. However, in the Dan-NICAD 1 population [11], 40% of the patients with "coronary CTA stenosis" underwent revascularization due to treatment requiring stenosis. As the inclusion criteria for this trial is similar with Dan-NICAD 1, we expect about the same occurrence of need for revascularization.

Symptoms of angina are ultimately caused by oxygen supply-demand mismatch caused by diminished blood-flow to the myocardium. However, FFR is a pressure-only index used as surrogate for coronary flow measurements.[61] Recent data document that abnormal FFR values may coexist with normal coronary flow reserve (CFR) measurements while normal FFR can coexist with abnormal CFR measurements.[62] Hence, CFR and IMR may clarify potential mismatches between CMRI, ^{82}Rb-PET and FFR measurements. Further, the global uptake of FFR-guided revascularization remains low.[63] This has led to the development of quantitative flow ratio (QFR) for a less invasive, cheaper and safer assessment of coronary artery stenosis. In the initial studies, QFR showed good agreement to FFR. However, the approximately 20 % disagreement has not yet been evaluated using a third ischemia test as reference standard. The results of Dan-NICAD 2 may help determine whether disagreement between FFR and QFR is caused by methodological errors, natural variation or if one test may be superior to the other.

4. Perspective

The results of the Dan-NICAD 2 study are expected to contribute to the improvement of diagnostic strategies for patients suspected of CAD in three different steps; risk-stratification prior to coronary CTA, diagnostic strategy after coronary CTA and invasive wireless QFR analysis as an alternative to ICA-FFR.

5. Study status

The study is ongoing. The first patient was enrolled on January 24th 2018. Enrolment completion is expected in June 2020.

6. References

1. **National Institute for Health and Care Excellence: *Chest pain.* NICE pathway. Manchester, NICE, 2017.** [https://pathways.nice.org.uk/pathways/chest-pain]
2. Nielsen LH, Botker HE, Sorensen HT, Schmidt M, Pedersen L, Sand NP, Jensen JM, Steffensen FH, Tilsted HH, Bottcher M *et al*: **Prognostic assessment of stable coronary artery disease as determined by coronary computed tomography angiography: a Danish multicentre cohort study**. *Eur Heart J* 2017, **38**(6):413-421.
3. Cheng V, Gutstein A, Wolak A, Suzuki Y, Dey D, Gransar H, Thomson LE, Hayes SW, Friedman JD, Berman DS: **Moving beyond binary grading of coronary arterial stenoses on coronary computed tomographic angiography: insights for the imager and referring clinician**. *JACC Cardiovasc Imaging* 2008, **1**(4):460-471.
4. Coronary Revascularization Writing G, Patel MR, Dehmer GJ, Hirshfeld JW, Smith PK, Spertus JA, Technical P, Masoudi FA, Dehmer GJ, Patel MR *et al*: **ACCF/SCAI/STS/AATS/AHA/ASNC/HFSA/SCCT 2012 appropriate use criteria for coronary revascularization focused update: a report of the American College of Cardiology Foundation Appropriate Use Criteria Task Force, Society for Cardiovascular Angiography and Interventions, Society of Thoracic Surgeons, American Association for Thoracic Surgery, American Heart Association, American Society of Nuclear Cardiology, and the Society of Cardiovascular Computed Tomography**. *J Thorac Cardiovasc Surg* 2012, **143**(4):780-803.
5. Tonino PA, Fearon WF, De Bruyne B, Oldroyd KG, Leesar MA, Ver Lee PN, Maccarthy PA, Van't Veer M, Pijls NH: **Angiographic versus functional severity of coronary artery stenoses in the FAME study fractional flow reserve versus angiography in multivessel evaluation**. *J Am Coll Cardiol* 2010, **55**(25):2816-2821.
6. Task Force on Myocardial Revascularization of the European Society of C, the European Association for Cardio-Thoracic S, European Association for Percutaneous Cardiovascular I, Kolh P, Wijns W, Danchin N, Di Mario C, Falk V, Folliguet T, Garg S *et al*: **Guidelines on myocardial revascularization**. *Eur J Cardiothorac Surg* 2010, **38 Suppl**:S1-S52.
7. Thomas JL, Winther S, Wilson RF, Bottcher M: **A novel approach to diagnosing coronary artery disease: acoustic detection of coronary turbulence**. *Int J Cardiovasc Imaging* 2017, **33**(1):129-136.
8. Schmidt SE, Holst-Hansen C, Graff C, Toft E, Struijk JJ: **Segmentation of heart sound recordings by a duration-dependent hidden Markov model**. *Physiol Meas* 2010, **31**(4):513-529.
9. Winther S, Nissen L, Schmidt SE, Westra JS, Rasmussen LD, Knudsen LL, Madsen LH, Kirk Johansen J, Larsen BS, Struijk JJ *et al*: **Diagnostic performance of an acoustic-based system for coronary artery disease risk stratification**. *Heart* 2018, **104**(11):928-935.
10. Fihn SD, Blankenship JC, Alexander KP, Bittl JA, Byrne JG, Fletcher BJ, Fonarow GC, Lange RA, Levine GN, Maddox TM *et al*: **2014 ACC/AHA/AATS/PCNA/SCAI/STS focused update of the guideline for the diagnosis and management of patients with stable ischemic heart disease: a report of the American College of Cardiology/American Heart Association Task**

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Force on Practice Guidelines, and the American Association for Thoracic Surgery, Preventive Cardiovascular Nurses Association, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. *J Thorac Cardiovasc Surg* 2015, **149**(3):e5-23.

11. Nissen L, Winther S, Isaksen C, Ejlersen JA, Brix L, Urbonaviciene G, Frost L, Madsen LH, Knudsen LL, Schmidt SE *et al*: **Danish study of Non-Invasive testing in Coronary Artery Disease (Dan-NICAD): study protocol for a randomised controlled trial**. *Trials* 2016, **17**(1):262.
12. Nissen L, Winther S, Westra J, Ejlersen JA, Isaksen C, Rossi A, Holm NR, Urbonaviciene G, Gormsen LC, Madsen LH *et al*: **Diagnosing coronary artery disease after a positive coronary computed tomography angiography: the Dan-NICAD open label, parallel, head to head, randomized controlled diagnostic accuracy trial of cardiovascular magnetic resonance and myocardial perfusion scintigraphy**. *Eur Heart J Cardiovasc Imaging* 2018, **19**(4):369-377.
13. Task Force M, Montalescot G, Sechtem U, Achenbach S, Andreotti F, Arden C, Budaj A, Bugiardini R, Crea F, Cuisset T *et al*: **2013 ESC guidelines on the management of stable coronary artery disease: the Task Force on the management of stable coronary artery disease of the European Society of Cardiology**. *Eur Heart J* 2013, **34**(38):2949-3003.
14. Norgaard BL, Terkelsen CJ, Mathiassen ON, Grove EL, Botker HE, Parner E, Leipsic J, Steffensen FH, Riis AH, Pedersen K *et al*: **Coronary CT Angiographic and Flow Reserve-Guided Management of Patients With Stable Ischemic Heart Disease**. *J Am Coll Cardiol* 2018, **72**(18):2123-2134.
15. Min JK, Leipsic J, Pencina MJ, Berman DS, Koo BK, van Mieghem C, Erglis A, Lin FY, Dunning AM, Apruzzese P *et al*: **Diagnostic accuracy of fractional flow reserve from anatomic CT angiography**. *JAMA* 2012, **308**(12):1237-1245.
16. Tu S, Westra J, Yang J, von Birgelen C, Ferrara A, Pellicano M, Nef H, Tebaldi M, Murasato Y, Lansky A *et al*: **Diagnostic Accuracy of Fast Computational Approaches to Derive Fractional Flow Reserve From Diagnostic Coronary Angiography: The International Multicenter FAVOR Pilot Study**. *JACC Cardiovasc Interv* 2016, **9**(19):2024-2035.
17. Westra J, Tu S, Winther S, Nissen L, Vestergaard MB, Andersen BK, Holck EN, Fox Maule C, Johansen JK, Andreasen LN *et al*: **Evaluation of Coronary Artery Stenosis by Quantitative Flow Ratio During Invasive Coronary Angiography: The WIFI II Study (Wire-Free Functional Imaging II)**. *Circ Cardiovasc Imaging* 2018, **11**(3):e007107.
18. Xu B, Tu S, Qiao S, Qu X, Chen Y, Yang J, Guo L, Sun Z, Li Z, Tian F *et al*: **Diagnostic Accuracy of Angiography-Based Quantitative Flow Ratio Measurements for Online Assessment of Coronary Stenosis**. *J Am Coll Cardiol* 2017, **70**(25):3077-3087.
19. Genders TS, Steyerberg EW, Alkadhi H, Leschka S, Desbiolles L, Nieman K, Galema TW, Meijboom WB, Mollet NR, de Feyter PJ *et al*: **A clinical prediction rule for the diagnosis of coronary artery disease: validation, updating, and extension**. *Eur Heart J* 2011, **32**(11):1316-1330.
20. Raff GL, Abidov A, Achenbach S, Berman DS, Boxt LM, Budoff MJ, Cheng V, DeFrance T, Hellinger JC, Karlsberg RP *et al*: **SCCT guidelines for the interpretation and reporting of coronary computed tomographic angiography**. *J Cardiovasc Comput Tomogr* 2009, **3**(2):122-136.

ACCEPTED MANUSCRIPT

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21. Nesterov SV, Deshayes E, Sciagra R, Settimo L, Declerck JM, Pan XB, Yoshinaga K, Katoh C, Slomka PJ, Germano G *et al*: **Quantification of myocardial blood flow in absolute terms using (82)Rb PET imaging: the RUBY-10 Study**. *JACC Cardiovasc Imaging* 2014, **7**(11):1119-1127.
22. Cerqueira MD, Weissman NJ, Dilsizian V, Jacobs AK, Kaul S, Laskey WK, Pennell DJ, Rumberger JA, Ryan T, Verani MS *et al*: **Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart. A statement for healthcare professionals from the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association**. *Int J Cardiovasc Imaging* 2002, **18**(1):539-542.
23. Tilkemeier PL, Cooke CD, Ficaro EP, Glover DK, Hansen CL, McCallister BD, Jr., American Society of Nuclear C: **American Society of Nuclear Cardiology information statement: Standardized reporting matrix for radionuclide myocardial perfusion imaging**. *J Nucl Cardiol* 2006, **13**(6):e157-171.
24. Lortie M, Beanlands RS, Yoshinaga K, Klein R, Dasilva JN, DeKemp RA: **Quantification of myocardial blood flow with 82Rb dynamic PET imaging**. *Eur J Nucl Med Mol Imaging* 2007, **34**(11):1765-1774.
25. Hsu LY, Rhoads KL, Holly JE, Kellman P, Aletras AH, Arai AE: **Quantitative myocardial perfusion analysis with a dual-bolus contrast-enhanced first-pass MRI technique in humans**. *J Magn Reson Imaging* 2006, **23**(3):315-322.
26. Ishida M, Schuster A, Morton G, Chiribiri A, Hussain S, Paul M, Merkle N, Steen H, Lossnitzer D, Schnackenburg B *et al*: **Development of a universal dual-bolus injection scheme for the quantitative assessment of myocardial perfusion cardiovascular magnetic resonance**. *J Cardiovasc Magn Reson* 2011, **13**:28.
27. Schuster A, Sinclair M, Zarinabad N, Ishida M, van den Wijngaard JP, Paul M, van Horssen P, Hussain ST, Perera D, Schaeffter T *et al*: **A quantitative high resolution voxel-wise assessment of myocardial blood flow from contrast-enhanced first-pass magnetic resonance perfusion imaging: microsphere validation in a magnetic resonance compatible free beating explanted pig heart model**. *Eur Heart J Cardiovasc Imaging* 2015, **16**(10):1082-1092.
28. Morton G, Chiribiri A, Ishida M, Hussain ST, Schuster A, Indermuehle A, Perera D, Knuuti J, Baker S, Hedstrom E *et al*: **Quantification of absolute myocardial perfusion in patients with coronary artery disease: comparison between cardiovascular magnetic resonance and positron emission tomography**. *J Am Coll Cardiol* 2012, **60**(16):1546-1555.
29. Sammut EC, Villa ADM, Di Giovine G, Dancy L, Bosio F, Gibbs T, Jeyabraba S, Schwenke S, Williams SE, Marber M *et al*: **Prognostic Value of Quantitative Stress Perfusion Cardiac Magnetic Resonance**. *JACC Cardiovasc Imaging* 2017.
30. Westra J, Andersen BK, Campo G, Matsuo H, Koltowski L, Eftekhari A, Liu T, Di Serafino L, Di Girolamo D, Escaned J *et al*: **Diagnostic Performance of In-Procedure Angiography-Derived Quantitative Flow Reserve Compared to Pressure-Derived Fractional Flow Reserve: The FAVOR II Europe-Japan Study**. *J Am Heart Assoc* 2018, **7**(14).
31. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG: **Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support**. *J Biomed Inform* 2009, **42**(2):377-381.
32. Kosinski AS: **A weighted generalized score statistic for comparison of predictive values of diagnostic tests**. *Stat Med* 2013, **32**(6):964-977.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

33. Lee JM, Kim CH, Koo BK, Hwang D, Park J, Zhang J, Tong Y, Jeon KH, Bang JI, Suh M *et al*: **Integrated Myocardial Perfusion Imaging Diagnostics Improve Detection of Functionally Significant Coronary Artery Stenosis by 13N-ammonia Positron Emission Tomography**. *Circ Cardiovasc Imaging* 2016, **9**(9).
34. Budoff MJ, Dowe D, Jollis JG, Gitter M, Sutherland J, Halamert E, Scherer M, Bellinger R, Martin A, Benton R *et al*: **Diagnostic performance of 64-multidetector row coronary computed tomographic angiography for evaluation of coronary artery stenosis in individuals without known coronary artery disease: results from the prospective multicenter ACCURACY (Assessment by Coronary Computed Tomographic Angiography of Individuals Undergoing Invasive Coronary Angiography) trial**. *J Am Coll Cardiol* 2008, **52**(21):1724-1732.
35. Meijboom WB, Meijs MF, Schuijf JD, Cramer MJ, Mollet NR, van Mieghem CA, Nieman K, van Werkhoven JM, Pundziute G, Weustink AC *et al*: **Diagnostic accuracy of 64-slice computed tomography coronary angiography: a prospective, multicenter, multivendor study**. *J Am Coll Cardiol* 2008, **52**(25):2135-2144.
36. Rochitte CE, George RT, Chen MY, Arbab-Zadeh A, Dewey M, Miller JM, Niinuma H, Yoshioka K, Kitagawa K, Nakamori S *et al*: **Computed tomography angiography and perfusion to assess coronary artery stenosis causing perfusion defects by single photon emission computed tomography: the CORE320 study**. *Eur Heart J* 2014, **35**(17):1120-1130.
37. Patel MR, Peterson ED, Dai D, Brennan JM, Redberg RF, Anderson HV, Brindis RG, Douglas PS: **Low diagnostic yield of elective coronary angiography**. *N Engl J Med* 2010, **362**(10):886-895.
38. Winther S, Schmidt SE, Holm NR, Toft E, Struijk JJ, Botker HE, Bottcher M: **Diagnosing coronary artery disease by sound analysis from coronary stenosis induced turbulent blood flow: diagnostic performance in patients with stable angina pectoris**. *Int J Cardiovasc Imaging* 2016, **32**(2):235-245.
39. Maddahi J, Packard RR: **PET should replace SPECT in cardiac imaging for diagnosis and risk assessment of patients with known or suspected CAD: Pro**. *J Nucl Cardiol* 2017.
40. Parker MW, Iskandar A, Limone B, Perugini A, Kim H, Jones C, Calamari B, Coleman CI, Heller GV: **Diagnostic accuracy of cardiac positron emission tomography versus single photon emission computed tomography for coronary artery disease: a bivariate meta-analysis**. *Circ Cardiovasc Imaging* 2012, **5**(6):700-707.
41. Danad I, Raijmakers PG, Driessen RS, Leipsic J, Raju R, Naoum C, Knuuti J, Maki M, Underwood RS, Min JK *et al*: **Comparison of Coronary CT Angiography, SPECT, PET, and Hybrid Imaging for Diagnosis of Ischemic Heart Disease Determined by Fractional Flow Reserve**. *JAMA Cardiol* 2017.
42. Greenwood JP, Maredia N, Younger JF, Brown JM, Nixon J, Everett CC, Bijsterveld P, Ridgway JP, Radjenovic A, Dickinson CJ *et al*: **Cardiovascular magnetic resonance and single-photon emission computed tomography for diagnosis of coronary heart disease (CE-MARC): a prospective trial**. *Lancet* 2012, **379**(9814):453-460.
43. Feuchtner G, Goetti R, Plass A, Wieser M, Scheffel H, Wyss C, Stolzmann P, Donati O, Schnabl J, Falk V *et al*: **Adenosine stress high-pitch 128-slice dual-source myocardial computed tomography perfusion for imaging of reversible myocardial ischemia: comparison with magnetic resonance imaging**. *Circ Cardiovasc Imaging* 2011, **4**(5):540-549.

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44. Qayyum AA, Kuhl JT, Mathiasen AB, Ahtarovski KA, Vejlstrup NG, Kofoed KF, Kastrup J: **Value of cardiac 320-multidetector computed tomography and cardiac magnetic resonance imaging for assessment of myocardial perfusion defects in patients with known chronic ischemic heart disease**. *Int J Cardiovasc Imaging* 2013, **29**(7):1585-1593.
45. Ishida N, Sakuma H, Motoyasu M, Okinaka T, Isaka N, Nakano T, Takeda K: **Noninfarcted myocardium: correlation between dynamic first-pass contrast-enhanced myocardial MR imaging and quantitative coronary angiography**. *Radiology* 2003, **229**(1):209-216.
46. Sakuma H, Suzawa N, Ichikawa Y, Makino K, Hirano T, Kitagawa K, Takeda K: **Diagnostic accuracy of stress first-pass contrast-enhanced myocardial perfusion MRI compared with stress myocardial perfusion scintigraphy**. *AJR Am J Roentgenol* 2005, **185**(1):95-102.
47. Hamon M, Fau G, Nee G, Ehtisham J, Morello R, Hamon M: **Meta-analysis of the diagnostic performance of stress perfusion cardiovascular magnetic resonance for detection of coronary artery disease**. *J Cardiovasc Magn Reson* 2010, **12**:29.
48. Melikian N, De Bondt P, Tonino P, De Winter O, Wyffels E, Bartunek J, Heyndrickx GR, Fearon WF, Pijls NH, Wijns W *et al*: **Fractional flow reserve and myocardial perfusion imaging in patients with angiographic multivessel coronary artery disease**. *JACC Cardiovasc Interv* 2010, **3**(3):307-314.
49. Li M, Zhou T, Yang LF, Peng ZH, Ding J, Sun G: **Diagnostic accuracy of myocardial magnetic resonance perfusion to diagnose ischemic stenosis with fractional flow reserve as reference: systematic review and meta-analysis**. *JACC Cardiovasc Imaging* 2014, **7**(11):1098-1105.
50. Chiribiri A, Villa AD, Sammut E, Breeuwer M, Nagel E: **Perfusion dyssynchrony analysis**. *Eur Heart J Cardiovasc Imaging* 2016, **17**(12):1414-1423.
51. Taylor CA, Fonte TA, Min JK: **Computational fluid dynamics applied to cardiac computed tomography for noninvasive quantification of fractional flow reserve: scientific basis**. *J Am Coll Cardiol* 2013, **61**(22):2233-2241.
52. Koo BK, Erglis A, Doh JH, Daniels DV, Jegere S, Kim HS, Dunning A, DeFrance T, Lansky A, Leipsic J *et al*: **Diagnosis of ischemia-causing coronary stenoses by noninvasive fractional flow reserve computed from coronary computed tomographic angiograms. Results from the prospective multicenter DISCOVER-FLOW (Diagnosis of Ischemia-Causing Stenoses Obtained Via Noninvasive Fractional Flow Reserve) study**. *J Am Coll Cardiol* 2011, **58**(19):1989-1997.
53. Norgaard BL, Leipsic J, Gaur S, Seneviratne S, Ko BS, Ito H, Jensen JM, Mauri L, De Bruyne B, Bezerra H *et al*: **Diagnostic performance of noninvasive fractional flow reserve derived from coronary computed tomography angiography in suspected coronary artery disease: the NXT trial (Analysis of Coronary Blood Flow Using CT Angiography: Next Steps)**. *J Am Coll Cardiol* 2014, **63**(12):1145-1155.
54. investigators S-H: **CT coronary angiography in patients with suspected angina due to coronary heart disease (SCOT-HEART): an open-label, parallel-group, multicentre trial**. *Lancet* 2015, **385**(9985):2383-2391.
55. Douglas PS, Hoffmann U, Patel MR, Mark DB, Al-Khalidi HR, Cavanaugh B, Cole J, Dolor RJ, Fordyce CB, Huang M *et al*: **Outcomes of anatomical versus functional testing for coronary artery disease**. *N Engl J Med* 2015, **372**(14):1291-1300.
56. Meijboom WB, Van Mieghem CA, van Pelt N, Weustink A, Pugliese F, Mollet NR, Boersma E, Regar E, van Geuns RJ, de Jaegere PJ *et al*: **Comprehensive assessment of coronary

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artery stenoses: computed tomography coronary angiography versus conventional coronary angiography and correlation with fractional flow reserve in patients with stable angina. *J Am Coll Cardiol* 2008, **52**(8):636-643.

57. Norgaard BL, Gormsen LC, Botker HE, Parner E, Nielsen LH, Mathiassen ON, Grove EL, Ovrehus KA, Gaur S, Leipsic J *et al*: **Myocardial Perfusion Imaging Versus Computed Tomography Angiography-Derived Fractional Flow Reserve Testing in Stable Patients With Intermediate-Range Coronary Lesions: Influence on Downstream Diagnostic Workflows and Invasive Angiography Findings**. *J Am Heart Assoc* 2017, **6**(8).
58. Sand NPR, Veien KT, Nielsen SS, Norgaard BL, Larsen P, Johansen A, Hess S, Deibjerg L, Husain M, Junker A *et al*: **Prospective Comparison of FFR Derived From Coronary CT Angiography With SPECT Perfusion Imaging in Stable Coronary Artery Disease: The ReASSESS Study**. *JACC Cardiovasc Imaging* 2018, **11**(11):1640-1650.
59. Rizvi A, Hartaigh BO, Knaapen P, Leipsic J, Shaw LJ, Andreini D, Pontone G, Raman S, Khan MA, Ridner M *et al*: **Rationale and Design of the CREDENCE Trial: computed TomogRaphic evaluation of atherosclerotic DEtermiNants of myocardial IsChEmia**. *BMC Cardiovasc Disord* 2016, **16**(1):190.
60. Vavere AL, Arbab-Zadeh A, Rochitte CE, Dewey M, Niinuma H, Gottlieb I, Clouse ME, Bush DE, Hoe JW, de Roos A *et al*: **Coronary artery stenoses: accuracy of 64-detector row CT angiography in segments with mild, moderate, or severe calcification--a subanalysis of the CORE-64 trial**. *Radiology* 2011, **261**(1):100-108.
61. Pijls NH, van Son JA, Kirkeeide RL, De Bruyne B, Gould KL: **Experimental basis of determining maximum coronary, myocardial, and collateral blood flow by pressure measurements for assessing functional stenosis severity before and after percutaneous transluminal coronary angioplasty**. *Circulation* 1993, **87**(4):1354-1367.
62. Cook CM, Jeremias A, Petraco R, Sen S, Nijjer S, Shun-Shin MJ, Ahmad Y, de Waard G, van de Hoef T, Echavarria-Pinto M *et al*: **Fractional Flow Reserve/Instantaneous Wave-Free Ratio Discordance in Angiographically Intermediate Coronary Stenoses: An Analysis Using Doppler-Derived Coronary Flow Measurements**. *JACC Cardiovasc Interv* 2017, **10**(24):2514-2524.
63. Gotberg M, Cook CM, Sen S, Nijjer S, Escaned J, Davies JE: **The Evolving Future of Instantaneous Wave-Free Ratio and Fractional Flow Reserve**. *J Am Coll Cardiol* 2017, **70**(11):1379-1402.

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Figure legends

Figure 1: Dan-NICAD 2 patient flowchart.

Numbers (n) in the figure are the estimated flow in patients. Abbreviations: CAD, coronary artery disease; CTA, coronary computed tomography angiography; FFR_{CT}: CT-derived fractional flow reserve; CMRI, cardiac magnetic resonance imaging; ^{82}Rb-PET, rubidium-82 positron emission tomography; ICA-FFR, invasive coronary angiography with fractional flow reserve; QFR, quantitative flow reserve; IMR, index of microvascular resistance; 2D QCA, two-dimensional quantitative coronary angiography.

Figure 2: Image modalities and examination set-up in Dan-NICAD 2 study.

Abbreviations: ECG, electrocardiogram; CAD, coronary artery disease; CTA, coronary computed tomography angiography; FFR_{CT}: CT-derived fractional flow reserve; CMRI, cardiac magnetic resonance imaging; ^{82}Rb-PET, rubidium-82 positron emission tomography; ICA-FFR, invasive coronary angiography with fractional flow reserve; CFR, coronary flow reserve; IMR, index of microvascular resistance, QFR, quantitative flow reserve; 2D QCA, two-dimensional quantitative coronary angiography.

Highlights

- The poor positive predictive value of coronary CT scanning facilitates unnecessary invasive angiographies. An optimized diagnostic CAD algorithm would be preferable.

- The Dan-NICAD 2 is a prospective, multicenter, cross-sectional study including 2,000 patients with low to intermediate pre-test probability of CAD. Patients are tested using a rule-out sound-based device before clinical coronary CTA. If the coronary CTA does not rule-out CAD, patients are examined with 3T CMRI, 82Rb-PET, FFRCT, ICA with FFR, CFR, IMR and QFR measurements.

- No previous study has compared head-to-head the more advanced myocardial perfusion examinations and FFRCT as second-line investigations following a positive coronary CTA with ICA-FFR as reference. Moreover, the study will analyze the differences between FFR and QFR using gold standard nuclear technique 82Rb-PET as reference.

- The results of the Dan-NICAD 2 study are expected to contribute to the improvement of diagnostic strategies for patients suspected of CAD in three different ways; 1) evaluate risk-stratification prior to coronary CTA using acoustic detection, 2) evaluate diagnostic strategies after coronary CTA and 3) evaluate invasive wireless QFR analysis as an alternative to ICA-FFR.

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Table 1: Study enrollment criteria

Criteria for inclusion
- Patients with low to intermediate pre-test probability of CAD with an indication for CCTA
- Qualified patients who have signed a written informed consent form
Criteria for exclusion
Demography and co-existing morbidity specific
- Age below 30 years
- Acute coronary syndrome and unstable angina pectoris
- Previous revascularization or known ischemic heart disease
- Patients having a heart transplantation, a mechanic heart, or mechanical heart pump.
- Patients not able to breath-hold (COPD/asthma)
CADScor®System specific
- Damaged skin in the area for application of the CADScor®Patch
- Known allergy to polyacrylate adhesives
- Significant scar tissue or bodily deformation in left IC4 (Left 4th Inter Costal region)
- Use of vasodilating agents at the same day and prior to CAD-score measurements
Scan specific
CCTA:
- Pregnant women, including women who are potentially pregnant or lactating
- Reduced kidney function, with an estimated glomerular filtration rate (eGFR) < 40 mL/min
- Allergy to X-ray contrast medium
CMRI and PET:
- Unstable CAD at CCTA.
- Contra-indication for adenosine (severe asthma, advanced AV block, or critical aorta stenosis)
- Contra-indications for MRI (implanted medicinal pumps or nerve stimulators, magnetic foreign objects in sensitive areas, i.e. the eye
- Patients having an ICD or pacemaker, a cochlea implant, or metal clips evaluated by the including physician

Study enrollment criteria in the Dan-NICAD 2 study.

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Table 2: Definitions of abnormal examinations

Blinded analysis					
CADScor®System	Coronary CTA*	FFR_{CT}	^{82}Rb PET	CMRI	ICA with FFR*
CAD-score value >20	≥50% diameter stenosis *or* Non-evaluable segments due to low examination quality	Na	Significant reduction in the isotope distribution in > 10% of the entire myocardium of the left ventricle during stress (SSS≥4 in ≥ 2 contiguous segments) *and/or* locally reduced blood flow <2 ml/g/min during adenosine stress *or* Myocardial flow reserve ≤1.8 x global rest blood flow corrected to rate-pressure product 8000 *and/or*	Significant perfusion defect, either subendocardial or transmural signal changes, in ≥ 2 contiguous segments *and/or* LGE in ≥ 2 contiguous segments *and/or* Wall motion abnormalities in ≥ 2 contiguous segments *or* Non-evaluable examination due to low examination quality	High-grade stenosis (> 90 % diameter stenosis) by visual assessment *or* ICA-FFR ≤ 0.80 in a vessel with a diameter stenosis of 30-90% *or* QCA-based diameter stenosis (≥50 % diameter) if ICA-FFR not performed due to e.g. technically not possible

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			Transient ischemic dilation ratio >1.13 + stress-EF < rest-EF *or* Non-evaluable examination due to low examination quality		
Prior knowledge analysis (Not blinded to patient data and the CCTA)					
Na	Na	$\text{FFR}_{CT} \leq 0.80^{*\Delta}$	Significant reduction in the isotope distribution in > 10% of the entire myocardium of the left ventricle during stress (SSS≥4 in ≥ 2 contiguous segments) in an area of the myocardium corresponding to coronary stenosis at CCTA *and/or* locally reduced blood flow <2 ml/g/min during adenosine stress in an area of the myocardium corresponding to coronary stenosis at CCTA *and/or* Myocardial flow reserve ≤1.8 x global rest blood flow corrected to rate-pressure product 8000 *and/or* Transient ischemic dilation ratio >1.13 + stress-EF < rest-EF + multivessel disease present at the coronary CTA *or* Non-evaluable examination due to low examination quality	Significant perfusion defect, either subendocardial or transmural signal changes, in ≥2 contiguous segments corresponding to coronary stenosis at coronary CTA *and/or* LGE in ≥ 2 contiguous segments corresponding to coronary stenosis at coronary CTA *and/or* Wall motion abnormalities in ≥ 2 contiguous segments corresponding to coronary stenosis at coronary CTA *or* Non-evaluable examination due to low examination quality	Na

Definitions of abnormal examinations in the Dan-NICAD 2 study. *In coronary vessel ≥ 2.0 mm in diameter. $^{\Delta}$still blinded to patient information. Abbreviations: coronary CTA, coronary computed tomography angiography; CT-derived fractional flow reserve, FFR_{CT}; CMRI, cardiac magnetic resonance imaging; ^{82}RbPET, 82 rubidium positron emission tomography; SSS, summed stress score;; ICA-FFR, invasive coronary angiography with fractional flow reserve measurements.; quantitative coronary angiography, QCA; 2D QCA, two-dimensional quantitative coronary angiography; LGE, late gadolinium enhancement; LVEF, left ventricle ejection fraction.

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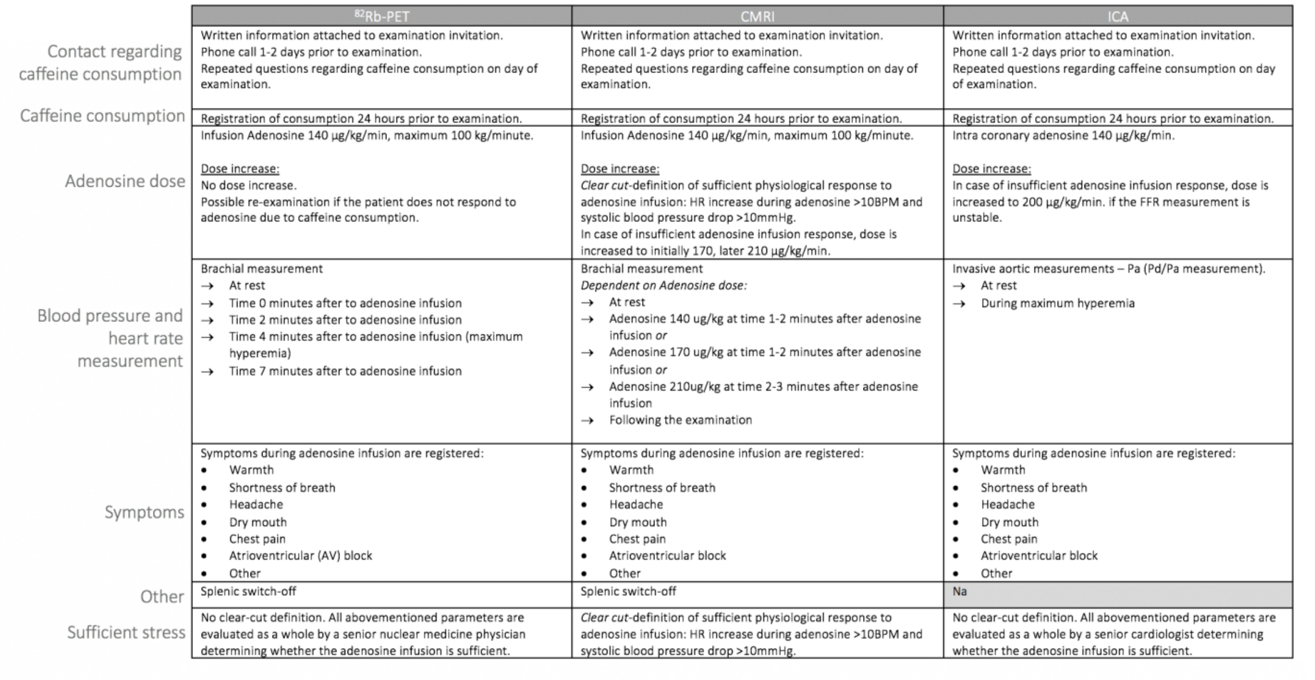
Table 3: Sufficient adenosine stress



	^{82}Rb-PET	CMRI	ICA
Contact regarding caffeine consumption	Written information attached to examination invitation. Phone call 1-2 days prior to examination. Repeated questions regarding caffeine consumption on day of examination.	Written information attached to examination invitation. Phone call 1-2 days prior to examination. Repeated questions regarding caffeine consumption on day of examination.	Written information attached to examination invitation. Phone call 1-2 days prior to examination. Repeated questions regarding caffeine consumption on day of examination.
Caffeine consumption	Registration of consumption 24 hours prior to examination.	Registration of consumption 24 hours prior to examination.	Registration of consumption 24 hours prior to examination.
Adenosine dose	Infusion Adenosine 140 µg/kg/min, maximum 100 kg/minute. Dose increase: No dose increase. Possible re-examination if the patient does not respond to adenosine due to caffeine consumption.	Infusion Adenosine 140 µg/kg/min, maximum 100 kg/minute. Dose increase: *Clear cut*-definition of sufficient physiological response to adenosine infusion: HR increase during adenosine >10BPM and systolic blood pressure drop >10mmHg. In case of insufficient adenosine infusion response, dose is increased to initially 170, later 210 µg/kg/min.	Intra coronary adenosine 140 µg/kg/min. Dose increase: In case of insufficient adenosine infusion response, dose is increased to 200 µg/kg/min. if the FFR measurement is unstable.
Blood pressure and heart rate measurement	Brachial measurement → At rest → Time 0 minutes after to adenosine infusion → Time 2 minutes after to adenosine infusion → Time 4 minutes after to adenosine infusion (maximum hyperemia) → Time 7 minutes after to adenosine infusion	Brachial measurement *Dependent on Adenosine dose:* → At rest → Adenosine 140 ug/kg at time 1-2 minutes after adenosine infusion *or* → Adenosine 170 ug/kg at time 1-2 minutes after adenosine infusion *or* → Adenosine 210ug/kg at time 2-3 minutes after adenosine infusion → Following the examination	Invasive aortic measurements – Pa (Pd/Pa measurement). → At rest → During maximum hyperemia
Symptoms	Symptoms during adenosine infusion are registered: • Warmth • Shortness of breath • Headache • Dry mouth • Chest pain • Atrioventricular (AV) block • Other	Symptoms during adenosine infusion are registered: • Warmth • Shortness of breath • Headache • Dry mouth • Chest pain • Atrioventricular block • Other	Symptoms during adenosine infusion are registered: • Warmth • Shortness of breath • Headache • Dry mouth • Chest pain • Atrioventricular block • Other
Other	Splenic switch-off	Splenic switch-off	Na
Sufficient stress	No clear-cut definition. All abovementioned parameters are evaluated as a whole by a senior nuclear medicine physician determining whether the adenosine infusion is sufficient.	*Clear cut*-definition of sufficient physiological response to adenosine infusion: HR increase during adenosine >10BPM and systolic blood pressure drop >10mmHg.	No clear-cut definition. All abovementioned parameters are evaluated as a whole by a senior cardiologist determining whether the adenosine infusion is sufficient.

Criteria for and initiatives to sufficient adenosine stress in the Dan-NICAD 2 study. Abbreviations: PET, positron emission tomography; CMRI, cardiac magnetic resonance imaging; ICA-FFR, invasive coronary angiography with fractional flow reserve measurements.

Addendum

Data collected during the project and registered in a dedicated eCRF:

- Demography – age, sex, ethical origin and pregnancy test.
- Co-morbidity.
- Risk factors for ischemic heart disease—genetic disposition, smoking status, diabetes mellitus, hypertension, hypercholesterolemia, blood pressure, heart rate, weight, height, hip to waist ratio and electrocardiogram (ECG).
- Symptoms—typical, atypical, or unspecific chest pain, New York Heart Association (NYHA) functional class, and Canadian Cardiovascular Society (CCS) score of angina pectoris.
- Seattle Angina Questionnaire at baseline, and 3 and 12 months post inclusion.
- Biochemistry – Cholesterol, glucose levels and creatinine.
- Protein measurements on serum and plasma.

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- Blood sample test results from the biobank
- Echocardiography—left ventricle ejection fraction (LVEF) and significant valve disease.
- CAD score—registration of the CAD-score and recording time.
- CCTA—scan quality, Agatston calcium score, anatomy, plaque type, degree of stenosis and radiation exposure.
- ^{82}Rb-PET and CMRI—Scan quality, function, response to the injection of adenosine and presence of perfusion defects. Regional and segmental MPR values.
- ICA-FFR—data concerning anatomy, localization of stenosis, visual evaluation of stenosis, QCA and QFR measurements, FFR, IMR and CFR measurements, and whether treatment by PCI or CABG is performed.
- Adverse Events (AE)—all adverse events occurring in the study period are registered.
- Follow-up 10 years —mortality, myocardial infarction, revascularization, comorbidity, medicinal receipts.

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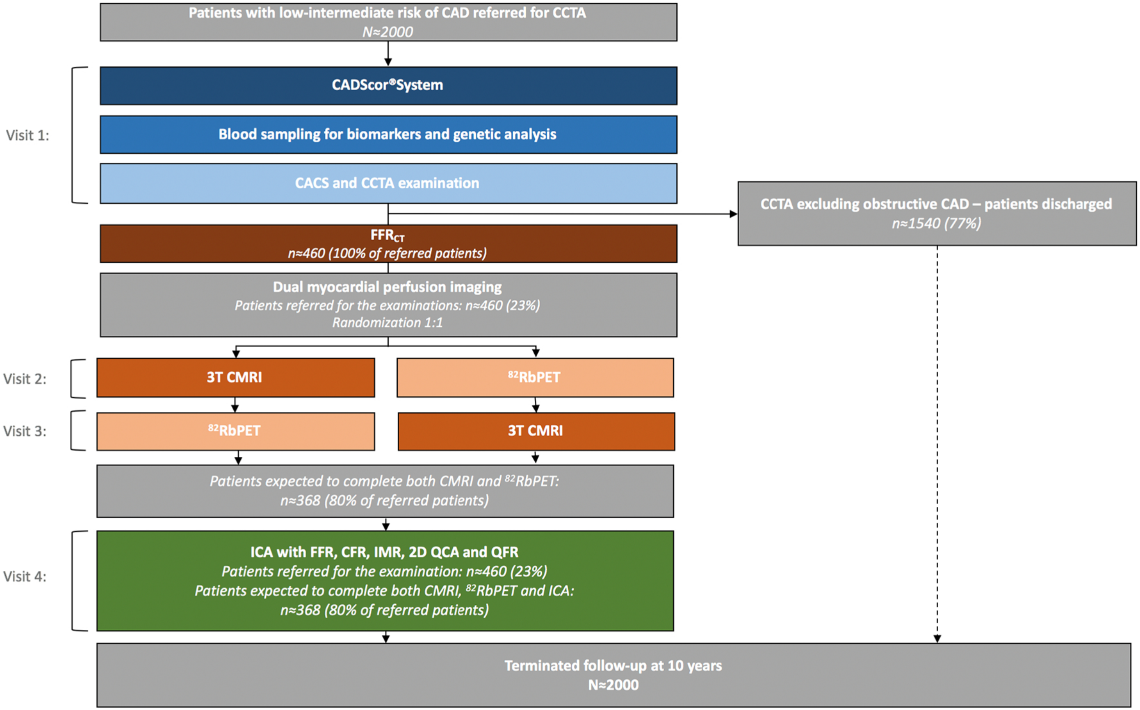
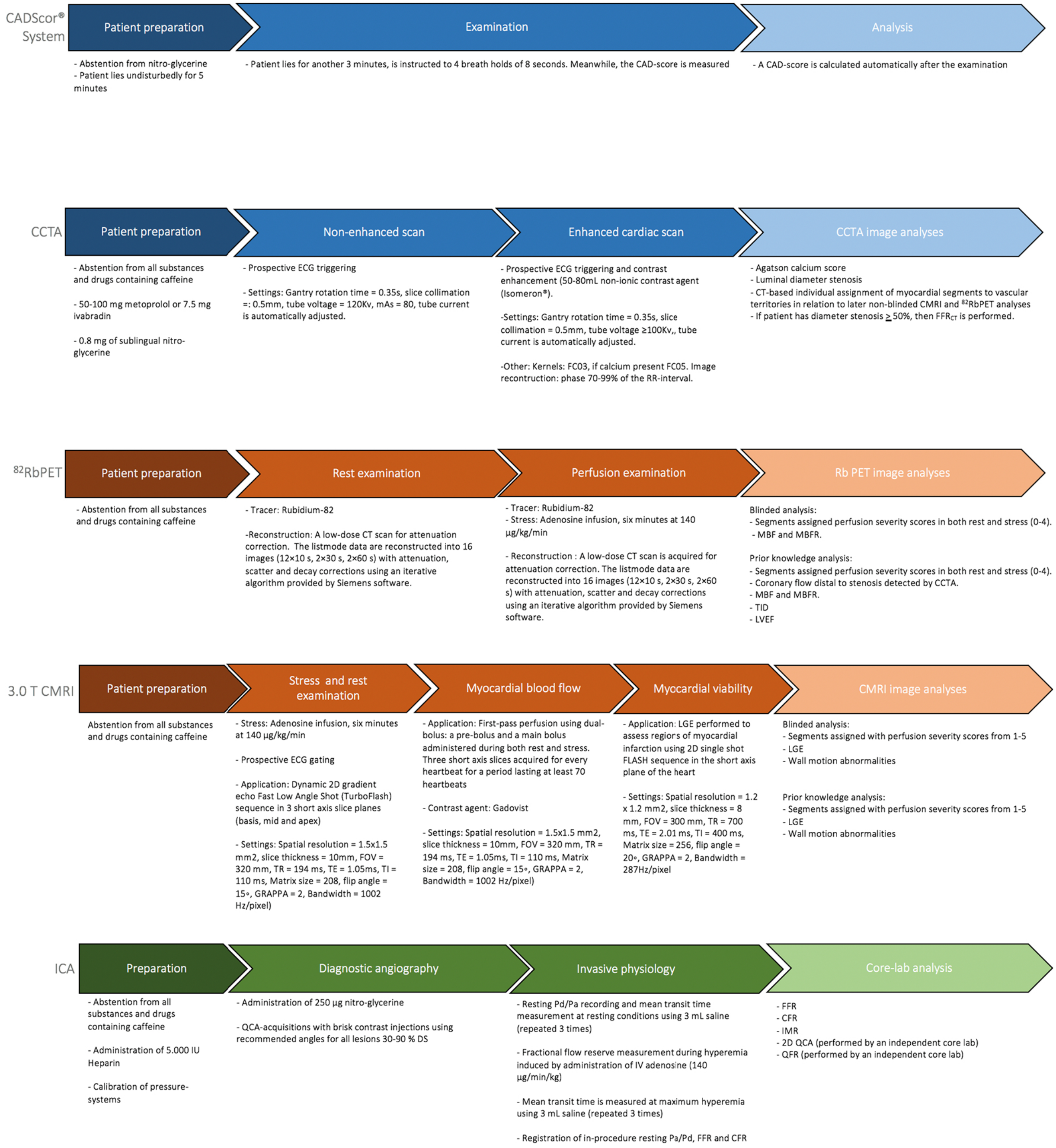

Patients with low-intermediate risk of CAD referred for CCTA
N≈2000
Visit 1:
CADScor®System
Blood sampling for biomarkers and genetic analysis
CACS and CCTA examination
CCTA excluding obstructive CAD – patients discharged
n≈1540 (77%)
FFR$_{CT}$
n≈460 (100% of referred patients)
Dual myocardial perfusion imaging
Patients referred for the examinations: n≈460 (23%)
Randomization 1:1
Visit 2:
3T CMRI
^{82}RbPET
Visit 3:
^{82}RbPET
3T CMRI
Patients expected to complete both CMRI and ^{82}RbPET:
n≈368 (80% of referred patients)
Visit 4:
ICA with FFR, CFR, IMR, 2D QCA and QFR
Patients referred for the examination: n≈460 (23%)
Patients expected to complete both CMRI, ^{82}RbPET and ICA:
n≈368 (80% of referred patients)
Terminated follow-up at 10 years
N≈2000

Figure 1



CADScor® System

Patient preparation
- Abstention from nitro-glycerine
- Patient lies undisturbedly for 5 minutes

Examination
- Patient lies for another 3 minutes, is instructed to 4 breath holds of 8 seconds. Meanwhile, the CAD-score is measured

Analysis
- A CAD-score is calculated automatically after the examination

CCTA

Patient preparation
- Abstention from all substances and drugs containing caffeine

- 50-100 mg metoprolol or 7.5 mg ivabradin

- 0.8 mg of sublingual nitro-glycerine

Non-enhanced scan
- Prospective ECG triggering

- Settings: Gantry rotation time = 0.35s, slice collimation =: 0.5mm, tube voltage = 120Kv, mAs = 80, tube current is automatically adjusted.

Enhanced cardiac scan
- Prospective ECG triggering and contrast enhancement (50-80mL non-ionic contrast agent (Isomeron®).

-Settings: Gantry rotation time = 0.35s, slice collimation = 0.5mm, tube voltage ≥100Kv,, tube current is automatically adjusted.

-Other: Kernels: FC03, if calcium present FC05. Image recontruction: phase 70-99% of the RR-interval.

CCTA image analyses
- Agatson calcium score
- Luminal diameter stenosis
- CT-based individual assignment of myocardial segments to vascular territories in relation to later non-blinded CMRI and ^{82}RbPET analyses
- If patient has diameter stenosis $\geq$ 50%, then FFR$_{CT}$ is performed.

^{82}RbPET

Patient preparation
- Abstention from all substances and drugs containing caffeine

Rest examination
- Tracer: Rubidium-82

-Reconstruction: A low-dose CT scan for attenuation correction. The listmode data are reconstructed into 16 images (12×10 s, 2×30 s, 2×60 s) with attenuation, scatter and decay corrections using an iterative algorithm provided by Siemens software.

Perfusion examination
- Tracer: Rubidium-82
- Stress: Adenosine infusion, six minutes at 140 µg/kg/min

- Reconstruction : A low-dose CT scan is acquired for attenuation correction. The listmode data are reconstructed into 16 images (12×10 s, 2×30 s, 2×60 s) with attenuation, scatter and decay corrections using an iterative algorithm provided by Siemens software.

Rb PET image analyses

Blinded analysis:
- Segments assigned perfusion severity scores in both rest and stress (0-4).
- MBF and MBFR.

Prior knowledge analysis:
- Segments assigned perfusion severity scores in both rest and stress (0-4).
- Coronary flow distal to stenosis detected by CCTA.
- MBF and MBFR.
- TID
- LVEF

3.0 T CMRI

Patient preparation

Abstention from all substances and drugs containing caffeine

Stress and rest examination
- Stress: Adenosine infusion, six minutes at 140 µg/kg/min

- Prospective ECG gating

- Application: Dynamic 2D gradient echo Fast Low Angle Shot (TurboFlash) sequence in 3 short axis slice planes (basis, mid and apex)

- Settings: Spatial resolution = 1.5x1.5 mm2, slice thickness = 10mm, FOV = 320 mm, TR = 194 ms, TE = 1.05ms, TI = 110 ms, Matrix size = 208, flip angle = 15◦, GRAPPA = 2, Bandwidth = 1002 Hz/pixel)

Myocardial blood flow
- Application: First-pass perfusion using dual-bolus: a pre-bolus and a main bolus administered during both rest and stress. Three short axis slices acquired for every heartbeat for a period lasting at least 70 heartbeats

- Contrast agent: Gadovist

- Settings: Spatial resolution = 1.5x1.5 mm2, slice thickness = 10mm, FOV = 320 mm, TR = 194 ms, TE = 1.05ms, TI = 110 ms, Matrix size = 208, flip angle = 15◦, GRAPPA = 2, Bandwidth = 1002 Hz/pixel)

Myocardial viability
- Application: LGE performed to assess regions of myocardial infarction using 2D single shot FLASH sequence in the short axis plane of the heart

- Settings: Spatial resolution = 1.2 x 1.2 mm2, slice thickness = 8 mm, FOV = 300 mm, TR = 700 ms, TE = 2.01 ms, TI = 400 ms, Matrix size = 256, flip angle = 20◦, GRAPPA = 2, Bandwidth = 287Hz/pixel

CMRI image analyses

Blinded analysis:
- Segments assigned with perfusion severity scores from 1-5
- LGE
- Wall motion abnormalities

Prior knowledge analysis:
- Segments assigned with perfusion severity scores from 1-5
- LGE
- Wall motion abnormalities

ICA

Preparation
- Abstention from all substances and drugs containing caffeine

- Administration of 5.000 IU Heparin

- Calibration of pressure-systems

Diagnostic angiography
- Administration of 250 µg nitro-glycerine

- QCA-acquisitions with brisk contrast injections using recommended angles for all lesions 30-90 % DS

Invasive physiology
- Resting Pd/Pa recording and mean transit time measurement at resting conditions using 3 mL saline (repeated 3 times)

- Fractional flow reserve measurement during hyperemia induced by administration of IV adenosine (140 µg/min/kg)

- Mean transit time is measured at maximum hyperemia using 3 mL saline (repeated 3 times)

- Registration of in-procedure resting Pa/Pd, FFR and CFR

Core-lab analysis
- FFR
- CFR
- IMR
- 2D QCA (performed by an independent core lab)
- QFR (performed by an independent core lab)

Figure 2