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

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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 (ICAs) 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 3 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 3T 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 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 coronary CTA because of symptoms suggestive of CAD, as evaluated by a cardiologist. Patient interviews, sound recordings, and blood

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Authors' contributions: L. D. R., S. W., and M. B. participated in the conception and design of the study. S. E. S. is responsible for the CADScor®System algorithm. C. I. obtains the CMRI scans. L. .B aids with the CMRI scans. C. I., A. C., and S. E. P. analyze the CMRI scans. J. A. E. obtains the 82Rb-PET scans. J. A. E. and L. G. analyze the 82Rb-PET scans. L. H. M. and L. L. K. enroll participants and analyze the CTA scans at the Hospital Unit West, Herning. L. F. coordinates the study at Silkeborg Regional Hospital. J. K. and G. U. enroll patients and analyze the CTA scans at the Silkeborg Regional Hospital. H. M. S. enrolls patients and analyzes the CTA scans at the Viborg Regional Hospital. O. H. enrolls patients and analyzes the CTA scans at the Randers Regional Hospital. N. R. H. and J. W. carry out the study regarding QFR. A. E., E. H. C., and H. E. B. conduct the ICA and FFR measurements at the Department of Cardiology, Aarhus University Hospital. M. N. 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.

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samples are obtained in connection with the coronary CTA. If coronary CTA does not rule out obstructive CAD, patients will be examined by 3T CMRI ^{82}Rb-PET, FFR_{CT}, ICA, and FFR. Reference standard is ICA-FFR. Obstructive CAD is defined as an FFR ≤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 3 different steps: risk stratification prior to coronary CTA, diagnostic strategy after coronary CTA, and invasive wireless QFR analysis as an alternative to ICA-FFR. (Am Heart J 2019;215:114-28.)

Background

An increasing number of patients are referred for evaluation of suspected obstructive coronary artery disease (CAD). Coronary computed tomographic angiography (CTA) is currently recommended by the National Institute for Health and Care Excellence 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 implies 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 detection of coronary stenosis from automatically recorded and analyzed heart sounds is a newly developed technology potentially useful for pre-test probability stratification before, for example, 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.

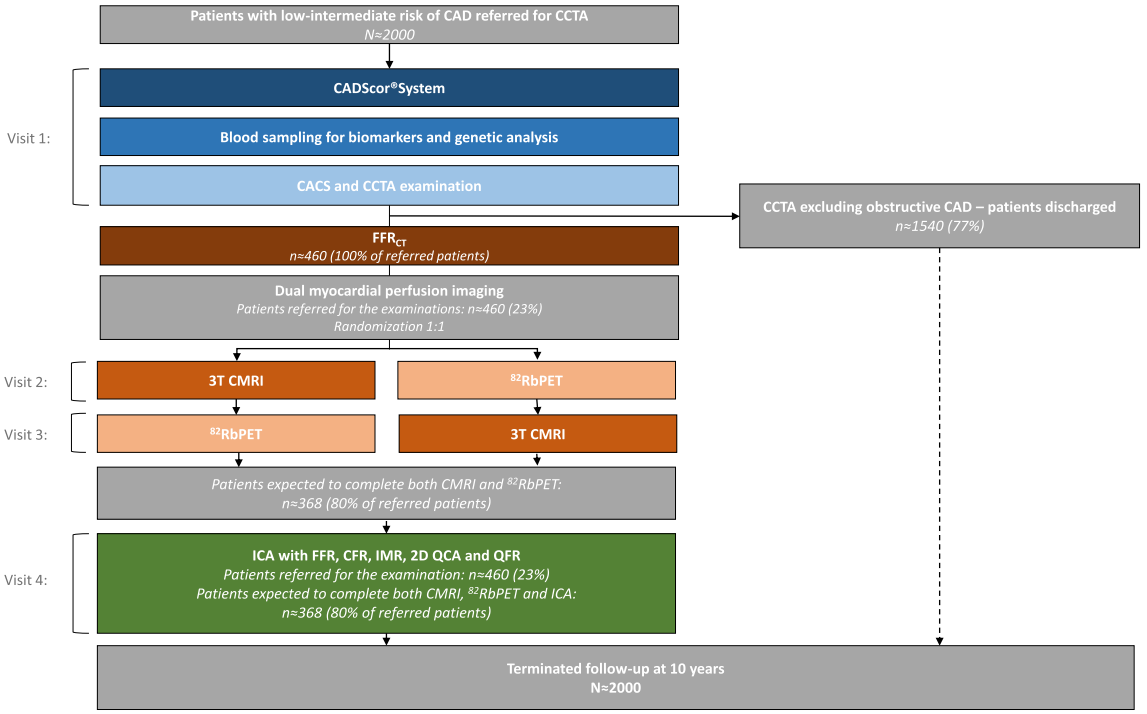
In patients with suspicion of coronary stenosis detected by coronary CTA, current guidelines recommend performing functional tests to verify the presence of inducible myocardial ischemia to reduce unnecessary ICAs.[10] Diagnostic accuracy of secondary non-invasive imaging with single photon emission computed tomography (SPECT) and 1.5-Tesla cardiac magnetic resonance imaging (CMRI) was 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 use 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 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 (quantitative coronary angiography [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

Figure 1



Dan-NICAD 2 patient flowchart.Numbers (n) in the figure are the estimated flow in patients.

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.

Method

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 and blood sampled for a biobank, and finally will undergo a nonenhanced 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 core laboratory following an abnormal coronary CTA. ICA and invasive measurements will be conducted at Aarhus University Hospital.

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 regard 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 tests. 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

Table I. 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 coexisting morbidity specific
- Age below 30 y
- 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 intercostal 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
- Contraindication for adenosine (severe asthma, advanced AV block, or critical aorta stenosis)
- Contraindications for MRI (implanted medicinal pumps or nerve stimulators, magnetic foreign objects in sensitive areas, ie, 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.

CTA, but not ICA, results. Results are recalculated with information on coronary CTA findings and symptoms to mimic the clinical situation. The unblinded procedures are secondary investigations.

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 I. All enrolled patients are systematically interviewed and undergo an acoustic CAD score examination alongside blood sampling stored in a biobank and coronary CTA.

Biobank

From all participants consenting to the study, 5 blood samples are drawn prior to coronary CTA contrast administration. Within 2 hours, 3 of the samples are centrifuged and processed into EDTA plasma, heparin plasma, and serum, which are aliquoted into individual matrix tubes and placed at −20°C. Two 4-mL 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 −80°C. 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 subphenotypes recorded in the trial.

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 fourth intercostal space just left to the sternum using a dedicated patch. The CAD-score examination is performed in accordance with the manufacturers' 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 II).

Imaging

Cardiac CTA

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, 50-100 mg atenolol, or 7.5-15 mg ivabradin the night before and 2 hours prior to coronary CTA to reduce the heart rate to <65 beat/min. If not contraindicated, patients with persistent heart rate >65 beat/min 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: coronary 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

Figure 2

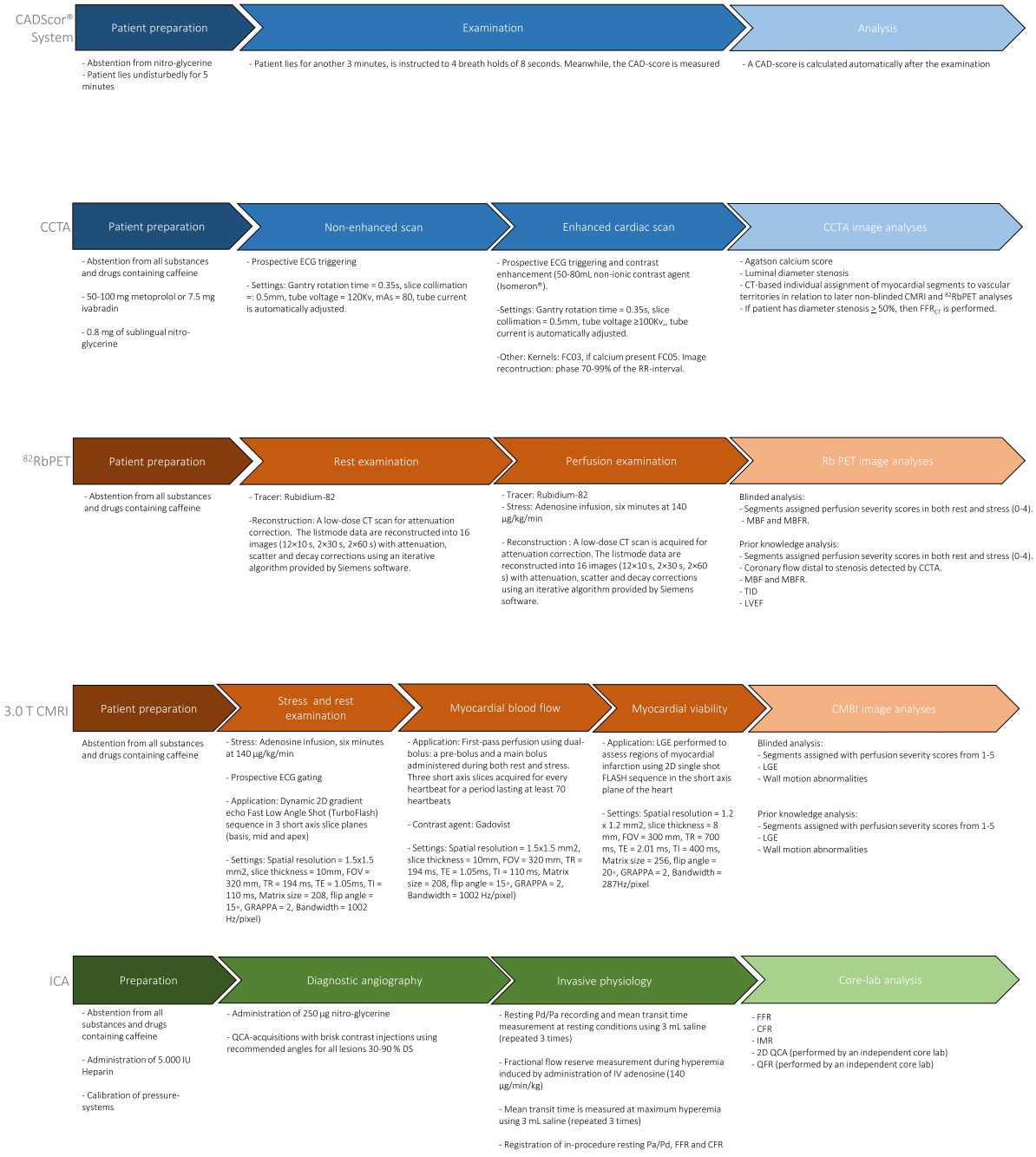


Image modalities and examination set-up in Dan-NICAD 2 study.

examination and (2) a contrast-enhanced cardiac 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, or Syngo.Via, Siemens Healthcare, Erlangen, Germany).

Imaging analyses: coronary CTA. All coronary CTA analyses are performed by an experienced cardiologist. An Agatson calcium score is initially calculated using dedicated

Table II. 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 × global rest blood flow corrected to rate-pressure product 8000 *and/or* Transient ischemic dilation ratio >1.13 + stress-EF < rest-EF *or* Nonevaluable examination due to low examination quality	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* Nonevaluable 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, eg, technically not possible
Prior knowledge analysis (not blinded to patient data and the CCTA)					
Na	Na	FFR_{CT} ≤ 0.80*†	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 × 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* Nonevaluable 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* Nonevaluable 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.
† Still blinded to patient information.

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 is classified as follows: no stenosis—0% diameter reduction (≈0% area reduction); mild stenosis—1%-29% diameter reduction (≈1%-50% area reduction); moderate

Table III. Sufficient adenosine stress

	^{82}Rb-PET	CMRI	ICA
Contact regarding caffeine consumption	Written information attached to examination invitation. Phone call 1-2 d prior to examination. Repeated questions regarding caffeine consumption on day of examination.	Written information attached to examination invitation. Phone call 1-2 d prior to examination. Repeated questions regarding caffeine consumption on day of examination.	Written information attached to examination invitation. Phone call 1-2 d prior to examination. Repeated questions regarding caffeine consumption on day of examination.
Caffeine consumption	Registration of consumption 24 h prior to examination.	Registration of consumption 24 h prior to examination.	Registration of consumption 24 h prior to examination.
Adenosine dose	Infusion adenosine 140 μg/(kg min), maximum 100 kg/min. 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/mine. Dose increase: *Clear-cut* definition of sufficient physiological response to adenosine infusion: HR increase during adenosine >10 beat/min and systolic blood pressure drop >10 mm Hg. In case of insufficient adenosine infusion response, dose is increased to initially 170, later 210 μg/(kg min).	Intracoronary 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 min after to adenosine infusion → Time 2 min after to adenosine infusion → Time 4 min after to adenosine infusion (maximum hyperemia) → Time 7 min after to adenosine infusion	Brachial measurement *Dependent on adenosine dose:* → At rest → Adenosine 140 μg/kg at time 1-2 min after adenosine infusion *or* → Adenosine 170 μg/kg at time 1-2 min after adenosine infusion *or* → Adenosine 210 μg/kg at time 2-3 min 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 >10 beat/min and systolic blood pressure drop >10 mm Hg.	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.

stenosis—30%-49 % diameter reduction (≈50%-69% area reduction); and severe stenosis—50%-100% diameter reduction (≈70%-100 % area reduction). The criteria for an abnormal coronary CTA are shown in Table II.

FFR_{CT} is performed using dedicated software. FFR_{CT} values are determined 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 III.

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 are obtained in list mode with a Siemens Biograph mCT/64 PET-scanner (Siemens Healthcare, Knoxville,

TN). The participants undergo 2 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 III.

Imaging analysis: PET. Imaging analyses are performed by an independent core laboratory 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, CA).[21]

The quality of the stress and rest images is evaluated semiquantitatively 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 are 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) a SDS $\geq$ 4 involving $\geq$ 2 contiguous segments (reversible ischemia) and $\geq$ 1 segment with a stress severity score $\geq$ 2; (2) a SRS $\geq$ 4 involving $\geq$ 2 contiguous segments and $\geq$ 1 segment with a stress severity score $\geq$ 2 (irreversible ischemia); (3) an SSS $\geq$ 4 involving $\geq$ 2 contiguous segments and $\geq$ 1 segment with a stress severity score $\geq$ 2 (combination of reversible and irreversible ischemia, mixed ischemia); or (4) poor image quality (score 3, nondiagnostic). 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) are 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* +defined as MBF during maximal hyperemia divided by MBF at rest will be reported for the 3 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 reevaluated 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 III.

Cardiac magnetic resonance imaging

Patient preparation: 3T 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: 3T 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® Veris® monitoring system (Bayer Healthcare LCC, USA). All sequences are ECG gated, whereas 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 2-dimensional (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 I. 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 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: 3T CMRI. CMRI analyses are carried out in an independent core laboratory blinded for additional patient information and results. Image quality regarding artifacts and image homogeneity are scored qualitatively using a scale from 1 to 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/nondiagnostic image quality).

Several volume measurements are carried out including left ventricular end-diastolic volume, left ventricular end-systolic volume, left ventricular stroke volume, left ventricular ejection fraction (LVEF), and left ventricular mass.

Regional wall motion abnormalities will be scored using a scale from 1 to 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 5 dynamic cardiac cycles in ≥2 contiguous segments compared with normal remote myocardium. Each AHA segment will be subdivided 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 III.

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

Invasive coronary angiography and invasive physiological examination: ICA, FFR, CFR, and 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 intracoronary 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 >2 mm are considered for physiological assessment. Angiographic acquisitions are performed at 15 frames per second in at least 2 projections more than 25° apart allowing for 2D QCA and QFR analysis. Coronary artery overlap, foreshortening, zooming, and planning are avoided if possible. All vessels are visualized in their full length if possible.

Patient preparation for physiological examination. Anticoagulation (5,000 IU heparin) is administrated prior the pressure measurement. The pressure-wire (PressureWire X Guidewire, Abbott, Chicago, IL) and CoroFlow systems are used according to manufacturer 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.

FFR, CFR, and IMR. Hyperemia is induced using a 1-mg/mL concentration of adenosine at 140 μg/(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, 3 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.

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

Image analysis: ICA. All physiologic core laboratory 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 core laboratory (Institute of Clinical Medicine, Aarhus University, Denmark). 2D-QCA is performed in an independent core laboratory (ClinFact, Leiden, the Netherlands).

The criteria for an abnormal ICA are shown in Table II.

Quantitative flow ratio analysis. QFR and 3D-QCA core laboratory analyses are performed in a core laboratory setting (Aarhus University Hospital, Skejby, Denmark) using the latest version of the software (QAngio

XA 3D, Medis medical imaging system, 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 cutoff value.

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 and the National Patient Registry. 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 the 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.

Data collection and recordings

All study data are recorded in a secure Web-based electronic case record form (eCRF) (Research Electronic Data Capture[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 core laboratory analyses have limited access in regard of blinding procedures. Data collected and registered in the dedicated eCRF are listed in the Addendum. The study is monitored according to ICH Harmonized Tripartite Guideline for Good Clinical Practice.

End points and statistical analysis

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

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 (ROC-AUC) 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 (1) obstructive CAD: ≥50% diameter stenosis by ICA-2D-QCA or (2) 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, and negative predicative value where the CAD-scores are dichotomized 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.

Analysis of value of noninvasive 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, and positive and negative predictive value is tested using McNemar 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; and (4) the diagnostic precision of CMRI dyssynchrony analysis of coronary stenoses.

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 cutpoint 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 versus mismatched FFR and QFR, (2) agreement of the 2 groups with ^{82}Rb-PET and separately with 3T CMRI, and (3) stenosis morphology as assessed with 2D and 3D-QCA compared for the 2 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 2-sided P value < .05 is considered statistically significant, and 95 % CIs are

reported when appropriate. Statistical analysis is performed using dedicated statistical software (STATA, StataCorp, College Station, TX).

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 and ^{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 (1) the AUC for CAD score is 0.72 and the AUC for the Diamond-Forrester score is 0.66, (2) the covariance between the CAD score and the Diamond-Forrester score is 0.46, and (3) the CAD prevalence is 10%. Based on these assumptions a minimum of 157 CAD patients and 1,413 patients without CAD are to be included in the analysis for the primary CAD score objective.

QFR has not previously been compared to noninvasive 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 2-sided CI is used. With an expected dropout rate of 0.20, $\alpha = .05$, 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.

Ethical considerations

The study follows the principles outlined in the Declaration of Helsinki and ISO 14155:2011. The study's additional radiation exposure in regard of the ^{82}Rb-PET examination increases the cumulated risk over a lifetime of dying from cancer from approximately 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).

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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Discussion

Dan-NICAD 2 is a multipurpose study (1) assessing the diagnostic performance of the CADScor®System, (2) assessing 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, for example, ICA, where patients are not revascularized. The need for more specific strategies for noninvasive diagnostic workup 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 noninvasive 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 aims 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] Furthermore, 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 DANNICAD 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 toward a lower prevalence in populations undergoing noninvasive 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 are a limitation in the current study. However, the impact of this potential workup bias is considered low due to the high sensitivity of the coronary CTA.

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 and 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 compared 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 noninvasive, nonradioactive, 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 have primarily been conducted with ICA diameter stenosis as reference standard.[5,45-48] However, using ICA-FFR as reference standard, a recent meta-analysis found CMRI having an average sensitivity of 90% and a specificity of 87%.[49] This meta-analysis, however, only included 3 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 noninvasive 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 on head-to-head comparison of FFR$_{CT}$ and advanced myocardial perfusion examinations are lacking. In a 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]

The National Institute for Health and Care Excellence 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 nondiagnostic coronary CTA have intermediate coronary stenoses.[3,60] Hence, a population based on nondiagnostic coronary CTA has 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 are 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 CFR measurements, whereas 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.

Furthermore, the global uptake of FFR-guided revascularization remains low.[63] This has led to the development of 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 or natural variation or if one test may be superior to the other.

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 3 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 status

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

Addendum

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

- Demography—age, sex, ethical origin, and pregnancy test.
- Comorbidity.
- 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 ECG.
- Symptoms—typical, atypical, or unspecific chest pain; New York Heart Association functional class; and Canadian Cardiovascular Society score of angina pectoris.
- Seattle Angina Questionnaire at baseline and 3 and 12 months postinclusion.
- Biochemistry—cholesterol, glucose levels, and creatinine.
- Protein measurements on serum and plasma.
- Blood sample test results from the biobank
- Echocardiography—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—All adverse events occurring in the study period are registered.
- Follow-up 10 years—mortality, myocardial infarction, revascularization, comorbidity, and medicinal receipts.

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