Dynamic CT myocardial perfusion imaging: comparison of clinical analysis methods for the detection of vessel-specific ischaemia

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Abstract (247 words)

Background

The clinical analysis of myocardial dynamic computed tomography myocardial perfusion imaging (CT-MPI) lacks standardization. The objective of this prospective study was to compare different analysis approaches to diagnose ischaemia in patients with stable angina referred for invasive coronary angiography.

Methods and Results

Patients referred for evaluation of stable angina symptoms underwent adenosine-stress dynamic CT-MPI with a second-generation dual-source scanner. Quantitative perfusion parameters such as blood flow were calculated by parametric deconvolution for each myocardial voxel. Initially, perfusion parameters were extracted according to standard 17-segment-model of the left ventricle (fully automatic analysis). These were then manually sampled by an operator (semi-automatic analysis). Areas under the receiver-operating characteristic curves (AUCs) of the two different approaches were compared. Invasive fractional flow reserve =<0.80, or diameter stenosis >=80% on quantitative coronary angiography (QCA) were used as reference standard to define ischaemia.

We enrolled 115 patients (88 men; age 57±9 years). There were 72/286 (25%) vessels causing ischaemia in 52/115 (45%) patients. The semi-automatic analysis method was better than the fully automatic method at predicting ischaemia (AUCs: 0.87 vs. 0.69; p<0.001) with readings obtained in the endocardial myocardium performing better than those in the epicardial myocardium (AUCs: 0.87 vs. 0.72; p<0.001). The difference in performance between blood flow, expressed as relative to remote myocardium, and absolute blood flow was not statistically significant (AUCs: 0.90 vs. 0.87; p=ns).

Conclusions

Endocardial perfusion parameters obtained by semi-automatic analysis of dynamic CT-MPI may permit robust discrimination between coronary vessels causing ischaemia vs. not causing ischaemia.

Key words: image interpretation; imaging; computed tomography; perfusion imaging.

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INTRODUCTION

Dynamic computed tomography myocardial perfusion imaging (CT-MPI) using adenosine-mediated vasodilation allows for quantitative measurement of perfusion parameters such as myocardial blood flow, perfused capillary blood volume and first pass distribution volume.

The feasibility of dynamic CT-MPI was shown in animal studies [1-6] and clinical studies [7-17]. Our group as well as others demonstrated previously that myocardial blood flow from dynamic CT-MPI added value to anatomical computed tomography coronary angiography (CTCA) in the detection of vessel-specific ischaemia [12].

In dynamic CT-MPI, time-resolved attenuation curves are constructed as contrast agent passes through the heart. A signal-deconvolution step is then required to calculate perfusion parameters. Commercially available software packages can automatically display data as a polar map and provide segmental readings of perfusion parameters. The accuracy of these in the detection of regional ischaemia in clinical settings, however, remains uncertain. As with any new diagnostic test, the requirements for understanding the potential clinical value and clinical adoption cannot be fulfilled in the absence of standardized analysis methods. At the present time there is no consensus regarding the optimal method of dynamic CT-MPI interpretation.

The aims of this study were: 1) to evaluate if a fully automatic analysis method is clinically accurate; 2) to compare endocardial and transmural CT-MPI findings for their ability to identify vessel-specific ischaemia; 3) to compare the diagnostic performances of various absolute perfusion parameters with relative perfusion parameters. We used invasive coronary angiography (ICA) and fractional flow reserve (FFR) [18] as reference standards to define vessel-specific ischaemia in a prospective cohort of patients with stable angina.

METHODS

Study population

The Research Ethics Committee approved the study protocol and all patients gave written informed consent. Between April 2011 and September 2015, 215 patients with stable chest pain clinically referred to ICA at a single institution were screened for inclusion in this prospective study (*NIHR Clinical Research Network 10590)*. Study exclusion criteria were acute coronary syndrome, previous percutaneous or surgical coronary revascularization, severely impaired left ventricular ejection fraction (≤35%), estimated glomerular filtration rate <60ml/min, documented or suspected allergy to contrast and contraindications to adenosine infusion (history of severe asthma or obstructive lung disease, second or third degree atrio-ventricular block and systolic blood pressure <90mmHg) (**Figure 1**).

Imaging protocol

Patients underwent CTCA followed by adenosine-stress dynamic CT-MPI one to four weeks before ICA. A second-generation dual-source CT scanner (Somatom Definition Flash, Siemens, Forchheim, Germany) was used. We allowed 10-15min-delay between CTCA and CT-MPI to minimize cross-contamination of contrast on CT-MPI. The CT-MPI scan was acquired using the ECG-triggered axial shuttle mode, where the table moves between two alternate positions to sample dynamic data. The dynamic dataset consisted of 13-14 volumes of the left ventricle acquired over 30 s, each volume consisting of thirty-four 3mm-thick images. The total contrast volume used for the combined CTCA/CT-MPI protocol was 135ml. A flow-chart detailing patient preparation, scan and contrast injection protocols is given in **Supplemental figure 1.**

For CTCA, the median (IQR) dose length product (DLP) was 235 (120-372) mGy*cm (using a conversion factor for the chest of 0.014 this corresponds to 3.3mSv). For CT-MPI, the DLP was 734 (627-804) mGy*cm (10.3mSv) using 100kV/300mAs, and 430 (398-515) mGy*cm (6.0mSv) using 80kV/370mAs.

CT-MPI data post-processing

Firstly, dynamic CT-MPI datasets underwent post-processing using commercial software (Volume Perfusion CT Body, Siemens) by an operator with 5 years experience of CT-MPI. The left ventricular myocardium was segmented by placing a volume of interest (VOI) using a method of blood pool removal combined with thresholding based on attenuation values. A motion correction algorithm was applied when needed. The arterial input function (AIF) was sampled by drawing regions of interest (ROIs) on the descending aorta in both dynamic image stacks. Time-attenuation curves (TACs) were created for each myocardial volumetric image element (voxel) within the VOI. Dedicated parametric deconvolution based on 2-compartment model of intra- and extra-vascular space was applied to fit the TACs and compute myocardial blood flow, perfused capillary blood

volume and first pass distribution volume. Perfused capillary blood volume (ml/100ml) was obtained directly from the model as a function of contrast concentration in each voxel of the myocardium. Myocardial blood flow (ml/100ml/min) was calculated as the ratio between maximum slope of the fit curve/maximum AIF. First pass distribution volume (ml/100ml) was calculated as the ratio between peak of the fit curve/maximum AIF [3, 12] (**Figure 2**).

Parametric data were then processed by a second independent operator (also of 5 years experience of CT-MPI), blinded to the ICA/FFR results, using prototype software (Cardiac Functional Analysis Protocol Build Data; Siemens) [19, 20]. The software employed automatic segmentation of the left ventricle based on a heart model that includes the four cardiac chambers as well as other anatomical landmarks (cardiac valves and ventricular septum) as control points. Endocardial and epicardial contours of the left ventricle were segmented automatically, with the option of manual contour correction. From the resulting segmentation, polar maps were generated (bulls-eye plots based on 17-segment AHA model [21]) for each perfusion parameter.

CT-MPI clinical analysis methods

From this point onward, we applied two pre-specified clinical analysis methods in all cases, i.e. fully automatic and semi-automatic (**Figure 2**). The fully automatic analysis was followed by the semi-automatic analysis, with a time interval of 12 weeks between analyses. Anonymized datasets in random order were analyzed by both approaches. Parametric look-up table (LUT) display settings had range 0-200 ml/100ml/min for blood flow, 0-15 ml/100ml for perfused capillary blood volume, 0-20 ml/100ml for first-pass distribution volume.

For the fully automatic analysis, the polar map provided segmental values for each perfusion parameter. These were derived from the endocardial layer of the myocardium, from the epicardial layer as well as transmural (total) myocardium. Within each vascular territory (LAD, LCx, and RCA) [21], the segment with the lowest value was selected and used in this analysis.

For the semi-automatic analysis, VOIs of at least 0.5cm^3 were drawn manually on the perfusion polar maps, guided by the color-coded scale. For each VOI, transmural, endocardial and epicardial values for each parameter were provided. The endocardial/epicardial (endo/epi) ratio was calculated for each parameter. Additionally, relative parameters were calculated as the ratio between the absolute parameter obtained from the manually drawn VOI and the segmental parameter corresponding to the 75% percentile of the fully automatic segmental analysis in the same patient, the latter inputted as denominator (‘remote myocardium’).

To ensure accurate matching of coronary distribution and associated myocardial territories, the patient specific coronary anatomy on CTCA (right, left or balanced dominance, length of LAD) was used to decide which vessel (RCA, LCA or both) supplied the inferior and infero-septal segments in the myocardial polar map. CTCA multi-planar reconstructions and perfusion polar maps were

inspected side by side. CTCA was not used to decide whether or not a coronary vessel was causing ischaemia, but only to guide the positioning of VOIs on the polar map. The reader was blinded to ICA/FFR results.

For the assessment of intra- and inter-observer agreement, 37 anonymized CT-MPI studies were randomly chosen and re-analyzed by same operator after a time interval of 12 weeks, and by a second blinded operator.

ICA/FFR

During ICA two interventional cardiologists (10 years experience) visually identified intermediate coronary lesions with diameter narrowing between 30% and 90%. FFR was measured in these lesions (if deemed safe) using a sensor-tipped 0.014-inch guidewire (Pressure Wire, Radi Medical Systems, Uppsala, Sweden). The pressure sensor was positioned just distal to the lesion. FFR was calculated as the ratio of mean distal pressure measured by the pressure wire divided by the mean proximal pressure measured by the guiding catheter during rest and during maximal myocardial hyperemia induced by a continuous intravenous infusion of adenosine (140µg/kg/min for a minimum of 2min).

ICA images were further analyzed offline on multiple projections by a single observer (7 years experience) blinded to the CT-MPI results. The most severely diseased segment in each coronary vessel was identified to derive the percentage diameter narrowing using validated quantitative coronary angiography (QCA) software (QAngio® XA, 7.3, Medis, Leiden, the Netherlands).

Pre-specified reference standard

Lesions producing 30-90% visual coronary narrowing on ICA were classified as ischaemic or non-ischaemic based on FFR findings. Vessels with FFR ≤0.80 were called ischaemic (i.e. haemodynamically significant), those with FFR >0.80 were called non-ischaemic.

Lesions where FFR could not be obtained due to safety reasons were classified as follows based on QCA. Lesions with ≥80% diameter narrowing on QCA were adjudicated as ischaemic. Lesions with <30% diameter narrowing on QCA were adjudicated as non-ischaemic [18]. This was based on the observation that a QCA 80% stenosis is likely to correspond to a 90% visual stenosis [22]. Lesions without an FFR producing QCA 30-80% diameter narrowing were excluded from the analysis.

Statistical analysis

Data were analyzed using commercial software (*IBM SPSS Statistics for Macintosh*, Version 22.0; Armonk, NY: IBM Corp. and *STATA Statistical Software*: release 14. College Station, TX: StataCorp LP). Results were reported in accordance with the STARD criteria [23]. Continuous variables were presented as means ± standard deviations (SD) or medians with interquartile ranges (IQR). Categorical variables were shown as frequencies and percentages.

The diagnostic performances of the different analysis methods were determined against the reference standard FFR/QCA. We obtained receiver operating characteristic (ROC) curves for: a) fully automatic and semi-automatic analyses; b) transmural, epicardial and endocardial analyses; c) myocardial blood flow, perfused capillary blood volume and first pass distribution volume; d) absolute and relative perfusion parameters. Areas under the curve (AUCs) were compared using the DeLong test and p-values were adjusted for multiple comparisons using Bonferroni correction. Optimal cut-off values were identified for each parameter using the Youden index. A vessel based analysis was performed; therefore, the clustered nature of the data (three vessels per patient, or two in left coronary dominance) was adjusted for using logistic generalized estimating equations (GEE's) [24].

Perfusion parameters were plotted against invasive FFR and differences in the median perfusion parameters among the five FFR ranges were tested using the Kruskal Wallis test and Mann-Whitney U test.

Intra- and inter-observer agreement was evaluated using intra-class correlation coefficients (ICC's).

A p-value of less than 0.05 was considered statistically significant.

RESULTS

Baseline characteristics and ICA/FFR

The study included 115 patients (88 men; age 57±9 years) who underwent CT-MPI prior to ICA. No severe adverse reactions to adenosine or iodinated contrast agent were observed. In 15 patients with left coronary dominance, the RCA was short and not included in the analysis. Forty-four vessels 30-80% narrowing on QCA (in 40 patients) were excluded from the analysis because FFR measurements were not performed due to safety reasons. Therefore 286 coronary vessels and corresponding myocardial territories were available for inclusion in this analysis **(Figure 1)**. Based on ICA/FFR there were 72/286 (25%) vessels causing ischaemia in 52/115 (45%) patients. Ninety-six/286 (34%) vessels were directly interrogated with FFR (**Table 1**).

Fully automatic and semi-automatic CT-MPI analyses

The time needed to post-process a CT-MPI dataset was 6-8 min. For the semi-automatic analysis the operator visually inspected the polar maps and manually positioned the VOIs, which required an additional 2-3 min per case. The fully automatic analysis required no additional time. The fully automatic analysis was found to have worse performance when compared to the semi-automatic analysis. Myocardial blood flow had moderate performance (AUC 0.69; 0.62-0.76) in the fully automatic analysis, which improved with semi-automatic analysis (AUC 0.87; 0.83-0.92) ($p<0.001$). Similar findings were observed for the other two parameters (**Table 2**).

Endocardial and epicardial measurements

Myocardial blood flow sampled in the endocardial layer of the myocardium (AUC 0.87; 0.83-0.92) performed better than transmural blood flow (AUC 0.82; 0.76-0.87) and epicardial blood flow (AUC 0.72; 0.65-0.79) ($p<0.05$). The endo/epicardial ratio did not improve performance (AUC 0.76; 0.68-0.83) (**Table 3**).

Absolute and relative perfusion parameters

Absolute perfusion parameters performed well (AUC range 0.87-0.89). Although there was no statistically significant improvement in diagnostic performance with the use of relative perfusion parameters (**Table 4**), when considering only intermediate lesions the AUC of relative blood flow was 9% larger than that of absolute blood flow (**Table 5**). Relative blood flow had the largest AUC (AUC 0.90; 0.85-0.95).

Relative perfused capillary blood volume was the parameter with the lowest ratio ischaemic/remote myocardium (cut-offs to identify ischaemia 0.64 in all vessels; 0.54 in vessels with intermediate lesions). This parameter had higher visual contrast compared to the other parameters (**Figure 2**).

A significant decrease in all perfusion parameters was observed below an FFR value of 0.80 ($p<0.05$) (**Figure 3**).

Intra- and inter-observer agreement

The semi-automatic analysis of 97 myocardial territories (37 patients) yielded intra- and inter-observer ICC's (95% CI) of 0.975 (0.963-0.983) and 0.945 (0.919-0.963) for blood flow; 0.938 (0.908-0.958) and 0.906 (0.862-0.936) for perfused capillary blood volume; 0.977 (0.965-0.984) and 0.944 (0.918-0.962) for first pass distribution volume, respectively.

DISCUSSION

The main findings of this study were: 1) a semi-automatic analysis of dynamic CT-MPI with minimal additional operator time performed better than fully automatic analysis (17-segment model) in the diagnosis of myocardial ischaemia; 2) sampling perfusion in the endocardial layer of the myocardium made perfusion defects more conspicuous, likely a reflection of the pathophysiological wave-front phenomenon of ischaemia. The endo/epicardial ratio did not improve diagnostic performance; 3) Relative blood flow had the best AUC; 4) a significant decrease in perfusion was observed below an invasive FFR of 0.80.

Previous studies have provided validation of dynamic CT-MPI in patients using a variety of reference standards [7-15]. The analysis methods used, however, were heterogeneous. Some groups used axial images of the thorax and manually drew ROIs on the myocardium to sample blood flow [7], while others manually re-sliced the dataset into short and long axis views of the left ventricle according to classic cardiac planes [9-15]. These approaches were operator-dependent and time consuming. Availability of a standardized analysis method is a pre-requisite to define broadly applicable thresholds for the differentiation of normal and abnormal perfusion, and implement CT-MPI in clinical practice.

Automatic software with limited user interference should benefit the standardization of the CT-MPI analysis procedure. Several commercial software packages offer segmentation of the left ventricle from CT data and the construction of polar maps (bulls-eye plots) according to standard AHA myocardial 17-segment anatomy [19]. In this study, we found that perfusion values of myocardial segments derived fully automatically did not perform as well as measurements obtained by an operator who identified areas of reduced perfusion on the color-coded polar maps. This may be explained by the fully automatic approach possibly diluting perfusion defects within a segment (partial volume effect), especially when perfusion defects are located at the border of two or more adjacent myocardial segments. It is likely that the operator corrected for this. Also, the quantification of perfusion parameters relies on precise demarcation of the myocardial territory downstream to a coronary vessel. Myocardial segments are usually assigned to vascular segments following assumptions based on the most frequent vascular distribution pattern. Coronary anatomy may vary affecting the boundaries of vascular territories. The standard assignment of myocardial segments to vascular territories proposed by the AHA 17-segment model may lead in some cases to incorrect identification of the target vessel [25-27]. CT can provide integrated coronary artery and myocardial anatomy. The length of vessels such as the RCA (coronary dominance), the LAD and the number of diagonal and marginal branches are accepted determinants of myocardial segment reclassification [26].

Presence of a transmural blood flow gradient through the myocardium is a well-known phenomenon [28]. The endocardial layer of the myocardium is more sensitive to ischaemia than the epicardial layer

due to lower auto-regulatory pressure limits, higher metabolic demand and higher oxygen consumption . This principle lies at the basis of the assessment of the transmurality [29, 30] or the transmural attenuation ratio applied in static CT-MPI to identify ischaemia [31-34]. While the endocardial analysis made perfusion defects more conspicuous, in this study the endo/epicardial ratio did not improve diagnostic performance.

Previous physiological studies using electron beam computed tomography (EBCT) and positron emission tomography (PET) [35, 36] showed that myocardial blood flow was a direct index of myocardial perfusion. In this study, we evaluated two additional parameters. First pass distribution volume reflects the kinetics of iodinated contrast agents, which exit the vascular space and distribute throughout the intravascular and extra-vascular spaces. The perfused capillary blood volume quantifies the intravascular contrast agent component only [37].

Perfused capillary blood volume was associated with the lowest ischaemic/remote myocardium ratio and the highest image contrast. A potential explanation may be that this parameter directly reflects the intra-vascular space (not extra-vascular) during hyperaemic stress, which is characterized by fast flow. This parameter may be more sensitive than others to the functionally recruitable component of myocardial capillary vessels [38]. We hypothesize that this parameter may allow perfusion changes to be more conspicuous and easily appreciated by the operator, which may be useful for the accurate sampling of perfusion on color-coded polar maps.

Clinical implementation of dynamic CT-MPI

In dynamic CT-MPI the heart is imaged repeatedly to capture the arrival of the contrast bolus and its wash-out during first-pass circulation [1-4]. Based on physiological models temporal changes in myocardial attenuation, normalized to the enhancement of the blood pool, allow for calculation of quantitative measures of myocardial perfusion. In static CT-MPI, a snapshot of myocardial attenuation is acquired as a single dataset displaying the instantaneous variation in myocardial attenuation as a result of differences in perfusion. The detection of regional perfusion defects is qualitative or normalized to attenuation in the remote myocardium or left ventricular cavity [39].

Quantitative perfusion may be advantageous for uncovering balanced ischaemia associated with global left ventricular reduction of blood flow, which may be disguised by qualitative approaches. Studies using PET [40, 41] have shown the adverse prognostic value of (homogenously) reduced myocardial blood flow in the context of microvascular disease [42]. The demonstration of coronary anatomy by CTCA in conjunction with quantitative CT-MPI could allow for a comprehensive evaluation of epicardial coronary artery disease and microvascular dysfunction.

However, there are also challenges to the clinical implementation of dynamic CT-MPI. First, while values of myocardial blood flow in healthy subjects during hyperaemic stress were found in the range of 200–500 ml/100 g/min by PET [43], the values in this as well as other CT-MPI studies were

lower. Ranges of normal and abnormal blood flow obtained by PET [44, 45] and magnetic resonance imaging [46] were also different. This may be related to study design, samples sizes, image acquisition and post-processing methods, reference standards, as well as age and gender, coronary risk profile and prevalence of coronary artery disease. Also, limitation of CT in the temporal sampling of the hyperaemic intra-capillary first pass of contrast may partly explain the difference. The first-pass extraction of iodine into the normal myocardium under hyperaemia is low and purely intravascular transit times are short. Measuring these more correctly would require a sampling rate that would significantly increase radiation exposure [47]. The underestimation predominantly affects absolute values in the normal myocardium and as our results show, this does not appear to strongly diminish the discriminating power of CT-MPI based blood flow. It is noteworthy that the CT-MPI specific parameter perfused capillary blood volume, which has a smaller dependence on temporal sampling, therefore also exhibits a substantially higher ischaemic contrast. Underestimation of hyperaemic perfusion may also occur in the event of suboptimal adenosine vasodilator response. Based on the hypothesis that blood flow, once normalized to remote myocardium, should be less affected by these factors, Kono et al. [48] and Wichmann et al. [49] demonstrated a better diagnostic performance of relative blood flow compared to absolute blood flow. Our study confirmed this in territories downstream to intermediate lesions. Intermediate lesions may lead to a milder decline in perfusion compared to severe stenoses. Also, intermediate lesions may have an FFR value which sits within the known “grey zone” for FFR with high test-retest variability [50].

Secondly, adding CT-MPI to CTCA increases patient radiation and contrast agent exposure. The risk of contrast induced nephropathy can be minimized by excluding patients with impaired kidney function. In our study, decreasing tube voltage from 100kV to 80kV was associated with 40% decrease in effective dose. Further developments e.g. improved detector technology, lower tube voltage using more powerful X-ray generators, and iterative reconstruction algorithms are expected to further decrease radiation exposure.

Study limitations

We acknowledge some study limitations. FFR was not performed in angiographically normal or near-normal vessels and in vessels with ≥80% stenosis at QCA. This is in keeping with clinical standards. An 80% stenosis at QCA is likely to correspond to a 90% visual stenosis (“oculo-stenotic reflex”) [22]. By applying this conservative approach in the study, we were unlikely to functionally misclassify anatomically severe lesions [18, 22]. Although regarded as the clinical standard of reference for functional classification of coronary disease, FFR is a measure of pressure and reflects the functional consequence of narrowing in epicardial coronary vessels. Myocardial perfusion depends on epicardial coronary disease as well as microvasculature within the myocardium. In our study, the presence of significant microvascular dysfunction was largely avoided by excluding patients with severely impaired left ventricular ejection fraction. Thresholds were derived in this study using the Youden index and require validation in external cohorts. The effect played by age, gender, cardiovascular risk factors and other potential confounders also require definition in larger studies.

Lastly, our study did not include patients with known coronary artery disease and previous myocardial infarction. In a patient cohort with known myocardial infarction, Bamberg et al. [8] demonstrated that first pass distribution volume was significantly lower in infarcted myocardial segments compared to ischaemic but viable myocardium. Infarcted tissue is characterized by a lower density of capillary vessels compared to non-infarcted myocardium. Because first pass distribution volume is the ratio between the peak of the myocardial time attenuation curve and the peak of the arterial input function, in infarcted areas this may translate into lower peak enhancement and lower first pass distribution volume [8]. This observation potentially broadens the ability of CT-MPI to characterize not only ischaemic vs. non-ischaemic, but also viable vs. non-viable myocardium from a single dynamic scan.

Conclusions

Our study generates the hypothesis that endocardial perfusion parameters obtained by semi-automatic analysis of dynamic CT-MPI may permit robust discrimination between coronary vessels causing ischaemia vs. not causing ischaemia.

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DISCLOSURES

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Figure legends

Figure 1. Inclusion procedure

Patients with symptoms suggestive of coronary artery disease were referred for ICA and prospectively enrolled to undergo CT-MPI before ICA.

Footnote: Partial data from 45 patients included in the current study were reported previously in a proof-of-principle study [12]. That report however did not evaluate multi-parametric data and analysis methods.

Figure 2. Post-processing, fully automatic and semi-automatic analyses of dynamic CT-MPI

Panel A: attenuation changes in the aorta and in the myocardium were used to construct arterial input function (red curve) and tissue time attenuation curves (blue line), respectively. Curves were fit to a dedicated two-compartment model (intra- and extra-vascular spaces). Perfused capillary blood volume (PCBV) was derived from the model. Myocardial blood flow (BF) and first-pass distribution volume (FPDV) were calculated from the fit curves, as shown.
Panel B: fully automatic analysis of BF, PCBV, and FPDV. Parameter values were available for each myocardial segment of the polar map according to the 17-segment American Heart Association (AHA) model. In each vascular territory of the myocardium (LAD, LCx and RCA), the segment with the lowest value for each parameter was picked and compared to ICA/FFR for the detection of ischaemia.
Panel C: in the semi-automatic analysis, volumes of interest (VOIs, circles) were placed manually on the polar map, guided by color-coding regardless of the segmental grid imposed by the 17-segment model.

Footnote: Color scales: BF: 0-200 ml/100ml/min; PCBV: 0-15 ml/100ml; FPDV: 0-20 ml/100ml.

Figure 3. Relationship between perfusion parameters and FFR

Panel A: scatterplots show relative myocardial blood flow, perfused capillary blood volume and first pass distribution volume by invasive FFR. This study could not demonstrate significant differences between men *(dark dots)* and women *(white dots).*
Panel B: box and whisker plots show median values (interquartile ranges) of relative blood flow, perfused capillary blood volume and first pass distribution volume for FFR ranges, as shown. A significant decrease in perfusion was observed below an FFR value of 0.80 (all p-values <0.05).

Footnote: * p-value from Kruskal Wallis test; ** p-value from Mann-Whitney U test.

TABLES

Table 1. Baseline characteristics and main ICA/FFR findings (n=115).

Characteristics	Total (n=115)
Men	88 (77%)
Age (years)	57 ± 9
Body mass index (kg/m^2)	29 ± 5
Risk factors	
Diabetes mellitus *	39 (34%)
Hypertension †	66 (57%)
Dyslipidemia ‡	94 (82%)
Current smoker	72 (63%)
Family history of coronary artery disease §	50 (43%)
Agatston calcium score: median (IQR)	140 (21-467)
Right dominant coronary system	92 (80%)
Heart rate (beats/min)	
Baseline	68 ± 11
During adenosine stress	91 ± 15
Systolic blood pressure (mmHg)	
Baseline	141 ± 22
During adenosine stress	137 ± 21
Diastolic blood pressure (mmHg)	
Baseline	79 ± 10
During adenosine stress	75 ± 13
Diameter narrowing on QCA: median (IQR)	
Mild (≤30%) coronary lesions (n=174)	18% (11-24%)
Intermediate (30-80%) coronary lesions (n=75)	49% (40-59%)
Severe (≥80%) coronary lesions (n=37)	94% (85-100%)
Fractional flow reserve: median (IQR)	0.83 (0.75-0.88)
FFR range	0.46-0.99
Patients with functionally significant coronary lesion causing ischaemia ‖	52 (45%)
One-vessel disease	36 (31%)
Two-vessel disease	12 (10%)
Three-vessel disease	4 (4%)
Number of vessel evaluated	286
Vessels with functionally significant coronary lesion causing ischaemia ‖	72/286 (25%)
Right coronary artery	22 (8%)
Left main/left anterior descending coronary artery	34 (12%)
Left circumflex artery	16 (5%)

Values are means ± standard deviations, or frequencies (percentages), unless otherwise specified.

* Treatment with oral anti-diabetic medication or insulin; † Blood pressure ≥140/90 mmHg or treatment for hypertension; ‡ Total cholesterol >180 mg/dl or treatment for hypercholesterolemia; § Family history of coronary artery disease having first- or second- degree relatives with premature coronary artery disease (age<55 years).

[ǁ] Functionally significant coronary lesion defined as FFR ≤0.80 or QCA diameter narrowing ≥80%.

Table 2. Fully automatic and semi-automatic analyses for vessel-specific ischaemia

	Vessels not causing ischaemia	Vessels causing ischaemia	AUC (95% CI)	p-value *
All vessels (n= 286)				
	n=214	**n=72**		
Myocardial blood flow; ml/100ml/min				
Fully automatic	143 (122-167)	118 (93-146)	0.69 (0.62-0.76)	**<0.001**
Semi-automatic	161 (126-191)	92 (74-109)	0.87 (0.83-0.92)	
Perfused capillary blood volume; ml/100ml				
Fully automatic	8.8 (7.0-10.6)	6.8 (4.7-8.6)	0.69 (0.62-0.77)	**<0.001**
Semi-automatic	10.7 (7.8-13.3)	4.1 (3.1-5.7)	0.89 (0.84-0.93)	
First pass distribution volume; ml/100ml				
Fully automatic	17.1 (14.9-19.0)	14.1 (10.7-16.6)	0.72 (0.64-0.79)	**<0.001**
Semi-automatic	18.9 (15.7-21.4)	11.3 (9.1-13.6)	0.89 (0.84-0.93)	
Vessels interrogated with FFR (n=96)				
	n=59	**n=37**		
Myocardial blood flow; ml/100ml/min				
Fully automatic	142 (119-159)	141 (122-159)	0.50 (0.38-0.62)	**<0.001**
Semi-automatic	150 (114-178)	102 (89-121)	0.76 (0.66-0.86)	
Perfused capillary blood volume; ml/100ml				
Fully automatic	9.0 (7.3-10.6)	8.4 (6.8-10.3)	0.55 (0.43-0.66)	**<0.001**
Semi-automatic	9.8 (6.1-12.8)	4.4 (2.7-7.2)	0.82 (0.73-0.91)	
First pass distribution volume; ml/100ml				
Fully- automatic	17.0 (15.6-18.5)	16.1 (14.4-17.9)	0.58 (0.46-0.70)	**<0.001**
Semi-automatic	18.6 (14.7-20.7)	13.3 (11.6-15.3)	0.79 (0.70-0.87)	

Values are medians (IQR). This study could not demonstrate significant differences between men and women.
AUC= area under the curve; CI= confidence intervals.
Results from endocardial analysis. Functionally significant coronary lesion defined as FFR ≤0.80 or QCA diameter narrowing ≥80%.
* p-value from DeLong test comparing AUC's of fully-automatic and semi-automatic analyses. Significant p-values in **bold.**

Table 3. Myocardial blood flow from transmural, endocardial, epicardial myocardium and endo/epicardial ratio

					p-value *												
	Vessels not causing ischaemia	**Vessels causing ischaemia**	**p-value †**	**AUC (95% CI)**	**Trans-mural**	**Endo**	**Epi**	**Endo/epi ratio**	**Youden index**	**Cut-off value ‡**	**TP**	**TN**	**FP**	**FN**	**Sens, % (95% CI)**	**Spec, % (95% CI)**	**PPV, % (95% CI)**	**NPV, % (95% CI)**
Myocardial blood flow; ml/100ml/min																		
All vessels (n=286)																		
	n=214	**n=72**																
Transmural	149 (118-177)	96 (80-120)	<0.001	0.82 (0.76-0.87)	-	**0.001**	**<0.001**	0.770	0.517	114	51	173	41	21	0.708 (0.572-0.815)	0.808 (0.732-0.867)	0.554 (0.436-0.667)	0.892 (0.826-0.935)
Endo	161 (126-191)	92 (74-109)	<0.001	0.87 (0.83-0.92)	**0.001**	-	**<0.001**	**0.017**	0.638	106	54	189	25	18	0.750 (0.625-0.844)	0.883 (0.816-0.928)	0.684 (0.551-0.792)	0.913 (0.856-0.949)
Epi	140 (109-169)	100 (82-140)	<0.001	0.72 (0.65-0.79)	**<0.001**	**<0.001**	-	1.000	0.372	118	47	153	61	25	0.653 (0.519-0.766)	0.715 (0.626-0.790)	0.435 (0.333-0.544)	0.860 (0.786-0.911)
Endo/epi ratio	1.13 (1.01-1.26)	0.88 (0.73-1.07)	<0.001	0.76 (0.68-0.83)	0.770	**0.017**	1.000	-	0.490	0.91	43	188	26	29	0.597 (0.489-0.697)	0.879 (0.822-0.919)	0.623 (0.496-0.735)	0.866 (0.815-0.905)
Vessels interrogated with FFR (n=96)																		
	n=59	**n=37**																
Transmural	149 (114-171)	113 (93-135)	0.001	0.70 (0.59-0.81)	-	0.120	**0.001**	1.000	0.397	146	31	33	26	6	0.838 (0.685-0.925)	0.559 (0.404-0.704)	0.544 (0.399-0.681)	0.846 (0.705-0.927)
Endo	150 (114-178)	102 (89-121)	<0.001	0.76 (0.66-0.86)	0.120	-	**0.008**	1.000	0.475	145	32	36	23	5	0.865 (0.724-0.940)	0.610 (0.462-0.741)	0.582 (0.437-0.714)	0.878 (0.737-0.949)
Epi	145 (109-167)	121 (93-148)	0.056	0.62 (0.50-0.73)	**0.001**	**0.008**	-	0.580	0.262	127	21	41	18	16	0.568 (0.372-0.744)	0.695 (0.536-0.818)	0.538 (0.370-0.698)	0.719 (0.563-0.836)
Endo/epi ratio	1.09 (0.96-1.19)	0.86 (0.76-1.00)	<0.001	0.74 (0.63-0.86)	1.000	1.000	0.580	-	0.533	0.93	26	47	12	11	0.703 (0.553-0.819)	0.797 (0.679-0.879)	0.684 (0.525-0.809)	0.810 (0.699-0.887)

Values are medians (IQR).
AUC= area under the curve; CI= confidence intervals; TP= true positive; TN= true negative; FP= false positive; FN= false negative; PPV= positive predictive value; NPV= negative predictive value.

Results from semi-automatic analysis. Functionally significant coronary lesion defined as FFR ≤0.80 or QCA diameter narrowing ≥80%.
* p-value from DeLong test comparing AUC's of different approaches. Significant p-values in **bold.**
† p-value from Mann-Whitney U test comparing vessels not causing ischaemia vs. vessels causing ischaemia.
‡ cut-off value calculated according to the Youden index.

Table 4. Absolute and relative myocardial blood flow, perfused capillary blood volume and first-pass distribution volume (n=286 vascular territories)

	Vessels not causing ischaemia (n=214)	Vessels causing ischaemia (n=72)	p-value †	AUC (95% CI)	p-value *						Youden index	Cut-off value ‡	TP	TN	FP	FN	Sens, % (95% CI)	Spec, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
					Myocardial blood flow		Perfused capillary blood volume		First pass distribution volume											
					Absolute	Relative	Absolute	Relative	Absolute	Relative										
Myocardial blood flow; ml/100ml/min																				
Absolute	161 (126-191)	92 (74-109)	<0.001	0.87 (0.83-0.92)	-	0.833	1.000	1.000	1.000	1.000	0.638	106	54	189	25	18	0.750 (0.624-0.844)	0.883 (0.816-0.928)	0.683 (0.551-0.792)	0.913 (0.856-0.949)
Relative	0.92 (0.79-0.99)	0.58 (0.45-0.68)	<0.001	0.90 (0.85-0.95)	0.833	-	1.000	0.197	1.000	1.000	0.725	0.73	63	179	35	9	0.875 (0.787-0.930)	0.836 (0.781-0.880)	0.643 (0.534-0.738)	0.952 (0.911-0.975)
Perfused capillary blood volume; ml/100ml																				
Absolute	10.7 (7.8-13.3)	4.1 (3.1-5.7)	<0.001	0.89 (0.84-0.93)	1.000	1.000	-	1.000	1.000	1.000	0.656	6.7	59	179	35	13	0.819 (0.712-0.893)	0.836 (0.769-0.887)	0.628 (0.503-0.737)	0.932 (0.884-0.961)
Relative	0.85 (0.69-0.99)	0.39 (0.28-0.56)	<0.001	0.88 (0.82-0.93)	1.000	0.197	1.000	-	1.000	1.000	0.665	0.64	62	170	44	10	0.861 (0.774-0.918)	0.794 (0.733-0.844)	0.585 (0.473-0.688)	0.944 (0.900-0.970)
First pass distribution volume; ml/100ml																				
Absolute	18.9 (15.7-21.4)	11.3 (9.1-13.6)	<0.001	0.89 (0.84-0.93)	1.000	1.000	1.000	1.000	-	1.000	0.641	15.6	63	161	53	9	0.875 (0.783-0.932)	0.752 (0.678-0.814)	0.543 (0.439-0.644)	0.947 (0.902-0.972)
Relative	0.94 (0.83-0.99)	0.63 (0.48-0.75)	<0.001	0.89 (0.85-0.94)	1.000	1.000	1.000	1.000	1.000	-	0.684	0.79	62	173	41	10	0.861 (0.772-0.919)	0.808 (0.750-0.856)	0.602 (0.496-0.699)	0.945 (0.902-0.970)
CTCA	-	-	-	0.79 (0.71-0.86)	-	-	-	-	-	-	-	≥70%	43	208	6	29	0.597 (0.476-0.707)	0.972 (0.924-0.990)	0.878 (0.717-0.953)	0.878 (0.825-0.916)

Values are medians (IQR). This study could not demonstrate significant differences between men and women.
CTCA= CT coronary angiography. AUC= area under the curve; CI= confidence intervals; TP= true positive; TN= true negative; FP= false positive; FN= false negative; PPV= positive predictive value; NPV= negative predictive value.

Results from semi-automatic endocardial analysis. Functionally significant coronary lesion defined as FFR ≤0.80 or quantitative coronary angiography diameter narrowing ≥80%.
* p-value from DeLong test comparing AUC's of perfusion parameters.
† p-value from Mann-Whitney U test comparing vessels not causing ischaemia vs. vessels causing ischaemia.
‡ cut-off value calculated according to the Youden index, except for CTCA (≥70% diameter reduction as visually assessed) whose diagnostic performance is reported for comparison.

Table 5. Absolute and relative myocardial blood flow, perfused capillary blood volume and first-pass distribution volume in intermediate lesions directly interrogated with FFR (n=96 vascular territories)

	Vessels not causing ischaemia (n=59)	Vessels causing ischaemia (n=37)	p-value †	AUC (95% CI)	p-value * Myocardial blood flow		Perfused capillary blood volume		First pass distribution volume		Youden index	Cut-off value ‡	TP	TN	FP	FN	Sens, % (95% CI)	Spec, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
					Absolute	Relative	Absolute	Relative	Absolute	Relative										
Myocardial blood flow; ml/100ml/min																				
Absolute	150 (114-178)	102 (89-121)	<0.001	0.76 (0.66-0.86)	-	0.064	0.266	0.359	1.000	0.100	0.475	145	32	36	23	5	0.865 (0.724-0.940)	0.610 (0.462-0.741)	0.582 (0.437-0.714)	0.878 (0.737-0.949)
Relative	0.91 (0.73-0.98)	0.62 (0.55-0.68)	<0.001	0.85 (0.76-0.94)	0.064	-	1.000	1.000	0.422	1.000	0.638	0.75	33	43	16	4	0.892 (0.763-0.955)	0.729 (0.594-0.831)	0.673 (0.520-0.797)	0.915 (0.797-0.967)
Perfused capillary blood volume; ml/100ml																				
Absolute	9.8 (6.1-12.8)	4.4 (2.7-7.2)	<0.001	0.82 (0.73-0.91)	0.266	1.000	-	1.000	1.000	1.000	0.570	8.3	33	39	20	4	0.892 (0.757-0.956)	0.661 (0.516-0.781)	0.623 (0.469-0.755)	0.907 (0.781-0.964)
Relative	0.81 (0.57-1.00)	0.39 (0.24-0.50)	<0.001	0.83 (0.74-0.92)	0.359	1.000	1.000	-	1.000	1.000	0.614	0.54	29	47	12	8	0.784 (0.619-0.890)	0.797 (0.665-0.886)	0.707 (0.535-0.835)	0.855 (0.729-0.928)
First pass distribution volume; ml/100ml																				
Absolute	18.6 (14.7-20.7)	13.3 (11.6-15.3)	<0.001	0.79 (0.70-0.89)	1.000	0.422	1.000	1.000	-	0.579	0.550	16.3	31	41	18	6	0.838 (0.692-0.922)	0.695 (0.547-0.811)	0.633 (0.481-0.762)	0.872 (0.745-0.941)
Relative	0.94 (0.79-0.99)	0.69 (0.59-0.76)	<0.001	0.84 (0.75-0.93)	0.100	1.000	1.000	01.000	0.579	-	0.601	0.79	31	43	16	6	0.838 (0.698-0.920)	0.729 (0.694-0.831)	0.660 (0.505-0.786)	0.878 (0.750-0.945)
CTCA	-	-	-	0.65 (0.53-0.77)	-	-	-	-	-	-	-	≥70%	13	56	3	24	0.351 (0.207-0.529)	0.949 (0.809-0.988)	0.813 (0.498-0.950)	0.700 (0.588-0.792)

Values are medians (IQR).
CTCA= CT coronary angiography. AUC= area under the curve; CI= confidence intervals; TP= true positive; TN= true negative; FP= false positive; FN= false negative; PPV= positive predictive value; NPV= negative predictive value.

Results from semi-automatic endocardial analysis. Functionally significant coronary lesion defined as FFR ≤0.80.
* p-value from DeLong test comparing AUC's of perfusion parameters.
† p-value from Mann-Whitney U test comparing vessels not causing ischaemia vs. vessels causing ischaemia.
‡ cut-off value calculated according to the Youden index, except for CTCA (≥70% diameter reduction as visually assessed) whose diagnostic performance is reported for comparison.