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Danish study of Non-Invasive Testing in Coronary Artery Disease 3 (Dan-NICAD 3)

study design of a controlled study on optimal diagnostic strategy

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Danish study of Non-Invasive Testing in Coronary Artery Disease 3 (Dan-NICAD 3): study design of a controlled study on optimal diagnostic strategy

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ABSTRACT

Introduction Current guideline recommend functional imaging for myocardial ischaemia if coronary CT angiography (CTA) has shown coronary artery disease (CAD) of uncertain functional significance. However, diagnostic accuracy of selective myocardial perfusion imaging after coronary CTA is currently unclear. The Danish study of Non-Invasive testing in Coronary Artery Disease 3 trial is designed to evaluate head to head the diagnostic accuracy of myocardial perfusion imaging with positron emission tomography (PET) using the tracers ^{82}Rubidium (^{82}Rb-PET) compared with oxygen-15 labelled water PET (^{15}O-water-PET) in patients with symptoms of obstructive CAD and a coronary CT scan with suspected obstructive CAD.

Methods and analysis This prospective, multicentre, cross-sectional study will include approximately 1000 symptomatic patients without previous CAD. Patients are included after referral to coronary CTA. All patients undergo a structured interview and blood is sampled for genetic and proteomic analysis and a coronary CTA. Patients with possible obstructive CAD at coronary CTA are examined with both ^{82}Rb-PET, ^{15}O-water-PET and invasive coronary angiography with three-vessel fractional flow reserve and thermodilution measurements of coronary flow reserve. After enrolment, patients are followed with Seattle Angina Questionnaires and follow-up PET scans in patients with an initially abnormal PET scan and for cardiovascular events in 10 years.

Ethics and dissemination Ethical approval was obtained from Danish regional committee on health research ethics. Written informed consent will be provided by all study participants. Results of this study will be disseminated via articles in international peer-reviewed journal.

Trial registration number NCT04707859.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The optimal algorithm for diagnosing coronary artery disease (CAD) is uncertain.
⇒ Functional imaging for diagnosing myocardial ischaemia is recommended if coronary CT angiography (CTA) has shown CAD but diagnostic performance is not investigated.

WHAT THIS STUDY ADDS

⇒ Head-to-head evaluation of the diagnostic performance of ^{82}rubidium-positron emission tomography (PET) compared with ^{15}O-water-PET.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The results are expected to add knowledge about diagnostic strategies after coronary CTA.

BACKGROUND

Approximately 1% of all contacts to general practitioners are related to chest discomfort.[1] Consequently, millions of diagnostic tests are performed worldwide to diagnose obstructive coronary artery disease (CAD) despite an overall low pretest probability of disease in these patients.[2 3] Coronary CT angiography (CTA) is an excellent test to rule out obstructive atherosclerotic CAD due to a very high negative predictive value and is recommended by the European and American guidelines as the initial diagnostic test to exclude obstructive CAD in the majority of patients with de novo suspicion of CAD.[4–6] However, due to the low positive predictive value of coronary CTA, current guidelines propose a selective myocardial perfusion imaging strategy after inconclusive/abnormal coronary CTA to non-invasively rule-in obstructive CAD.[4 5] The aim of using the selective myocardial perfusion imaging

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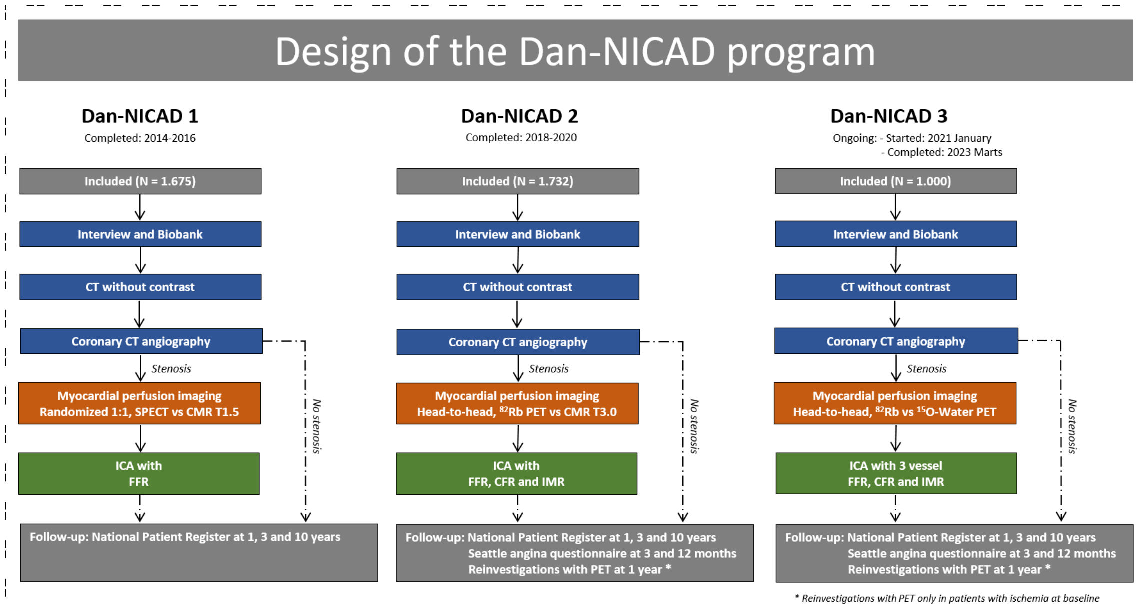

Figure 1 Design of the Dan-NICAD trial. Illustration of the three Dan-NICAD studies. The design is similar but the studies distinguishable with more advanced myocardial perfusion modalities, invasive investigation and follow-up in the later Dan-NICAD studies. CAD, coronary artery disease; CMR, cardiac MR; Dan-NICAD, Danish study of Non-Invasive testing in Coronary Artery Disease; FFR, fractional flow reserve; ICA, invasive coronary angiography; IMR, Index of Microvascular Resistance; ^{15}O-water-PET, Oxygen-15 labelled water positron emission tomography, ^{82}Rb-PET, rubidium-82 labelled positron emission tomography; SPECT, single-photon emission CT.

is to reduce the rates of unnecessary invasive coronary angiographies (ICAs) and guide potential revascularisation. Very few studies, however, have evaluated the clinical utility of a selective strategy of myocardial perfusion imaging in patients with suspected obstructive CAD at coronary CTA which is highlighted as a gap in evidence by the 2019 European Society of Cardiology (ESC) guidelines on chronic coronary syndrome.[4]

The Danish study of Non-Invasive testing in Coronary Artery Disease (Dan-NICAD) trial programme started in 2014 and aims to study the optimal individualised diagnostic strategy for diagnosing obstructive CAD (figure 1). In the Dan-NICAD programme, coronary CTA is the first-line diagnostic test for all patients with de novo suspicion of CAD. In patients where coronary CTA does not exclude obstructive CAD, the diagnostic accuracy of myocardial perfusion imaging tests has been investigated with ICA with fractional flow reserve (FFR) as reference. Hence, from 2014 to 2016, the Dan-NICAD 1 trial compared the diagnostic accuracy of single-photon emission CT (SPECT) versus 1.5 Tesla cardiac MRI using a randomised (1:1), controlled, open-labelled design.[7 8] Subsequently, from 2018 to 2020, the Dan-NICAD 2 trial compared the diagnostic accuracy of ^{82}rubidium positron emission tomography (^{82}Rb-PET) versus 3 Tesla cardiac MRI using a head-to-head comparison design.[9 10] Similarly, the present Dan-NICAD 3 study compares the diagnostic accuracy of ^{82}Rb-PET versus oxygen-15 labelled water PET (^{15}O-water-PET) using a head-to-head design. For both PET tracers applied in the Dan-NICAD 3 study, previous investigations have demonstrated high diagnostic performances in symptomatic patients with high

Box 1 Study enrolment criteria

Criteria for inclusion

⇒ Patients referred to coronary CTA due to symptoms suggestive of CAD.
⇒ Qualified patients who have signed a written informed consent form.

Criteria for exclusion

Demography and comorbidity
⇒ Age below 30 years—acute coronary syndrome or unstable angina pectoris.
⇒ Previous revascularisation or known ischaemic heart disease—patients having undergone a heart transplantation, or having a mechanic heart, or mechanical heart pump.
⇒ Patients not able to sufficient breath-hold (COPD/asthma).

Scan-specific exclusion criteria
Coronary CTA
⇒ Pregnant women, including women who are potentially pregnant or lactating. Reduced kidney function, with an estimated glomerular filtration rate <40 mL/min. Allergy to X-ray contrast medium.

PET
⇒ Very severe symptoms or critical 3 vessel or left main stem CAD at coronary CTA evaluated at the site reading.
⇒ Contraindication for adenosine (severe asthma, advanced AV block or critical aorta stenosis).

Study enrolment criteria in the Dan-NICAD 3 study.
AV, atrioventricular; CAD, coronary artery disease; COPD, chronic obstrucive pulmonary disease; CTA, CT angiography; PET, positron emission tomography.

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pretest probability of obstructive CAD.[11] However, no previous study has compared the diagnostic performance of the two PET tracers in a head-to-head design, and none of the tracers has been compared as part of a selective MPI strategy following a coronary CTA with suspected obstructive CAD. Hence, it is unknown whether the theoretical advantages of ^{15}O-water compared with ^{82}Rb is transformed into an increased diagnostic accuracy which may alter patient management.

Coronary CTA is an anatomy-based examination. In contrast, computation of FFR or Murray law-based quantitative flow ratio (uQFR) using specialised software from either coronary CTA or ICA datasets, respectively, are alternative methods to estimate the functional severity of coronary stenosis. CT-derived FFR (FFR-CT) is based on routinely acquired coronary CTA images from which FFR is estimated. Thus, FFR-CT is an alternative non-invasive strategy compared with selective MPI testing after coronary CTA. Similar to FFR-CT, uQFR is a wireless method based on one standard ICA image from which FFR is estimated. Thus, uQFR is an alternative to ICA-FFR aiming to reduce the use of intracoronary pressure wires. Both FFR-CT and uQFR have shown good agreement to ICA-FFR but large-scale studies comparing the clinical utility and prognostic value in head-to-head comparison with other diagnostic techniques are sparse uQFR.[12–15]

The aim of the Dan-NICAD 3 study is to (1) compare the diagnostic accuracy of ^{82}Rb-PET and ^{15}O-water-PET in patients with suspected obstructive CAD at coronary CTA with a reference of haemodynamically and anatomically obstructive stenosis based on ICA-FFR and ICA quantitative coronary angiography (QCA); (2) to evaluate the diagnostic accuracy of FFR-CT and uQFR for haemodynamically obstructive CAD identification and (3) to evaluate the prognostic value of all the diagnostic techniques by pooling all patients from the Dan-NICAD programme.

METHOD

Study design and cohort

This Dan-NICAD 3 study is an investigator-initiated, prospective, multicentre study conducted at hospitals in the Central Denmark Region. The study will include approximately 1000 patients without known CAD who are referred for diagnostic testing with coronary CTA due to symptoms suggestive of obstructive CAD as evaluated in an outpatient clinic. Patients are included on the day of the coronary CTA. The cohort will predominantly consist of patients with low/intermediate pretest probability of CAD according to current guideline recommendations.[4] Inclusion and exclusion criteria are listed in box 1.

All patients undergo a structured interview performed by dedicated research nurses to obtain detailed information about risk factors, chest discomfort and comorbidity. Blood samples are collected, processed and stored in a biobank for analyses of genetic and circulating biomarkers. After the interview, patients undergo both a non-enhanced CT and a contrast-enhanced coronary CTA. Based on previous trials, it is expected that 20%–25% of patients will have suspected obstructive CAD based on the site-reading of coronary CTA. These patients will be further examined with ^{82}Rb-PET, ^{15}O-water-PET and ICA with three-vessel FFR, coronary flow reserve (CFR) and index of microvascular resistance (IMR). FFR-CT and uQFR is subsequently computed based on coronary CTA and ICA images, respectively (figure 2).

Based on the previous Dan-NICAD studies, the inclusion rate of patients fulfilling the inclusion criteria is expected to be 70%–80%. In addition, we expect that 20% of the included patients will have an incomplete dataset. Patient inclusion is expected to be completed within 24 months.

Baseline information

In a structured interview, patients risk factors and baseline measurements including weight, height, hip to waist ratio, blood pressure and ECG is obtained (online supplemental addendum). The interview focus on the symptoms including categorisation into typical, atypical or unspecific chest pain categories. Typical chest pain was defined as constricting discomfort in the chest or neck, jaw, shoulder or arm provoked by exertion or emotional stress and relieved by rest or nitroglycerine. Atypical chest pain was defined as two of the previously mentioned criteria. If one or none of the criteria were present, chest pain symptoms were categorised as nonanginal chest pain. Dyspnoea was defined as having exertional dyspnoea as the primary symptom.

Biobank

From all included patients, blood samples are drawn prior to the coronary CTA contrast administration. Patients are non-fasting at the time of the blood sampling. Within 2 hours, three blood samples are centrifuged and processed into 3 mL EDTA plasma, 3 mL Heparin plasma and 3 mL serum, which are aliquoted into individual 1 mL matrix tubes and stored at −80°C. Two 3 mL blood samples in EDTA tubes are placed directly in the freezer for later extraction of genomic DNA. All biospecimens are transported on dry ice to the Dan-NICAD biobank, where all samples are stored at −80°C.

CT

Patient preparation

According to clinical routine, patients are instructed to abstain from all substances and drugs containing caffeine for at least 24 hours prior to the coronary CTA examination. Patients with elevated heart rate at the time of referral are instructed to take 50–100 mg metoprololsuccinat, 50–100 mg atenolol or 7.5 mg ivabradin the night before and 2 hours prior to coronary CTA to reduce the heart rate to <60 beats per minutes. If not contraindicated, patients with persistent elevated heart rate will receive 2.5–20 mg metoprolol tartrate intravenously. Just prior to the coronary CTA, all patients receive 0.8 mg of

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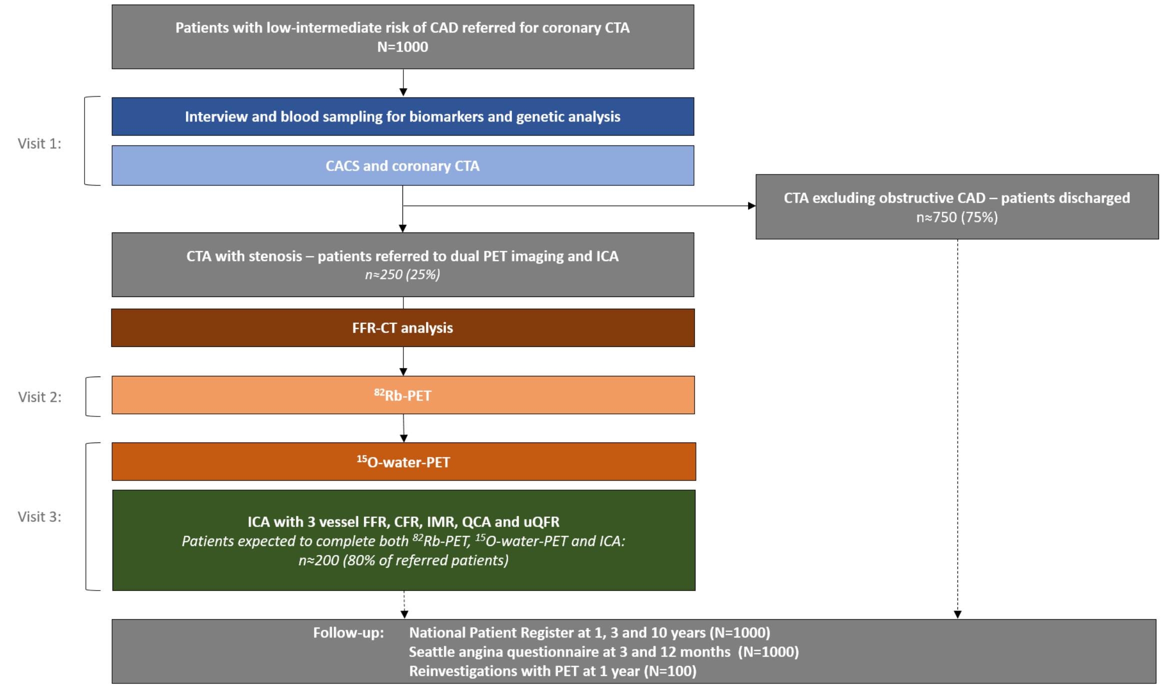

Figure 2 Dan-NICAD 3 patient flow chart. Numbers (n) in the figure are the estimated flow in patients. See figure 1. CFR, coronary flow reserve; CTA, CT angiography; Dan-NICAD, Danish study of Non-Invasive testing in Coronary Artery Disease; FFR-CT, CT angiography derived fractional flow reserve; ICA, invasive coronary angiography; PET, positron emission tomography; QCA, quantitative coronary angiography. uQFR, quantitative flow ratio.

sublingual nitroglycerin. The procedure is in accordance with normal clinical routine.

Imaging protocol

CT scans are performed with prospective ECG triggering using a multislice volume CT scanner (Aquilion One, Toshiba Medical Systems, Japan, or Revolution Apex, GE Healthcare, USA, or Siemens Flash, Siemens Healthcare, Germany). The CTA protocol is schematically shown in figure 3. The coronary CTA includes two different acquisition protocols: (1) a non-enhanced heart examination followed by (2) a contrast-enhanced coronary examination. The amount of contrast given is based on an individual assessment and follows clinical routine. A flow rate of 6 mL/s is recommended if possible and a chaser bolus of saline is administered. Following the enhanced examination, data are reconstructed in the cardiac diastolic phase which can be combined with the systolic phases if patient has tachycardia. The best phase images with low slice thickness are transferred to the image server for clinical site-reading.

Imaging analyses: CTA

All coronary CTA analyses are performed by an experienced cardiologist using dedicated software for reading depended on the CT scanner. An Agatston calcium score is initially calculated using dedicated workstations. Using the 18-segment model described by the Society of Cardiovascular CT, the luminal diameter stenosis is evaluated in each segment of the coronary tree.[16] By visually assessing and quantifying coronary lesions, the severity of coronary stenoses are classified as: no stenosis—0% diameter reduction (≈0% area reduction); mild stenosis—1%–29% diameter reduction (≈1%–50% area reduction); moderate stenosis—30%–49% diameter reduction (≈50%–69% area reduction) and severe stenosis—50%–100% diameter reduction (≈70%–100% area reduction). The criteria for diagnosing an abnormal coronary CTA are shown in table 1.

FFR-CT is performed using dedicated software. FFR-CT values are calculated in the major epicardial arteries with diameter >1.8 mm. Quantitative plaque analysis will be performed with commercially available software (Suite CT, Medis Medical Imaging, The Netherland).

Positron emission tomography

Patient preparation: PET

All ^{82}Rb PET and ^{15}O-water-PET examinations will be performed in accordance with clinical routine and recommendations from national and international societies. Participants are requested to abstain from intake of caffeine containing foods and beverages for 24 hours and are only allowed to drink tap water 2 hours prior to the PET scan. Medications with either antagonistic or agonistic effects on adenosine will be discontinued for 48 hours prior to the examination. Criteria for and initiatives to ensure sufficient adenosine stress are listed in table 2.

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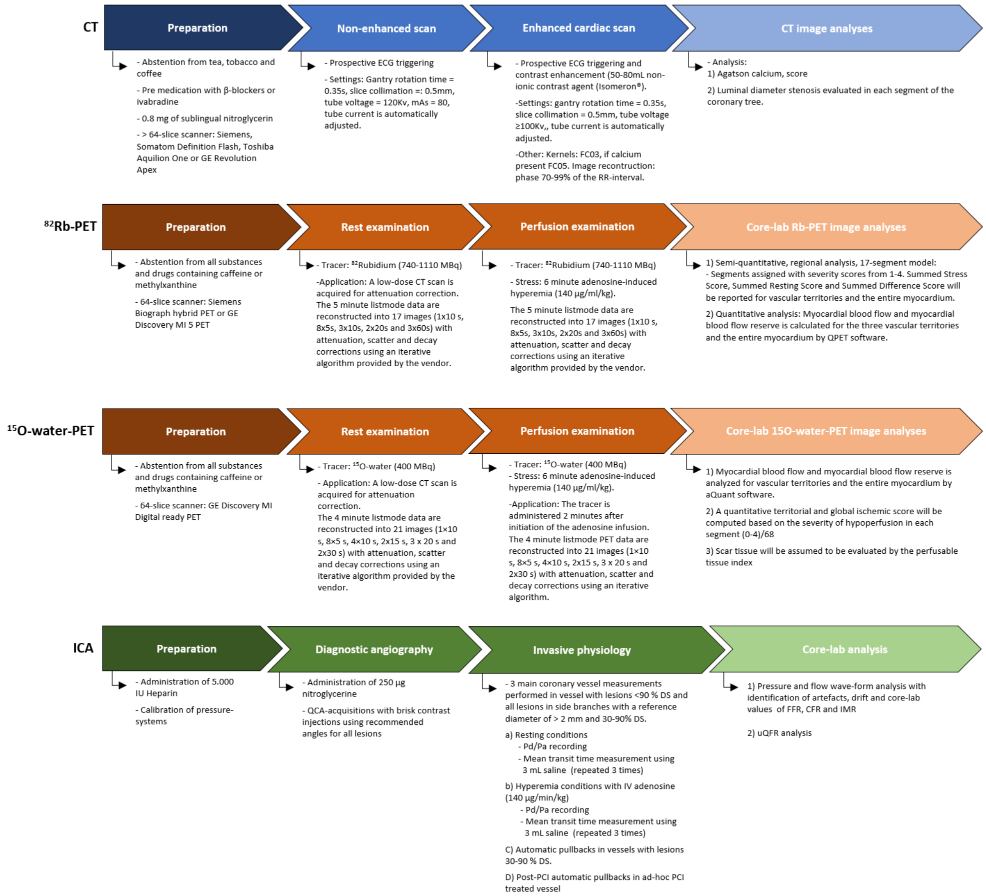

Figure 3 Image modalities and examination setup in Dan-NICAD 3 study. CAD, coronary artery disease; CFR, coronary flow reserve; CMR, cardiac MR; ICA-FFR, invasive coronary angiography with fractional flow reserve; IMR, Index of Microvascular Resistance; ^{15}O-water-PET, Oxygen-15 labelled water positron emission tomography; PCI, percuaneous coronary intervention; QCA, quantitative coronary angiography; ^{82}Rb-PET, rubidium-82 labelled positron emission tomography; SPECT, single-photon emission CT.

^{82}Rb-PET: imaging protocol, image reconstruction and image analysis

The ^{82}Rb-PET examinations are performed at one of two possible sites using a Siemens Biograph hybrid PET/64-slice CT scanner (Siemens Healthcare, Knoxville, Tennessee, USA) or a GE Discovery MI 5 PET/64-slice CT scanner (GE Healthcare Systems, USA), respectively. The acquisition protocol (figure 3) and the reconstructed data files are similar at the two sites. At both sites, PET data are reconstructed with the commercial software provided by Siemens/GE.

The ^{82}Rb-PET examination consists of two image acquisitions lasting 5 min each; the first at rest and the subsequent during hyperaemia induced by adenosine. Prior to the rest acquisition, a low-dose CT scan is acquired for attenuation correction. For each PET-acquisition, ^{82}Rb is eluted from a CardioGen-82 ^{82}strontium/^{82}Rb generator (Bracco Diagnostics, Princeton, New Jersey, USA). During hyperaemia, ^{82}Rb is infused 2 min after initiation of the 6 min adenosine infusion (140 µg/min/kg bodyweight). At site 1 (Siemens); 30 mCi / 1110 MBq ^{82}Rb is eluted for each image acquisition. At site 2 (GE); 20 mCi/ 740 MBq ^{82}Rb is eluted for each image acquisition if the body mass index is <30 kg/m^2 and 25 mCi / 925 MBq if the body mass index is ≥30 kg/m^2.

Image analyses are performed by an independent core lab 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, Cedars-Sinai Medical Center, Los Angeles, California).

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 artefacts; 2: moderate image quality, acceptable for clinical or research diagnosis; 3: poor image quality, diagnosing is impossible due to severe artefacts).

For segmental and vascular territory analyses, the summed perfusion images produced 150–300 s after 82Rb infusion are analysed visually with the recommended 17-segment American Heart Association model.[17] Segmental perfusion scores based on the average perfusion severity in a given segment is produced by the software and adjusted by an expert reader (0=normal; 1=mildly abnormal; 2=moderately abnormal; 3=severely abnormal;

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Table 1 Definitions of abnormal examinations

Blinded analysis			
CCTA*	^{82}Rb-PET	^{15}O-water-PET	ICA*
≥50% diameter stenosis or non-evaluable segments due to low image quality	Visually reduced isotope uptake in≥2 contiguous/17 segments during hyperaemia (summed stress score ≥4) or hyperaemic MBF<2.0 mL/g/min in ≥1 vessel territory or non-evaluable examination due to poor image quality	Hyperaemic MBF≤2.3 mL/g/min in ≥2 contiguous/17 segments or non-evaluable examination due to poor image quality	Haemodynamically obstructive CAD: High-grade stenosis (>90% diameter stenosis) by visual assessment *or* FFR≤0.80 in a vessel with a diameter stenosis of 30%–90% or QCA-based diameter stenosis (≥50% diameter) if FFR could not be performed due to for example, technical reasons Anatomically obstructive CAD: QCA-based diameter stenosis (≥70% diameter)
Prior knowledge analysis (not blinded to patient data and the CCTA)			
N/A	Visually reduced isotope uptake in≥2 contiguous/17 segments during hyperaemia (summed stress score ≥4) in an area of the myocardium corresponding to suspected coronary stenosis at coronary CTA or Hyperaemic MBF<2.0 mL/g/min ≥1 vessel territory corresponding to coronary stenosis at coronary CTA or non-evaluable examination due to poor image quality	Hyperaemic MBF≤2.3 mL/g/min in ≥2 contiguous/17 segments corresponding to coronary stenosis at coronary CTA or non-evaluable examination due to poor image quality	N/A

Definitions of abnormal examinations in the Dan-NICAD 3 study.
*In coronary vessel ≥2.0 mm in diameter.
CAD, coronary artery disease; CCTA, coronary CT angiography; Dan-NICAD, Danish study of Non-Invasive testing in Coronary Artery Disease; ICA-FFR, invasive coronary angiography-fractional flow reserve; MBF, myocardial blood flow; N/A, not available; PET, positron emission tomography; QCA, quantitative coronary angiography.

4=absent).[18] From the segmental scores, Summed Stress Score (SSS), Summed Resting Score (SRS) and Summed Difference Score (SDS) are calculated and reported for the three vascular territories, and the entire (global) left ventricular myocardium. Furthermore, the transient ischaemic dilation ratio (mean volume during hyperaemia/mean volume at rest) is calculated. From the gated images obtained 150–300 s after ^{82}Rb infusion, left ventricle ejection fraction during rest and hyperaemia is estimated.

Myocardial blood flow (MBF) is calculated by the QPET software from images acquired 0–300 s after the ^{82}Rb infusion using the model proposed by Lortie *et al.*[19] MBF and MBF reserve (MBFR, ratio: MBF during maximal hyperaemia/MBF at rest corrected for rate pressure product if above 10 000 will be reported for the three vascular territories and for the entire left ventricle.

The ^{82}Rb-PET scan results are categorised into (1) reversible ischaemia if SDS≥4 involving ≥2 contiguous segments or MBF<2.0 mL/g/min in ≥1 vessel territory; (2) irreversible ischaemia if SRS≥4 involving ≥2 contiguous segments; (3) combination of reversible and irreversible ischaemia (mixed ischaemia) if SSS≥4 due to increase of both SDS and SRS (4) poor image quality if the visual quality score is 3 or the scan is non-diagnostic. The exact criteria for classification of an abnormal ^{82}Rb-PET examinations are outlined in table 1.

Following the blinded analysis described above, the 'prior knowledge analysis' is performed, that is, the ^{82}Rb-PET images are re-evaluated taking clinical patient information and information from coronary CTA (anatomy of coronary vessels and possible stenosis) into account.

^{15}O-water PET: imaging protocol, image reconstruction and imaging analysis

All ^{15}O-water-PET scans will be performed on the same GE Discovery MI Digital ready PET/CT 64-slice system (GE Healthcare Systems, USA). The acquisition protocol is summarised in figure 3. A low-dose CT covering the heart will be acquired to correct for attenuation of both rest and stress PET studies. The ^{15}O-water radiotracer will be delivered by an automated generator/infusion system (Medtrace MT-100, Medtrace Pharma, Lyngby, Denmark) at a rate of 2 mL/s with subsequent flushing by 35 mL saline. Both the rest and hyperaemia studies are performed using 400 MBq ^{15}O-water delivered as a bolus with subsequent 4 min dynamic imaging. Approximately 4 min after completion of the rest study, maximal hyperaemia is obtained by infusing adenosine at a rate of 140 µg/kg/min for a total of 6 min. The ^{15}O-water bolus with subsequent 4 min dynamic imaging is administered 2 min after initiation of the adenosine infusion. The 4 min dynamic images from rest and hyperaemia will be reconstructed in a 3.27×3.27×3.27 mm matrix using all normal corrections (attenuation, scatter, dead time and randoms) and the VPFX-S reconstruction algorithm (PSF and ToF). For subsequent analysis, the dynamic scan will be divided into 21 frames (1×10, 8×5, 4×10, 2×15, 3×20 and 2×30 s).

The quality of the stress and rest images is evaluated by the semiquantitative visual scale similar to ^{82}Rb-PET (score 1–3).

Kinetic analyses of ^{15}O-water will be done using aQuant software (MedTrace Pharma, Lyngby, Denmark) using

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Table 2 Sufficient adenosine stress

	PET	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.
Caffeine consumption	Registration of consumption 24 hours prior to examination.	Registration of consumption 24 hours prior to examination.
Adenosine dose	Intravenous adenosine, dose adenosine 140 µg/kg/min, max 84 mg/6 min. Dose increase: No dose increase. Possible re-examination if the patient does not respond to adenosine, for example, due to caffeine consumption.	Intravenous adenosine, dose 140 µg/kg/min. Dose increase: In case of insufficient adenosine infusion response or if the FFR measurement is unstable, dose is increased to 200 µg/kg/min.
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 hyperaemia) ► Time 6–7 min after to adenosine infusion	Invasive aortic measurements—Pa (Pd/Pa measurement). ► At rest ► During maximum hyperaemia
Symptom	Symptoms during adenosine infusion are registered: ► Sensation of warmth ► Shortness of breath ► Headache ► Dry mouth ► Chest pain ► Atrioventricular block ► Other	Symptoms during adenosine infusion are registered: ► Sensation of warmth ► Shortness of breath ► Headache ► Dry mouth ► Chest pain ► Atrioventricular block ► Other
Other	Splenic switch-off (Only for Rb-PET) Increase in MBF during hyperaemia. If inadequate increase (MBFR<1.8) and adenosine stress is deemed sufficient the examination will be reported as being abnormal (table 1).	N/A
Sufficient stress	No clear-cut definition. All above-mentioned parameters are evaluated as a whole by a senior nuclear medicine physician determining whether the adenosine infusion is sufficient.	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 definitions of sufficient adenosine stress in the Dan-NICAD 3 study.
Dan-NICAD, Danish study of Non-Invasive testing in Coronary Artery Disease; ICA-FFR, invasive coronary angiography-fractional flow reserve; MBFR, myocardial blood flow reserve; N/A, not available; Pa, pressure aorta; Pd, pressure distal; Rb-PET, rubidium-positron emission tomography.

a validated one-tissue compartment model with image-derived input from cluster analysis, corrections for spill-over and automatic estimation of MBF and perfusable tissue fraction.[20] Resting and hyperaemic MBF (mL/g/min) will be assessed on both segmental and coronary artery territory level using the 17-segment American Heart Association[17] model. MBFR will be calculated as the ratio between hyperaemic and resting MBF corrected for rate pressure product if above 10 000. For each segment, a quantitative defect score (0–4) will be calculated based on the degree of hypoperfusion during hyperaemia MBF; 0: >2.3 mL/g/min, 1: 2.3–2.0 mL/g/min, 2: 2.0–1.7 mL/g/min, 3: 1.7–1.4 mL/g/min and 4: ≤ 1.4 mL/g/min. A hyperaemic MBF≤2.3 mL/g/min in two adjacent segments is considered abnormal.[21]

The combined ischaemic burden will then be calculated as summed quantitative defect score/68 (max score). Scar tissue will be estimated from perfusable tissue index values (PTI) with PTI<0.85 at rest indicating an irreversible perfusion defect.[22]

Criteria for an abnormal ^{15}O-water PET, in concordance with previous studies, are outlined in table 1.[21]

Following the blinded analysis described above, 'prior knowledge analysis' is performed, that is, the ^{15}O-water—PET images are re-evaluated taking clinical patient information and information from coronary CTA (anatomy of coronary vessels and possible stenosis) into account.

ICA and three-vessel invasive physiological examination: ICA, FFR, CFR and uQFR

Patient preparation

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

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Cardiac catheterisation protocol

Invasive coronary angiography

All diagnostic ICAs are performed according to present clinical guidelines through a radial or femoral access. Before acquisition of the ICA, the operator administrates anticoagulation (5000 IU heparin). The ICA protocol is schematically shown in figure 3.

Intracoronary nitroglycerine, 250 µg, is then administrated before the angiographic. Acquisitions are performed at 15 frames per second allowing for 2D and 3D QCA and uQFR analyses. Coronary artery overlap, foreshortening, zooming and planning are avoided if possible. All vessels are visualised in their full length if possible.

Invasive physiological examination

The pressure-wire (PressureWire X Guidewire, Abbott Chicago, USA) and CoroFlow (Coroventis, Uppsala, Sweden) are used according to manufacturer instructions for use. The pressure wire is advanced to the tip of the guiding catheter to equalise the pressure readings. Coronary physiological assessment including pressure and thermodilution measurements are performed in all main vessels (left anterior descending, right coronary artery and the left circumflex artery). In addition, all lesions in branches with a reference diameter of >2 mm and a diameter stenosis of 30%–90% by visual estimate are included for pressure and thermodilution measurements.

Resting pressure distal/pressure aorta and average mean resting transit time

The wire is advanced distal to all lesions in the vessels of interest and the wire position is documented. The pressure sensor is advanced distally to two-thirds of the vessel length for measurements in vessels without visual apparent disease. Resting pressure distal (Pd)/pressure aorta (Pa) is recorded as a minimum of 10 s with a stabilised Pa/Pa value after checking the pressure curves. Next, 3 mL of room-temperature saline is injected rapidly by hand three times to record mean transit time at baseline while the coronary system is not affected by adenosine.

FFR, CFR and IMR

Hyperaemia is induced using a 1 mg/mL concentration of adenosine at 140 ug/kg/min and the infusion rate is increased to 200 µg/ kg/min if a stable FFR value is not achieved. When maximum hyperaemia is achieved, 3 mL boluses of saline are injected to obtain hyperaemic thermodilution curves for hyperaemic mean transit time calculation. FFR, CFR and the IMR are instantly presented during the procedure. Pressure pullback curves are acquired in all vessels with FFR<0.80 for characterisation of diffuse versus focal disease.[23]

CFR is defined as the mean resting transit time by the mean hyperaemic transit time and describes the increase in flow to the myocardium during hyperaemia/stress. IMR is defined as the mean distal pressure multiplied by the mean hyperaemic transit time and indicates microcirculatory disease if increased.

Routine checks are made to ensure that ‘drift’ does not occur after the recordings. Absolute drift value of FFR≤±0.02 is accepted.

Postprocedural physiological examination

Resting Pd/Pa, FFR, IMR and CFR are measured following percutaneous coronary intervention treatment of diseased vessels. QCA projections are repeated for core-lab uQFR computation of the treated vessels.

Image analysis: ICA

All physiologic core-lab analyses are performed blinded to the coronary CTA and PET examinations. Invasive physiology analysis with dedicated software (Coroventis Research AB, Uppsala, Sweden) is performed in a suited core-lab (Institute of Clinical Medicine, Aarhus University, Denmark). The criteria for an abnormal ICA are shown in table 1.

QCA and uQFR analysis

Both QCA and uQFR core-lab analyses are performed in a core-lab setting (Interventional Imaging Core Laboratory, Aarhus University Hospital, Skejby, Denmark) using the latest version of the software (AngioPlus Core, Pulse Medical Imaging Technology, Shanghai, China). The Murray-based uQFR methodology was recently published.[15] uQFR≤0.80 is used as diagnostic cut-off value.

Follow-up

The follow-up period is 10 years from the coronary CTA examination. Data are extracted from the Civil Registration System, the National Patient Registry, the National Prescription Registry, the Laboratory Database and the Western Denmark Heart Registry. Cardiovascular events are adjudicated by an adjudication committee based on electronic patient files. The end-point in these follow-up trial is death and myocardial infarction according to the Fourth Universal Definition of Myocardial Infarction (2018).[24]

In addition, patients included in Dan-NICAD 3 study are followed with Seattle Angina Questionnaires at 3 and 12 months after the coronary CTA. Moreover, patients with hyperaemic MBF≤2.0 mL/g/min in ≥1 segment at baseline ^{82}Rb-PET are investigated 12 months after the procedure with a follow-up ^{82}Rb-PET similar to the index procedure.

Data collection and recordings

All study data are recorded in a secure web-based electronic case record form (eCRF)—Research Electronic Data Capture[25]—which enables logging of all data entries. All investigators have access to the eCRF. However, physicians performing imaging analyses have limited access in regards to the blinding procedures. Data collected and registered in the dedicated eCRF are listed in the online supplemental addendum.

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Endpoints and statistical analysis

Data analysis and reporting will follow the Standard Protocol Items: Recommendations for Standard for Reporting Diagnostic Accuracy Studies guidelines. Data are analysed by using appropriate statistical methods and for all statistical analyses, a two-sided $p<0.05$ is considered statistically significant, and 95% CIs are reported when appropriate. Statistical analysis is performed by using dedicated statistical software (STATA V16).

Diagnostic accuracy of non-invasive imaging

The main objective of this study is to investigate the diagnostic precision of ^{82}Rb-PET compared with ^{15}O-water-PET as secondary tests following a coronary CTA where obstructive CAD cannot be ruled out. ICA-FFR is used as reference standard as outlined in table 1.

The diagnostic accuracy is evaluated by sensitivity, specificity, positive and negative predictive value, and likelihood ratios. Comparison of sensitivity and specificity between diagnostic modalities is tested using McNemar's test and a weighted generalised score statistic for comparison of predictive values.[26]

Further, we will evaluate the diagnostic accuracy of FFR-CT compared with ^{82}Rb-PET and ^{15}O-water-PET and finally the impact of using additional CFR and IMR on the FFR-CT, ^{82}Rb-PET and ^{15}O-water-PET related diagnostic accuracy. The diagnostic performance of 2D-uQFR and 3D- uQFR is evaluated and compared with FFR as reference standard. The reproducibility and feasibility of 2D-uQFR and 3D-uQFR is compared. Patients with missing data on the index test and reference standard will be excluded in the primary analysis

Sample size

Based on the Dan-NICAD 1 and 2 trials, we expect that approximately 1000 patients are needed to be included and undergo coronary CTA. Following coronary CTA, we expect that 250 (25%) patients in whom coronary stenosis cannot be ruled out are eligible for continuing to the perfusion examinations and ICA part of the study. We expect 80% to complete both ^{82}Rb-PET and ^{15}O-water-PET and undergo ICA examination. By including 1000 patients, we are able to evaluate the predictive validity parameters (sensitivity, specificity, positive and negative predictive values) with a minimum of 8% absolute precision on both sides for the expected sensitivity (80%) and specificity (80%) for both ^{82}Rb-PET and ^{15}O-water-PET at a disease prevalence of 50% at ICA-FFR.

ETHICAL CONSIDERATIONS

The study follows the principles outlined in the Declaration of Helsinki and ISO 14155:2011. The additional radiation exposure by participation in the study in regard to the coronary CTA, ^{82}Rb-PET, ^{15}O-water-PET and ICA examination increases the cumulated risk over a lifetime of dying from cancer from approximately 25% to no more than 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.

DISCUSSION

With the Dan-NICAD 3 study, we aim to investigate and compare the diagnostic accuracy of ^{82}Rb-PET and ^{15}O-water-PET for obstructive CAD identification using the reference standard of ICA three-vessel coronary physiology as reference. Both radioactive isotope tracers are currently clinically available with ^{82}Rb-PET more widely used than ^{15}O-water-PET although ^{15}O-water has several potential advantages. However, no previous studies have compared the clinical utility of the tracers in a head-to-head study design.

The Dan-NICAD trial programme

The Dan-NICAD trial programme aims to investigate the optimal diagnostic strategy for patients without previous CAD but stable symptoms suggestive of obstructive CAD (figure 1). All patients are referred for coronary CTA from an outpatient cardiology clinic according to the clinical guidelines for patients with low to intermedia pretest probability of obstructive CAD.[2 4] In the region is exercise ECG and dobutamine stress Echo not used in the diagnostic management of patients with chronic coronary syndrome. If coronary CTA is not possible due ineligibility of the patient (eg, severe obesity, reduced renal function, server arrhythmia or inability to cooperate) the patient is generally referred directly to a myocardial perfusion scan or ICA. The prevalence of obstructive CAD observed in the previous Dan-NICAD 1 and 2 studies is comparable to the disease prevalence seen in national and international coronary CTA databases and prospective randomised studies.[2 27 28] Thus, the cohort seems to be representative for patients referred for coronary CTA in general. Importantly, all patients are included prior to the coronary CTA which avoids any selection bias based on the coronary CTA quality and results.

The coronary CTA is performed according to local standards and the initial interpretation of the coronary CTA is performed on-site. Based on this local interpretation, patients with abnormal coronary CTA are referred for ^{82}RbPET, ^{15}O-water-PET and ICA. Previous studies have shown the site-reading of coronary CTA tends to overestimate the presence of obstructive CAD compared with core-lab coronary CTA reading and findings at ICA.[29] On this basis, and in accordance with real-world practice, second-line testing with MPI is needed to rule-in patients for referral to ICA and revascularisation.

MPI and ICA

If the initial coronary CTA does not rule out obstructive CAD, the Dan-NICAD protocol refers patients to be

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investigated with MPI; in Dan-NICAD 1, patients were randomised to either 1.5T cardiac MRI or SPECT with a Technetium based tracer, in Dan-NICAD 2, with both 3T cardiac MRI and ^{82}Rb-PET. Following the selective MPIs, all referred patients are examined with ICA with FFR measurements in stenotic coronary vessels. Supplemental invasive measurements of CRF and IMR in stenotic coronary vessels were performed in the Dan-NICAD 2 trial. In the present Dan-NICAD 3 study, patients needing further diagnostic testing after coronary CTA undergo both ^{82}Rb-PET and ^{15}O-water-PET. Furthermore, the ICA investigation will include three-vessel invasive measurements of FFR, CRF and IMR as a supplement to the dedicated measurements in the stenotic vessel(s). Based on current data, and highlighted by the current 2019 ESC guidelines on chronic coronary syndrome as a gap in evidence, powered trials are needed to compare the effectiveness of different diagnostic strategies including myocardial perfusion techniques for obstructive CAD rule-in; this to evaluate how to best integrate diagnostic tests in patient care in terms of clinical outcomes and the use of healthcare resources.[4]

In contrast to the Dan-NICAD 1 and 2 studies, the three-vessel invasive measurements in the Dan-NICAD 3 study will enable invasive investigation of abnormal coronary flow patterns in non-obstructive vessels due to microvascular disease. Microvascular disease may explain abnormal myocardial perfusion at MPI in patients with non-obstructive coronary vessels at ICA with a sole reference of FFR.

Follow-up

All patients included in the three studies are followed using the national patients registers. Data regarding clinical endpoints, laboratory measurements and medical treatment and compliance are extracted from reimbursed medical prescriptions at Danish pharmacies.

In addition, patients included in Dan-NICAD 2 and 3 studies are followed with Seattle Angina Questionnaires at 3 and 12 months after the coronary CTA. Moreover, a 12 months follow-up, ^{82}Rb-PET scan is performed in patients with abnormal myocardial perfusion at the baseline ^{82}Rb-PET. Previous studies did not find differences in hard end-points using strategies of revascularisation compared with optimal medical treatment.[30] However, results on quality of life changes with revascularisation compared with optimal medical treatment are ambiguous,[31 32] and no previous studies have correlated changes in symptom burden with changes in myocardial ischaemia extent. Using the approach outlined, we will be able to investigate the correlation between the angina symptom burden and myocardial ischaemia reduction during follow-up. Importantly, follow-up 82Rb-PET was also performed in n=157 patients with hyperaemic MBF≤2.0 mL/g/min in≥1 segment at baseline 82Rb-PET in the Dan-NICAD 2 trial. Hence, the present Dan-NICAD 3 cohort can potentially validate findings from this study and increase the power of potential subanalyses.

Tracers: ^{82}Rb-PET versus ^{15}O-water-PET

Both ^{18}F-Flurpiridaz, ^{13}N-ammonia, ^{82}Rb and ^{15}O-water tracers can be used for PET myocardial perfusion assessment. However, for clinical use, ^{13}N-ammonia, ^{82}Rb-PET and ^{15}O-water-PET are of special interest as the tracers enable a rest–stress protocol scan-time completion within 30 min due to short physical half-life. ^{82}Rb can be produced without an on-site cyclotron, whereas production of ^{13}N-ammonia and ^{15}O-water requires an on-site cyclotron. However, ^{15}O-water is the reference standard for myocardial perfusion quantification due to ideal tracer kinetics and was used as the reference test in the original validation of FFR-based stenosis evaluation.[33] It is, therefore, likely that hypoperfused areas identified with ^{15}O-water-PET will be more concordant with coronary artery lesions measured by subsequent invasive FFR than has been the case for, for example, SPECT tracers and ^{82}Rb-PET.[8 10]

^{15}O-water is produced by irradiating natural nitrogen from basic air with deuterons using the ^{14}N(d,n)^{15}O reaction in an on-site cyclotron. Recent development of small, dedicated cyclotrons requiring limited shielding has lowered the cost of these ^{15}O-water cyclotrons. Hence, myocardial perfusion PET imaging with ^{15}O-water tracer may become feasible in less advanced nuclear medicine departments in the near future. In addition, ^{15}O-water PET software solutions using the same kinetics and base equations are currently becoming commercially available allowing for highly standardised MBF measurements and accurate cut-offs for pathology.

Although ^{82}Rb has the advantage of being produced and delivered by a simple ^{82}Strontium/^{82}Rb generator, it has a higher effective dose than ^{15}O-water-PET (effective dose of 2–3 mSv compared with 1–2 mSv). In addition, ^{82}Rb have several limitations compared with ^{15}O-water which may decrease the diagnostic accuracy making a head-to-head diagnostic accuracy study of special interest. First, the relationship between the extraction fraction of ^{82}Rb into the myocardium and MBF is not linear and blood flow above modest hyperaemia (~2 mL/g/min) is underestimated. Second, positrons from ^{82}Rb have higher energy with longer positron range resulting in lower spatial resolution and risk of partial volume effects. Thirdly, ^{82}Rb is also taken up by the lungs and gastric ventricle, which may result in lower image quality. Finally, the interpretation of ^{82}Rb-PET images is semi-quantitative.[34] However, whether these tracer limitations impact the diagnostic accuracy compared with ^{15}O-water-PET has not previously been investigated.

Diagnostic performance of ^{82}Rb-PET and ^{15}O-water-PET

To date, very limited data exist on the diagnostic performance of PET from larger high-quality prospective studies—in total, three studies (505 patients) with ^{82}Rb-PET and four studies (n=463) with ^{15}O-water-PET have been published (table 3).[35–40] In contrast, several retrospective studies have evaluated the diagnostic performance of myocardial perfusion PET compared with

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Table 3 Diagnostic accuracy studies

Prospective diagnostic accuracy studies of ^{82}Rb and ^{15}O-water PET														
Tracer	**Study**	**Year**	**No of patients**	**Design**	**Patients included**	**Patients with previous MI and revasc.**	**Prespecified cut-off for abnormal results**	**Comparison to other test**	**Disease prevalence**	**Disease ref. standard**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
^{82}Rb-PET	Stewart *et al* (Ann Arbor, Michigan, USA)[35]	1991	41	Prospective	Stable chest pain suspected of CAD referred for ICA	Excluded in this subanalysis	Qualitative	SPECT (head-to-head)	74	ICA-QCA DS>50%	75	75	NA	NA
	Sampson *et al* (Boston, Massachusetts, USA)[36]	2007	64	Prospective	Stable chest pain suspected of CAD	Not included	Qualitative	None	69	ICA visual DS>70%	93	50	80	77
	Rasmussen *et al* (Dan-NICAD 2) (Multicentre, Denmark)[10]	2022	372	Prospective	Stable chest pain with suspected CAD at CCTA (selected PET after CCTA)	Not included	Qualitative and/or hyperaemic MBF<2.0, CRF<1.8 and/or transient ischaemic dilation ratio 1.13+ stress EF<rest EF (QPET software)	CMR three tesla (head-to-head)	44 19	ICA-FFR ICA-QCA DS>70%	64 89	89 79	83 50	76 97
^{15}O-water-PET	Kajander *et al* (Turku, Finland)[37]	2010	107	Prospective	Stable chest pain suspected of CAD	Not included	Hyperaemic MBF<2.5, (Carimas software)	CCTA (head-to-head)	37	ICA-FFR	91	91	86	97
	Thomassen *et al* (Odense, Denmark)[38]	2013	44	Prospective	Stable chest pain suspected of CAD referred for ICA	Not included	Hyperaemic MBF<2.5, (Carimas software)	CCTA (head-to-head)	50	ICA-QCA DS>50%	91	86	87	90
	Joutsiniemi *et al* (Turku, Finland)[39]	2014	104	Prospective	Stable chest pain suspected of CAD	Not included	Hyperaemic MBF<2.5, (Carimas software)	None	34	ICA-FFR	95	89	84	97
	Danad *et al* (PACIFIC) (Amsterdam, The Netherlands)[40]	2017	208	Prospective	Stable chest pain suspected of CAD	Not included	Hyperaemic MBF≤2.3, (cardiac VUer software)	CCTA and SPECT (head-to-head)	44	ICA-FFR (3 vessel)	87	84	81	89
82Rb-PET vs 150-water-PET	Current study (Dan-NICAD 3) (Multicentre, Denmark)		Approx. 200	Prospective	Stable chest pain with suspected CAD at CCTA (selected PET after CCTA)	Not included	82Rb-PET Qualitative and/or Hyperaemic MBF<2.0, (QPET software) 150-water-PET Hyperaemic MBF≤2.3, prespecified (aQuant software)	^{82}Rb- PET vs ^{15}O-Water-PET (head-to-head)	Na	ICA-FFR (3 vessel) ICA-QCA DS>70%	The aim of current study			

List of prospective diagnostic accuracy studies investigating the performance of ^{82}Rb-PET and ^{15}O-water-PET with ICA as reference. Included are studies with predefined hyperaemic MBF (mL/min) or CRF cutoff, n>40, and which only includes patients without known CAD, previous myocardial infarction or revascularisation.
CAD, coronary artery disease; CCTA, coronary CT angiography; CMR, cardiac MR; CRF, case record form; Dan-NICAD, Danish study of Non-Invasive testing in Coronary Artery Disease; ICA, invasive coronary angiography; MBF, myocardial blood flow; NA, not available; NPV, negative predictive value; PPV, positive predictive value; QCA, quantitative coronary angiography; ^{82}Rb-PET, 82rubidium-positron emission tomography; SPECT, single-photon emission CT.

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a clinically indicated ICA performed after PET.[41–44] However, these studies were limited by bias as not all patients underwent ICA. Similarly, the Evaluation of Integrated Cardiac Imaging for the Detection and Characterisation of Ischaemic Heart Disease study compared coronary CTA and MPI to ICA but at least one test should be abnormal before ICA was required.[45] Finally, some studies were designed to define blood flow cut-offs and did not prespecify definitions of an abnormal test result or excluded patients with missing values.[21 46] In general, previous studies have found high diagnostic accuracy of PET but are hampered by a limited external validity, and the lack of head-to-head designs hinders a definitive conclusion of the PET-assessed diagnostic accuracy for obstructive CAD identification. Importantly, the definition of binary cut-off values has a major impact on test sensitivity and specificity.

In the Dan-NICAD programme, we investigate the performance of different MPI techniques as second-line diagnostic tests after an abnormal coronary CTA with suspected obstructive CAD. The strategy of 'selective MPI' after coronary CTA is recommended by the European and American guidelines to further stratify patients before ICA.[4 5] However, the diagnostic performance of second-line MPI have previously only been investigated in the Dan-NICAD trials and one other trial evaluating SPECT.[8 12 47] In contrast, a 'hybrid imaging' strategy where all patients undergo both coronary CTA and MPI has previously been investigated in several studies. This strategy, however, is currently not recommended since coronary CTA as first-line test exhibits excellent rule-out properties. Hence, the diagnostic performance of MPI may differ when tested in a 'selective MPI' strategy after coronary CTA compared with a 'hybrid imaging' strategy as only patients with an abnormal coronary CTA are included in the 'selective MPI' strategy. In a 'selective MPI' strategy, the lack of patients with no disease may reduce the specificity of the MPI examined. Finally, based on the previous Dan-NICAD 1 and 2 studies, inclusion of patients with primarily low/intermediate pretest probability of obstructive CAD referred to a primary coronary CTA compared with studies including patients referred for ICA potentially reduces the number of patients with very severe CAD (eg, three vessel disease and occluded vessels) and increase the number of patients with FFR values around 0.80 which may lower the sensitivity of MPI in the Dan-NICAD trials.

Computed estimation of FFR from coronary CTA and ICA

Calculated FFR values based on computational fluid dynamics from vessel contouring based on images produced from coronary CTA or ICA are highly interesting techniques.[48] Based on a high sensitivity for obstructive CAD identification and good prognostication, increasing evidence support the use of FFR-CT.[49 50] To date, FFR-CT is in clinical use with the method proposed by Heartflow, California, United States, but several prototypes of other software are tested.[51–54] However, studies comparing the diagnostic accuracy of FFR-CT to MPI tests are warranted. In Dan-NICAD 1, FFR-CT were compared head-to-head to CMR perfusion yielding similar overall diagnostic performance. Sensitivity for prediction of revascularisation was highest for FFR-CT, whereas specificity was highest for CMR.[55] Recently, FFR-CT was compared with ^{15}O-water-PET and SPECT in 208 patients without previously known CAD referred to ICA-FFR with an obstructive CAD prevalence of 44%.[13 40] This study showed improved performance of PET in the per-patients analysis but FFR-CT out-performed PET in detecting vessel-specific ischaemia. However, PET scans were analysed blinded to the coronary CTA results which does not mimic a strategy of 'selective MPI' after coronary CTA.

uQFR estimates FFR based on a ICA image using 2D-QCA analysis and Murrays fractal law. This technique can be performed without pressure wires and therefore reduces the patient risk and overall costs compared with ICA-FFR. uQFR has a diagnostic accuracy comparable to the 3D-based QFR model that is currently undergoing clinical testing. However, the need for 3-D reconstruction including acquisition of two high-quality images may hamper the clinical adaption of ICA-derived FFR.[56] Furthermore, the existing QFR model assumes linear tapering of vessel diameter and ignores side branches. Hence, estimation of FFR using the newly developed Murray-law based uQFR from a single angiographic view may improve the feasibility and reproducibility of angiography-derived FFR without compromising the diagnostic accuracy.

Within the Dan-NICAD studies, a total of more than 800 patients are investigated by ICA, all with abnormal coronary CTA and subsequent MPI. The cohort has the potential to compare these new techniques in a head-to-head design. With this sample size, a minimum of 4% absolute precision on both sides for the sensitivity (80%) and specificity (80%) can be achieved. In addition, the follow-up in the Dan-NICAD trials will enable studies of the impact of prognostic risk stratification using the new modalities.

Personalised medicine based on biomarkers

This study is also designed to investigate the potential use of biomarkers in risk stratification and diagnosis of obstructive CAD. Hence, all Dan-NICAD studies have similar designs enabling pooling of data. All patients included in Dan-NICAD 1 study have been whole genome-sequenced and patient included in Dan-NICAD 1 and 2 is genotyped and analysed using OLINK Explore proteomics panels for circulating biomarkers.

Knowledge about the impact of genetic variants related to CAD has increased dramatically over the past few years, and large genome-wide association studies of CAD have successfully identified more than 100 risk loci for CAD. Because each individual single nucleotide polymorphisms (SNPs) identified in genome-wide association studies have little effect on CAD risk (OR 1.1–1.2), methods have been developed to aggregate information

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on multiple SNPs into a single polygenetic risk score. The polygenetic risk score is able to identify 8% of the population as having three times greater risk of cardiac events compared with the background population.[57–60] We have previously demonstrated that a polygenetic risk score of CAD is correlated to an increased burden of coronary atherosclerosis rather than promoting specific plaque features, which may increase discrimination of CAD beyond clinical risk factors alone.[61 62]

Similar to obtaining genetic information, several research groups are now starting to combine circulating proteins in the same way as genetic risk variants.[63] A recent study tested 109 circulation protein biomarkers (proteomics) and found that combining the information from four proteins substantially improved risk prediction of CAD.[64] The integration of genetics and biomarkers to predict risk is under rapid development to discover new therapeutically targets which can change patients management and/or treatment.

In this study, genetic and circulating protein markers will be combined. While a polygenetic risk score represents the inherited risk, which in principle can be determined at birth, protein markers may reflect a mixture of vascular and myocardial factors such as injury, inflammation, abnormal glucose and fat metabolism, and an array of other processes.

To the best of our knowledge, this study will be the first to test the combination of clinical factors, biomarkers and genetic risk variants for a precise risk stratification score in patients with symptoms suggestive of CAD.

Perspective

The Dan-NICAD 3 study will evaluate the clinical benefit of using ^{15}O-water compared with ^{82}Rb tracers for PET myocardial perfusion. Hence, the study may guide hospitals in decisions regarding establishing on-site ^{15}O-water cyclotrons. The current study will furthermore increase the cohort size of the Dan-NICAD trial to approximately 4500 patients with structured interviews, biobank samples and coronary CTA images. Of these 4500 patients, 20–25% will have undergone MPI tests and ICA-FFR. On this basis, the Dan-NICAD programme will, to the best of our knowledge, be one of the largest cohorts with comprehensive anatomical and functional description of CAD extend.

Study status

The study is ongoing. The first patient was enrolled in January 2021; and as of March 2023, a total of 1000 patients are included and enrolment was completed.

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Patient consent for publication Not applicable.

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