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Precision of Myocardial Blood Flow and Flow Reserve measurement during CZT-SPECT perfusion imaging processing: intra- and inter-observer variability

Running title: SPECT MBF & MFR variability

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ABSTRACT

The aim of this study was to evaluate the reproducibility of myocardial blood flow (MBF) and flow reserve (MFR) measurement in patients referred for dynamic SPECT. **Methods**: We retrospectively analyzed patients referred for Myocardial Perfusion Imaging (MPI). SPECT data were acquired on a CZT-based pinhole cardiac camera (Discovery NM530c, GE Healthcare, Haïfa, Israël) in listmode using a stress (251 ± 15 MBq) / rest (512 ± 26 MBq) one-day Tc-99m-tetrofosmin protocol. Kinetic analyses were done with Corridor4DM^{TM} software using a 1-tissue-compartment model and converted to MBF using a previously determined extraction fraction correction. MFR was analyzed and compared globally and regionally. Motion detection was applied, but no attenuation correction. **Results**: 124 patients (64 male, 60 female) were included, and SPECT acquisitions were twice reconstructed by the same nuclear medicine board certified physician for 50 patients and by two different physicians for 74. Both intra- and inter-observer measurements of global MFR had no significant bias (-0.01 (p=0.94) and 0.01 (p=0.67) respectively). However, rMBF and sMBF were significantly different on global LV evaluation (p=0.001 and p=0.002 respectively) and on the anterior territory (p<0.0001) on the inter-user analysis. The average coefficient of variation was between 15% and 30% of the mean sMBF if the analysis is performed by the same or by two different nuclear medicine physicians, and around 20% of the mean MFR independently of the processing physician. Using the MFR threshold of 2, we noticed a good intra-user agreement, whereas it was moderate when the observers were different (kappa of 0.75, 95%IC: 0.56-0.94 vs 0.56, 95%IC:0.36-0.75 respectively). **Conclusion**: Repeated measurements of global MFR by the same or two different physicians are similar with an average coefficient of variation of 20%. Better reproducibility is achieved for intra-user MBF evaluation. An automatization of the processing is needed to improve the reproducibility.

Keywords: Myocardial blood flow; myocardial flow reserve; CZT-SPECT; variability

Clinical Trial Registration: CFR-OR trial (clinicaltrials.gov unique identifier NCT03586492). https://clinicaltrials.gov/ct2/show/NCT03586492

INTRODUCTION

Stress and Rest Myocardial Blood Flow (MBF, sMBF and rMBF) and Myocardial Flow Reserve (MFR) derived from Positron emission tomography (PET) perfusion imaging have shown to provide added diagnostic (*1*,*2*) and prognostic (*3*) information than relative perfusion analysis alone. Several studies have shown that clinical measurement of MBF and MFR using dynamic CZT-SPECT Myocardial Perfusion Imaging (MPI) with ^{99m}Tc radiopharmaceuticals is technically possible, resulting in similar MFR when compared to PET (*4*-*9*).

However, with the idea of a larger clinical use, there is a need to evaluate the precision and reproducibility of this measurement. A day-to-day test-retest precision in a group of 30 patients using a dedicated cardiac camera found that the standard deviation (SD) in the difference of the measured MBF values was around 30%, including physiological and processing variability (*10*). A recent simulation study was conducted to evaluate the impact of SPECT MFR imprecision on confidence of clinically relevant categorization. The authors concluded that current SPECT MFR precision as categorization with high confidence (> 80%) was only achieved for extreme MFR values (< 1.0 or > 2.5), with correct classification in only 15% of patients in a typical lab with MFR of 1.8 ± 0.5 (*11*). A third paper evaluated intra- and inter-observer repeatability of MBF and MFR values obtained by the same operator and two independent operators on 57 patients. This study showed quite good reproducibility in the whole myocardium, Left Anterior Descending artery (LAD), and Left Circumflex (LCx) vascular territories, but poor in the Right Coronary artery (RCA) territory (*12*).

In this study, we evaluated the intra- and inter-user processing repeatability of global and regional SPECT MBF and MFR in a larger cohort of patients.

MATERIALS AND METHODS

Patient Population

From October 2018 to January 2021, 128 patients referred for SPECT MPI with MBF and MFR quantification and addressed to two Nuclear Medicine departments, initially enrolled in the CFR-OR trial for coronary artery disease screening (*13*) (clinicaltrials.gov unique identifier NCT03586492), were retrospectively reconstructed and analyzed. The study protocol was approved by the institutional review board and the procedures were in accordance with the Declaration of Helsinki. Inclusion criteria was dynamic SPECT MPI. Every patient received information and gave written informed consent.

Exclusion criteria included missing files for new processing or technical issues.

Technical issues were reported for MBF and MFR measurement in 4 patients (late acquisition after injection). Flow chart of the study is displayed in FIGURE 1.

SPECT Acquisition

List-mode acquisitions were performed on two same Discovery NM530c cardiac CZT cameras (General Electric Healthcare, Haifa, Israel) in both departments. Initial injection of 37 MBq of ^{99m} Tc-tetrofosmin was used to center the patient's heart in the field of view. Pharmacological stress was then performed using either a regadenoson (400 µg) injection or a dipyridamole perfusion (0.56 mg/kg), immediately followed with a 250 MBq of ^{99m}Tc-tetrofosmin injection at hyperemia peak, then flushed by 50 mL of saline to ensure consistent delivery of a tight bolus. Rest dynamic acquisition was realized 3-hours later, with an injection of 500 MBq of ^{99m}Tc-tetrofosmin.

SPECT MBF and MFR Quantification

Dynamic SPECT were reconstructed using Corridor 4DM^{TM} software (INVIA, Ann Arbor, MI, USA) on a Xeleris workstation (General Electric Healthcare, Haifa, Israel). SPECT initial list-mode was resampled into 12 × 10-sec and 8 × 30-sec frames. Partial volume value was set to 0.6; the correction factor for myocardial density was set to 1. The spillover from the myocardium to the blood pool activity was assumed negligible and was set to 0. The uptake rate K1 was related to MBF using the Renkin-Crone equation according to Leppo (*14*), using a net retention model, where A=0.874 and B=0.443.

$$\mathrm{K1} = \mathrm{MBF} * (1 - \mathrm{A} * \mathrm{e}^{-\frac{B}{MBF}})$$

Residual activity subtraction on rest images sets after the stress dose was always applied. Because our previous results (*15*) showed no difference in terms of MFR whether attenuation correction was applied or not, we did not apply it in this study. All MBF and MFR values are presented without attenuation correction. However, motion was detected for each patient, and the operator could choose to perform motion correction. No significant movement had been detected, resulting in no correction of data. Double product (heart rate x blood pressure) correction was employed for MBF correction in all studies.

All 124 patients were reconstructed and analyzed by the same expert nuclear medicine physician for the second analysis. 50 patients had been initially reconstructed by the same physician (i.e. intra-user analysis). 74 patients

had been analyzed at first reading by another nuclear medicine physician (i.e. inter-user analysis). The mean elapsed time between the two analysis was 12.8 months.

When available, results from invasive coronary angiography were collected. Coronary angiograms were visually assessed by the experienced interventional cardiologist responsible for the procedure. The angiograms were assessed according to the clinical routine considering available clinical data and patient history. According to the recent guidelines defining very high-risk patients in need of secondary prevention intervention we considered all patients with significant coronary artery plaque (≥ 50%) according to the angiographer conclusion (*16*). We put in perspective the MFR variability with results of invasive coronary angiography, globally and regionally for each vessel with significant lesion.

Statistical Analysis

Continuous variables are presented as means ± SD. Categorical variables are provided as total numbers in percent. Gaussian distribution was assessed using D'Agostino-Pearson normality test. When analyzing differences between two groups, we applied independent samples t-test when comparing continuous variables, and the $\chi 2$ test or Fisher's exact test as appropriate when comparing categorical variables. Between two paired groups, due to the non-normal distributed variables, Wilcoxon matched pairs signed rank test was applied. Spearman's correlation coefficients were computed between variables. Bland-Altman was used to calculate the bias and the limits of agreement. The precision between the two measurements was determined as the coefficient of variation (COV) in the measured difference (COV = SD in the percent difference). Strength of agreement between the observers was evaluated using Fleiss Kappa. $p<0.05$ was considered statistically significant. All analysis were performed using Prism 9.

RESULTS

124 patients (61 male, 63 female) were included; both sub-populations were comparable in terms of gender, age, BMI, cardiovascular risk factors and technical parameters (Table 1). Both intra- and inter-observer measurements of global MFR had no significant bias (-0.01 ($p=0.94$) and 0.01 ($p=0.67$) respectively) (Table 2). Regarding regional MFR, no significant difference was found either for intra- or inter-observer analysis. On the inter-user analysis, sMBF was significantly different on global LV evaluation ($p=0.0002$) and on the anterior territory (LAD) ($p<0.0001$); rMBF was also significantly different. Lower differences were found for intra-user sMBF evaluation; only sMBF LAD was

significantly different (p=0.04). Considering rMBF, no significant difference was found for intra-observer analysis (p=0.15). Bland-Altman analysis showed that the variation in the difference between repeated analyses was consistent across the range of sMBF and MFR considered (FIGURE 2).

Bland-Altman of the intra-user analysis also showed better precision in terms of MBF evaluation (FIGURE 2 B). The COV between MFR measurements was similar both for intra-user and inter-user evaluations, respectively 20.2% vs 18.9% for global LV MFR. This COV was similar, around 20% for all regional MFR territories and analysis; however, the COV was significantly lower for MBF evaluation on the intra-observer analysis, in comparison to the inter-user analysis: 14.8% vs 32.2% for global sMBF ($p<0.001$). For intra-observer sub-population, 17 patients had BMI>30, and for inter-observer sub-population, 27 patients had BMI>30. Obesity did not impact COV: 21.4% for BMI<30 and 17.9% for BMI>30 for intra-observer analysis, 17.3% for BMI<30 and 22.5% for BMI>30 for inter-observer analysis.

Using the MFR threshold of 2, we noticed a good agreement when the two measurements were made by the same physician, with consistent classification of 27 patients with MFR>2 and 17 patients with MFR<2 (88% of observed agreements; kappa of 0.75, 95%IC: 0.56-0.94). Among the 6 patients differently classified, 4 patients had very similar results around 2, with less than 0.2 difference (1.89 and 2.01 for example). However, this agreement became moderate when the observers were different (kappa of 0.56, 95%IC:0.36-0.75), with consistent classification of 41 patients with MFR>2 and 18 patients with MFR<2 (79.73% of observed agreements). 15 patients were classified differently, with only 2 patients with similar MFR results around 2 and less than 0.2 difference.

34 patients underwent invasive coronary angiography within 3 months. 7 patients had no significant lesion, 4 of them had global and regional MFR values > 2, on both analyses. The other 3 had MFR < 2 on both analyses. Among the 27 patients with lesions, 55 significant plaques were found (24 on LAD, 15 on LCx and 16 on RCA). 7 out of these 55 vessel lesions (12.7%) had discrepant MFR values: one below 2 and one above; mean difference 0.43 (0.34, 0.93 and 0.04 respectively in LAD, LCx and RCA territories).

DISCUSSION

In this study, SPECT sMBF and MFR remained globally similar between different measurements, whether the analysis was performed by the same or by two different physicians, except for sMBF (global LV and LAD territory)

and rMBF where significant differences were found for inter-user evaluation. Using the MFR threshold of 2, we reported good agreement when the analysis was performed by the same user.

With the development of CZT cardiac-dedicated SPECT systems, SPECT MBF and MFR have been shown to have a certain diagnostic value for patients with suspected or known CAD and represents a useful supplement to the conventional qualitative diagnostic methods (*13,17,18*). Like in PET, MFR > 2 has been considered as a normal value, resulting in a very low rate of cardiac events (*3,19*). Recent studies evaluated the day-to-day test-retest precision of sMBF and MFR. Using 82Rb PET, test-retest methodological precision for serial quantitative global myocardial perfusion minutes apart is ±10% (mean delta of SD at rest ±0.09, at stress ±0.23 mL/min/g) and for days apart is ±21% (mean delta of SD at rest ±0.2, at stress ±0.46 mL/min/g) reflecting added biological variability (*20*). Recently, some authors evaluated the day-to-day test-retest precision of SPECT global MBF and MFR between 28% and 31% and 33% to 38% respectively, considering all the processing approaches (use of attenuation correction or not, use of manual motion correction or not) (*10*). The day-to-day test-retest precision in their study included both the methodological variability but also the physiological variability in the patient imaged during two separate sessions, multiple days apart. Though this study reported both methodological and physiological variation, the authors noticed a higher variability for SPECT evaluation. Wells et al advanced the following hypothesis to explain this greater variation: the low extraction fraction of tetrofosmin, the greater statistical noise in the dynamic images and reduced resolution compared with PET, the latter leading to increased partial-volume effects and a need for larger spill-over corrections, and the additional variability introduced from the manual registration of externally acquired CT images when attenuation correction was applied (due to the fact that most cardiac-dedicated CZT SPECT systems are not hybrid).

The impact of attenuation correction and motion correction on MBF accuracy had been evaluated previously (*21*). Wells et al. agreed that attenuation correction had only a small benefit, which may have been offset by the variability due to manually registering the attenuation map. In this study, we did not apply attenuation correction because, in our experience like in other studies, MFR wasn't different whether it was applied or not (*7,15,21*) and also because most of CZT-SPECT cameras are not equipped with CT, so it may not be achievable in routine. Regarding motion correction, we evaluated case-by-case the need for manual registration; but no correction was needed.

Our study only focused on the processing variability (not on the physiological individual one). We reported a lower SPECT MFR COV around 20% compared to Wells et al. (33% to 38% respectively (*10*)), who evaluated both physiological and processing variation. A previous study, focusing on analysis only, with the same initial dynamic

image series, on a conventional dual-head camera with sestamibi SPECT MBF using FlowQuant™ software, reported SD of the differences around 0.30 mL/min/g with an average MBF of 1.5 mL/min/g giving a COV of 20% (*22*). However, we noticed a significantly lower COV on rest and stress MBF measurements when the processing is performed by the same physician (between 18.8% and 14.8% vs 25.3% and 32.2% respectively for intra-user and inter-user). This lower variation wasn't noticed on MFR, probably because of the ratio, considering that the variability between two different observers remains the same on sMBF and rMBF reconstructions. Our limits of agreement for global MFR were also very close to the results of a recent simulation study (*11*).

Regarding regional MFR, unlike Cichocki et al. (*12*), we did not notice a poor repeatability of MBF and MFR in RCA territory. Indeed, we even observed lower limits of agreement in the RCA territory on the Bland-Altman analysis. COV remained similar. However, we noticed a greater variability on LAD territory when processing was performed by different physicians. This might also be explained by poor automatic orientation of the heart axis during post-processing. Better automatic heart orientation and introduction of automatic motion correction should be likely to drastically improve inter-observer repeatability.

There is a need to increase the analysis precision of SPECT MBF and MFR as integrated assessment of stress MBF and MFR helps to improve diagnostic performances (*23*,*24*). sMBF is 2.7 mL/min/g in young healthy subjects (*25*). Considering a precision of 15% and 32% for intra- and inter-user processing, the lower 95% confidence limit would be 1.9mL/min/g and 1.2mL/min/g respectively. This remains a major limit to identify patients with moderate reduction of stress flow. In a previous study with invasive coronary angiography correlation, we identified the best sMBF SPECT threshold around 1.28mL/min/g (*13*). In their simulation study, Renaud et al. showed correct classification of up to only 34% of patients when $1.5 \leq$ true MFR ≤ 2.0. Categorization with high confidence (> 80%) was only achieved for extreme MFR values (< 1.0 or > 2.5), with correct classification in only 15% of patients with MFR of 1.8 ± 0.5 (*11*). Our results showed a better agreement when the analysis is performed by the same expert nuclear medicine physician. However, 20% of the patients were classified differently using the MFR threshold of 2 in our inter-user analysis of 74 patients. Considering the results of invasive coronary angiography on a smaller size, only 13% of patients were classified differently on a vessel-based analysis. This result is interesting because it counteracts the 20% variability of the MFR result we observed. At this time, clinical interpretation should remain cautious for SPECT global MFR around 2, and even more for regional MFR.

In fact, SPECT MFR variability is higher than in PET because many steps of the processing remain manual. SPECT MBF is a promising technique, but further work to improve its precision would enhance its potential clinical value, and there is a need for automation and standardization in the processing and software used.

This remains difficult, partly because of the lower SPECT spatial resolution and artifacts at the edge of the field of view that makes it more difficult for the software to automatically identify the location, size, and orientation of the heart. At this point, automated motion correction software such as what was recently proposed for PET imaging (*26*) should reduce variability, as may improvements in image quality provided by more advanced reconstruction approaches (*27*,*28*).

Our study has also a major limitation that needs to be acknowledged; we have not compared our results to MFR calculated in PET, which remains the gold standard. But, as mentioned before, several studies have shown similar quantification of MBF and MFR using dynamic CZT-SPECT MPI with ^{99m}Tc-sestamibi compared to PET (*4*-*7*). Moreover, we only focused on a processing variation, with the same initial dynamic data. To our knowledge, this work represents the largest study focusing on the intra- and inter-user variability of dynamic SPECT, with clinical impact.

CONCLUSION

The sMBF analysis precision, measured as the SD in the difference of sMBF measured, was between 15% and 30% of the mean sMBF if the analysis was performed by the same or by two different nuclear medicine physicians. On the other hand, the MFR analysis precision was around 20% independently of the processing physician. MFR remained similar between different measurements, both on global LV and on regional artery territories, whether the analysis was performed by the same or by two different physicians. Regarding the MFR threshold of 2, we noticed a good agreement of patient's classification when processing by the same physician, whereas it was moderate if this wasn't the case. However, the limits of agreement seemed to be quite wide regarding the threshold of MFR. Though dynamic SPECT is a promising, further work is mandatory to improve its precision and enhance its potential value before applying this technique to a wide clinical use. The major key point is a need for automation and standardization in the processing and software used.

DISCLOSURE

None regarding this study. This study was supported by CHR d'Orleans.

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KEY POINTS

QUESTION:

How repeatable are MBF and MFR values measured during dynamic SPECT?

PERTINENT FINDINGS:

124 patients were twice processed. The MFR analysis precision was around 20% independently of the processing physician; for sMBF it was between 15% and 30% if the analysis was performed by the same or by two different nuclear medicine physicians. Despite this, we noticed a quite good agreement of patient's classification.

IMPLICATIONS FOR PATIENT CARE:

Clinical interpretation should remain cautious for SPECT MFR around 2. There is a need for automation and standardization in the processing and software used to improve the reliability of this promising technique.

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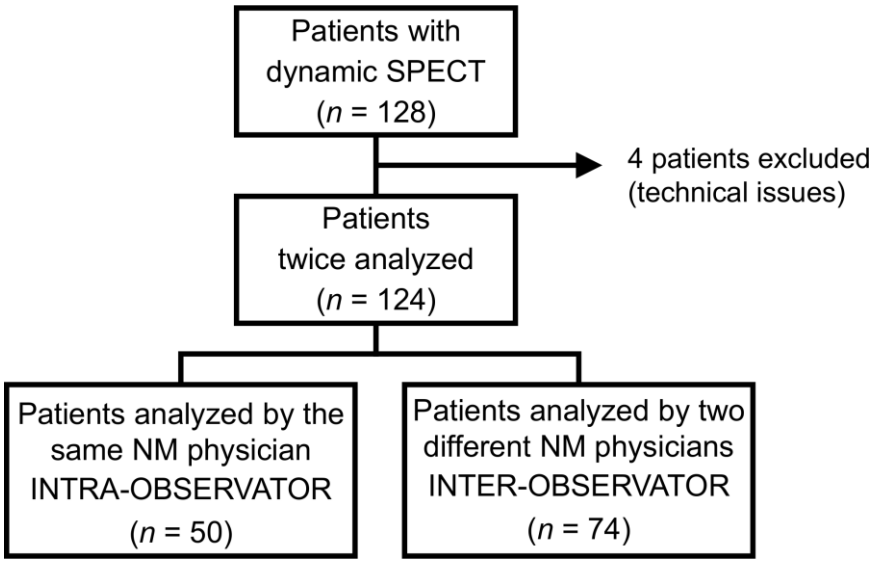

FIGURE 1 Flow chart of the study

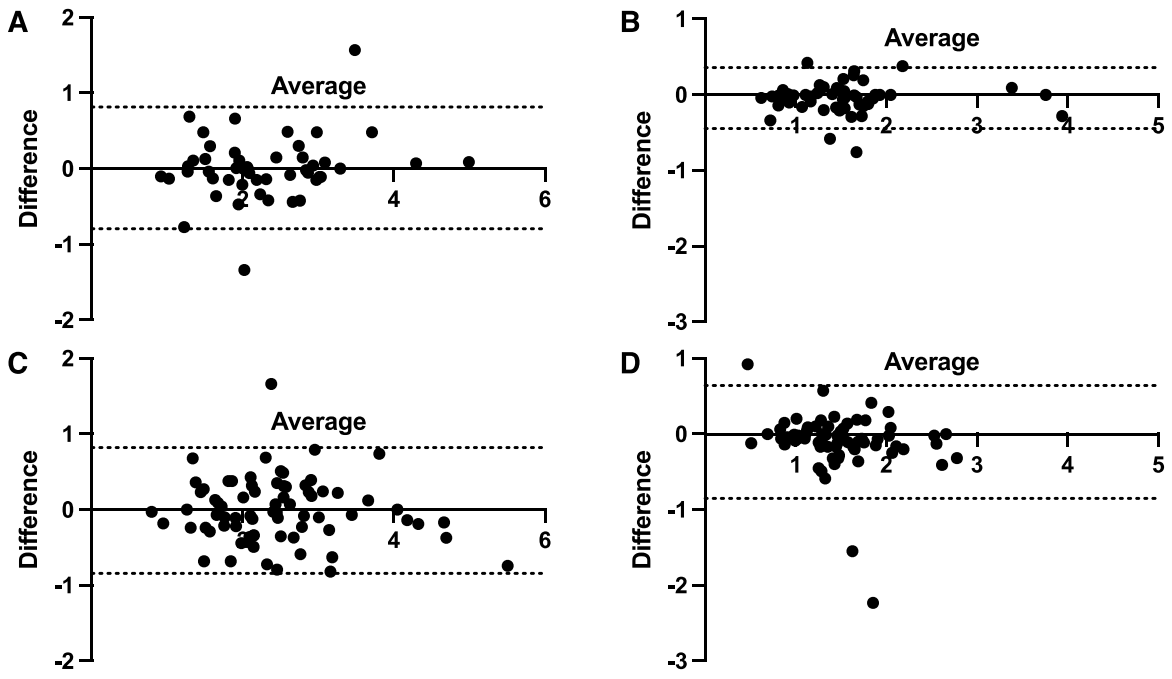

FIGURE 2 Differences in repeated measurements of myocardial flow reserve (MFR) and stress myocardial blood flow (sMBF) for intra-observator (**A** and **B** respectively) and inter-observator analysis (**C** and **D** respectively). 95% confidence limits are shown with dashed lines. Results are displayed for global left ventricle; results for regional analysis were similar.

GRAPHICAL ABSTRACT

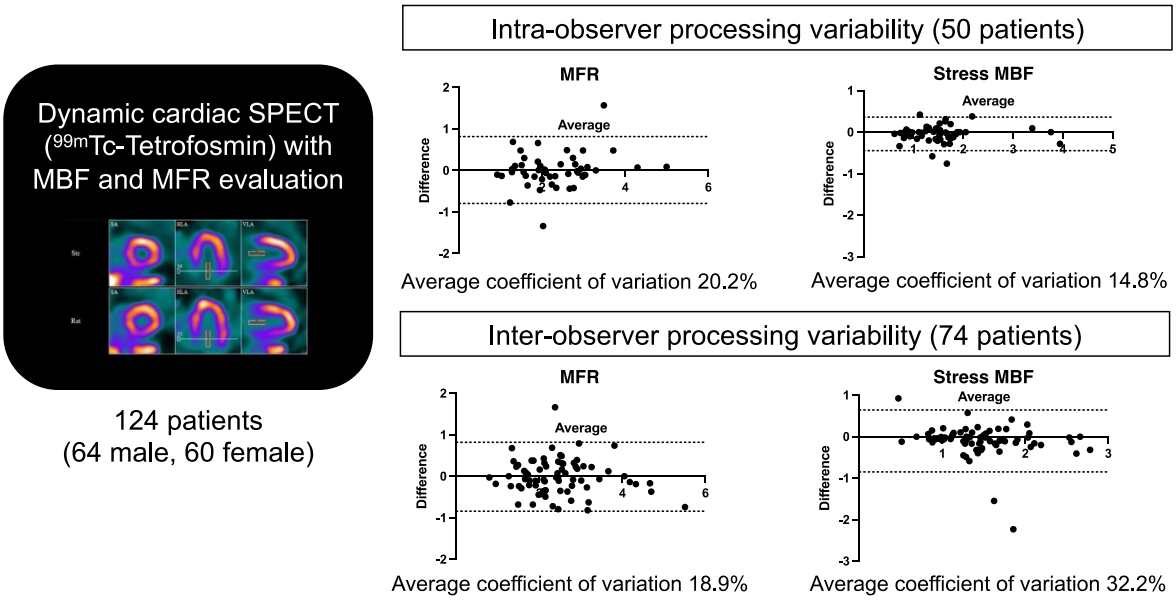

Table 1. Patient description

	Total	**Intra-observer**	**Inter-observer**	**p value**
Number of patients	124	50	74	
Gender Male / Female	61 (45%) / 63 (55%)	26 (52%) / 24 (48%)	35 (47%) / 39 (53%)	0.72
Mean age ± SD (years)	68 ± 9.3 (41 - 87)	69 ± 8.6 (41 - 87)	67 ± 10.5 (44 - 85)	0.99
BMI ± SD (kg/m^2)	28.3 ± 5.4 (15 - 44)	28.2 ± 5.5 (18 - 40)	29.4 ± 6.8 (15 - 44)	0.33
Stress activity ± SD (MBq)	261 ± 14 (240 – 294)	262 ± 13 (248 – 294)	258 ± 15 (240 – 287)	0.99
Rest activity ± SD (MBq)	519 ± 18 (468 – 545)	517 ± 17 (468 – 538)	522 ± 18 (478 – 545)	0.99
Positioning activity ± SD (MBq)	41 ± 5 (34 – 55)	41 ± 3 (38 – 53)	40 ± 5 (34 – 55)	0.99
Cardiovascular risk (CVR) factors				
▪ **Diabetes**	44 (35%)	17 (33%)	27 (36%)	0.87
▪ **Hypertension**	84 (68%)	35 (70%)	49 (66%)	0.75
▪ **Smoking**	61 (49%)	26 (51%)	35 (47%)	0.88
▪ **Dyslipidemia**	82 (66%)	32 (64%)	50 (68%)	0.84
▪ **Family history of coronary artery disease**	18 (15%)	8 (16%)	10 (14%)	0.92
Mean number of CVR factors	2.3 ± 1 (0 - 5)	2.4 ± 1 (0 - 5)	2.2 ± 0.8 (0 - 5)	0.71

Table 2. Differences in myocardial flow reserve (MFR) and stress myocardial blood flow (sMBF) between the two measurements, with statistical results.

	Measurement 1 Mean value ± SD	Measurement 2 Mean value ± SD	Mean Difference	COV	p value	Spearman r	Agreement (Bland-Altman)	
							Bias	95% limit of agreement
Intra-observator (n=50)								
sMBF LAD	1.72±0.74 mL/min/g	1.79±0.73 mL/min/g	-0.06 mL/min/g	15.1%	0.04	0.86	-0.06	-0.52 to 0.39 mL/min/g
sMBF LCx	1.44±0.62 mL/min/g	1.48±0.63 mL/min/g	-0.04 mL/min/g	16.8%	0.22	0.86	-0.04	-0.47 to 0.39 mL/min/g
sMBF RCA	1.29±0.75 mL/min/g	1.31±0.74 mL/min/g	-0.02 mL/min/g	13.9%	0.38	0.93	-0.02	-0.38 to 0.34 mL/min/g
sMBF global	1.51±0.68 mL/min/g	1.56±0.68 mL/min/g	-0.04 mL/min/g	14.8%	0.10	0.87	-0.04	-0.45 to 0.36 mL/min/g
rMBF global	0.72±0.34 mL/min/g	0.75±0.41 mL/min/g	-0.04 mL/min/g	18.8%	0.15	0.87	-0.04	-0.40 to 0.33 mL/min/g
MFR LAD	2.41±0.84	2.39±0.98	0.01	20.9%	0.64	0.90	0.01	-0.85 to 0.87
MFR LCx	2.34±0.92	2.37±0.97	-0.04	22.0%	0.79	0.84	-0.04	-0.96 to 0.88
MFR RCA	2.11±0.95	2.10±0.94	0.00	20.0%	0.94	0.88	0.003	-0.80 to 0.81
MFR global	2.29±0.81	2.29±0.89	-0.01	20.2%	0.94	0.88	0.01	-0.80 to 0.81
Inter-observator (n=74)								
sMBF LAD	1.58±0.56 mL/min/g	1.78±0.63 mL/min/g	-0.21 mL/min/g	23.1%	<0.0001	0.72	-0.21	-1.01 to 0.59 mL/min/g
sMBF LCx	1.48±0.49 mL/min/g	1.54±0.56 mL/min/g	-0.06 mL/min/g	22.2%	0.53	0.79	-0.06	-0.90 to 0.78 mL/min/g
sMBF RCA	1.14±0.48 mL/min/g	1.18±0.48 mL/min/g	-0.04 mL/min/g	21.8%	0.17	0.83	-0.04	-0.56 to 0.48 mL/min/g
sMBF global	1.42±0.48 mL/min/g	1.52±0.56 mL/min/g	-0.11 mL/min/g	32.2%	0.002	0.75	-0.11	-0.85 to 0.64 mL/min/g
rMBF global	0.62±0.25 mL/min/g	0.68±0.27 mL/min/g	-0.05 mL/min/g	25.3%	0.001	0.77	-0.05	-0.46 to 0.35 mL/min/g
MFR LAD	2.45±0.98	2.45±0.93	0.01	20.7%	0.87	0.82	-0.01	-0.88 to 0.86
MFR LCx	2.68±1.12	2.65±1.12	0.03	18.8%	0.40	0.86	0.03	-1.00 to 1.07
MFR RCA	2.33±1.06	2.28±0.97	0.05	19.4%	0.51	0.89	0.04	-0.74 to 0.83
MFR global	2.46±0.94	2.45±0.90	0.01	18.9%	0.67	0.84	-0.01	-0.84 to 0.82

sMBF: stress Myocardial Blood Flow, rMBF: rest Myocardial Blood Flow, MFR: LAD: Left Anterior Descending, LCx: Left Circumflex, RCA: Right Coronary Artery, COV: coefficient of variation