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Left ventricular function measurements in a mouse myocardial infarction model

Comparison between 3D-echocardiography and ECG-gated SPECT

Geert Hendrikx[1,2]; Matthias Bauwens[1,3]; Roel Wierts[1]; Felix M. Mottaghy[1,4]; Mark J. Post[2,5]

[1]Department of Nuclear Medicine, Maastricht University Medical Centre (MUMC+), Maastricht, The Netherlands; [2]Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, The Netherlands; [3]School of Nutrition and Translational Research in Metabolism (NUTRIM), Maastricht University, The Netherlands; [4]Department of Nuclear Medicine, University hospital, RWTH University, Aachen, Germany; [5]Department of Physiology, CARIM, Maastricht University, Maastricht, The Netherlands

Keywords
ECG-gated SPECT, 3D-echocardiography, left ventricular function, myocardial infarction

Summary
Aim: To assess the accuracy of ECG-gated micro (μ)-SPECT in a mouse myocardial infarction (MI) model in comparison to 3D-echocardiography. Animals, methods: In a mouse (Swiss mice) MI model we compared the accuracy of technetium-99m sestamibi (^{99m}Tc-sestamibi) myocardial perfusion, electrocardiogram (ECG) gated μSPECT to 3D-echocardiography in determining left ventricular function. 3D-echocardiography and myocardial perfusion ECG-gated μSPECT data were acquired in the same animal at baseline (n = 11) and 7 (n = 8) and 35 (n = 9) days post ligation of the left anterior descending coronary artery (LAD). Sham operated mice were used as a control (8, 6 and 7 mice respectively). Additionally, after day 35 μSPECT scans, hearts were harvested and 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) staining and autoradiography was performed to determine infarct size. Results: In both infarcted and sham-operated mice we consistently found comparable values for the end-diastolic volume (EDV), end-systolic volume (ESV) and ejection fraction (EF) obtained by 3D-echocardiography and ECG-gated μSPECT. Excellent correlations between measurements from 3D-echocardiography and ECG-gated μSPECT were found for EDV, ESV and EF (r = 0.9532, r = 0.9693 respectively and r = 0.9581) in infarcted mice. Furthermore, comparable infarct size values were found at day 35 post MI by TTC staining and autoradiography (27.71 ± 1.80% and 29.20 ± 1.18% with p = 0.43). Conclusion: We have demonstrated that ECG-gated μSPECT imaging provides reliable left ventricular function measurements in a mouse MI model. Obtained results were comparable to the highly accurate 3D-echocardiography. This, in addition to the opportunity to simultaneously image multiple biological processes during a single acquisition makes μSPECT imaging a serious option for studying cardiovascular disease in small animals.

Correspondence to:
Prof. Dr. Felix M. Mottaghy
Department of Nuclear Medicine, Maastricht University Medical Centre (MUMC+), Postbox 5800, 6202 AZ Maastricht, The Netherlands
Tel. +31/433 87 49 11
fmottaghy@ukaachen.de

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Schlüsselwörter
EKG getriggerte SPECT, 3D-Echokardiographie, linksventrikuläre Funktion, Myokardinfarkt

Zusammenfassung
Ziel: Um die Genauigkeit der EKG-getriggerte μSPECT in einem Maus-Myokardinfarkt(MI)-Modell im Vergleich zu 3D-Echokardiography zu bewerten. Methoden: Wir vergleichen die Genauigkeit von Technetium-99m-Sestamibi(^{99m}Tc-Sestamibi)-Myokardperfusion und EKG-getriggerte μSPECT mit der 3D-Echokardiographie zur Bestimmung der linksventrikulären Funktion. Beide Techniken wurden vor sowie 7 und 35 Tage nach Ligatur der linken anterioren Koronararterie (LAK) am selben Tier durchgeführt. Als Kontrolle wurden Mäuse mit Sham-Operation verwendet. Nach dem letzten μSPECT, wurden die Herzen entnommen. Zur Bestimmung der Infarktgröße wurden sie mit 2,3,5-Triphenyl-2H-tetrazolium chlorid (TTC) markiert und zum Vergleich wurde auch eine Autoradiographie durchgeführt. Ergebnisse: In beiden Gruppen (Infarkt- und Kontrolltiere) wurden vergleichbare Werte für das post-diastolische Volumen (PDV), das post-systolische Volumen (PSV) und die Ejektionsfraktion (EF) mit 3D-Echokardiographie und μSPECT ermittelt. Es zeigte sich eine exzellente Korrelation zwischen den beiden Methoden für PDV, PSV und SV (r = 0,95, r = 0,97 und r = 0,96) in Tieren mit Infarkt. Des Weiteren wurde kein signifikanter Unterschied der gemessenen Infarktgrößen post

mortem durch TTC und Autoradiographie festgestellt (27,71 ± 1,80% vs 29,20 ± 1,18%; p = 0,43). Schlussfolgerung: In dieser Studie zeigen wir, dass EKG-getriggerte µSPECT verlässliche Informationen zur linksventrikulären Funktion im Tiermodell bieten kann. Die Ergebnisse sind vergleichbar mit der 3D-Echokardiographie. Es besteht die Möglichkeit mit Multiple-Tracer-µSPECT verschiedene pathophysiologische Aspekte mit einem Scan zu evaluieren. µSPECT kann als ein wichtiges Tool zur Untersuchung kardiovaskulärer Erkrankungen bei Tiermodellen angesehen werden.

Patients suffering from cardiac diseases nowadays benefit from better understanding, diagnosis, new therapy strategies and continuously improving imaging equipment. In vivo, cardiac imaging in mouse models for cardiac disease has proven to be essential in this niche of translational medicine. Monitoring left ventricular (LV) function is a key factor in this research. In patients LV function can be measured non-invasively (14) by

- echocardiography,
- magnetic resonance imaging (MRI),
- equilibrium radionuclide angiography,
- myocardial perfusion electrocardiogram (ECG) gated single photon emission computed tomography (SPECT),
- positron emission computed tomography (PET).

Despite the challenging aspects (i.e. the small heart size and rapid heart rate) of cardiac imaging in the mouse, there are multiple dedicated preclinical imaging modalities available that enable detailed analysis of cardiac function. Successful studies have been reported for MRI (2, 7, 10, 17, 22), micro (µ) CT (3–6, 11), 2-dimensional (2D) (21) and 3-dimensional (3D) echocardiography (10), PET imaging (18) and µSPECT imaging (8, 9, 23).

While MRI is considered gold-standard for cardiac imaging in the mouse (6, 10) echocardiography has developed into one of the most commonly used techniques to measure murine cardiac function in cardiac research laboratories (19). Especially current echocardiography systems capable of acquiring 3D data are of superior quality compared to 2D echocardiography and have already for more than a decade been reported to approach the accuracy of MRI and histological examination for determining LV parameters and infarct size, respectively (10).

As previously reported, a new generation of µSPECT scanners has been developed, enabling high-resolution functional cardiac imaging (6, 13). These new generation scanners provide clear advantages over echocardiography as quantitative radiotracer distribution revealing multiple biochemical processes can be gathered within one single acquisition. Simultaneously acquiring data on biochemical processes like myocardial perfusion or neovascularization and functional cardiac data is a cost and time effective way to diagnose cardiac disease and to monitor therapy.

Here, we compared technetium-99m sestamibi (^{99m}Tc-sestamibi) myocardial perfusion ECG-gated µSPECT to routine 3D echocardiography for the assessment of LV function in a mouse model of myocardial infarction (MI). 3D-echocardiography and myocardial perfusion ECG-gated µSPECT data were acquired in the same animals at baseline and 7 and 35 days post ligation of the left anterior descending coronary artery (LAD). Infarct size was measured 35 days after surgically induced MI by 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) staining and autoradiography.

Animals, material, methods

Animal experiments

Animals were held under the guidelines of the animal care facility (Maastricht University) with unrestricted access to food and drinking water. All animal experiments were approved by the Committee for Animal Welfare of the Maastricht University conform the Directive 2010/63/EU of the European Parliament.

A flowchart overview (▶Fig. 1) shows the number of used Swiss mice per group and technique (i.e. 3D-echocardiography and ECG-gated µSPECT). Non-interpretable 3D-echocardiography and ECG-gated µSPECT scans were excluded from analysis. Non-interpretability of ECG-gated µSPECT images was usually a result of a distorted ECG-signal hampering image reconstruction at true EDV and ESV dimensions. This, together with animal drop-out in the MI group caused a variable number of animals to be available for paired analysis of left ventricular function parameters (▶Fig. 1, ▶Tab. 1). In total 7 mice died in the MI group during the study. Of them, 1 died immediately after the baseline echo (and before the first µSPECT scan), 5 died within a few days after the MI procedure but before the day 7 scans and 1 died during the day 35 echo. Furthermore, we were unable to perform 1 3D-echocardiography scan in the MI group at day 7 and 2 ECG-gated µSPECT scans in the MI group at day 7, accounting for the difference in available animals at day 7 and day 35.

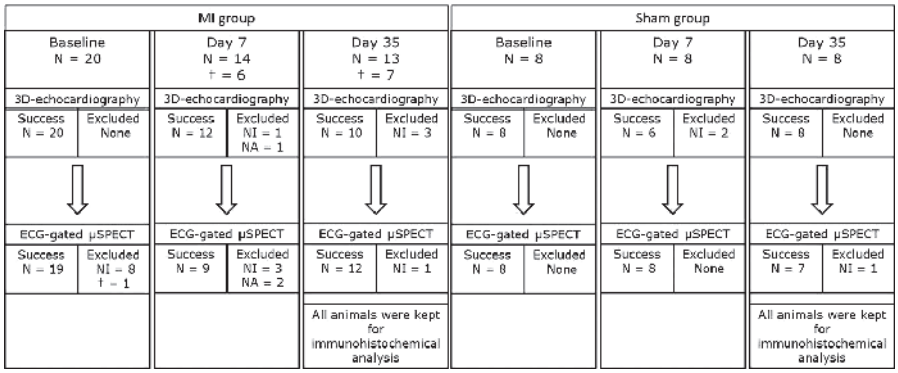

Fig. 1 Flowchart of the number of Swiss mice used in the MI and sham operated group (Success: successful scan; NI: non-interpretable; NA: not available for scan; †: animal(s) died)

Experimental protocol

All mice were subjected to baseline 3D-echocardiography measurements followed by a baseline ECG-gated SPECT scan 4 days before surgical intervention. Surgical intervention marked the start of the experiment (i.e. day 1). 3D-echocardiography measurements followed by ECG-gated SPECT scans were repeated at day 7 and 35 after surgical intervention.

Tab. 1 Number of Swiss mice used in the study

	paired analysis*					
time point	baseline		day 7		day 35	
procedure	MI	sham	MI	baseline	MI	baseline
3D-echocardiography	11	8	8	6	9	7
ECG-gated µSPECT	11	8	8	6	9	7

MI: myocardial infarction; * performed on interpretable images from animals that successfully underwent both 3D-echocardiography and ECG-gated µSPECT at the baseline, day 7 or day 35.

Left anterior descending coronary artery (LAD) ligation

Experimental MI and sham surgery were performed as previously described (18). In short, after induction of anaesthesia (ketamine/medetomidin 50–75 mg/kg + 0,3–1 mg/kg intra-peritoneally) mice were fixed in supine position, intubated and connected to a respiratory pump (210 cycles/min and a tidal volume of 220 µl). The heart was then exposed via an incision in the fourth intercostal space of the left thorax. After opening the pericardium the left ascending coronary artery (LAD) was ligated just proximal to its main bifurcation with a 6–0 prolene suture. The thorax was closed and the skin was sutured using 5–0 silk sutures. Animals recovered from surgery at 30 °C. Sham-operated animals were subjected to similar surgery, except that no ligature was placed.

3D-echocardiography

Mice were anesthetized with isoflurane (induction 2,5%; maintenance 1,5%) and their

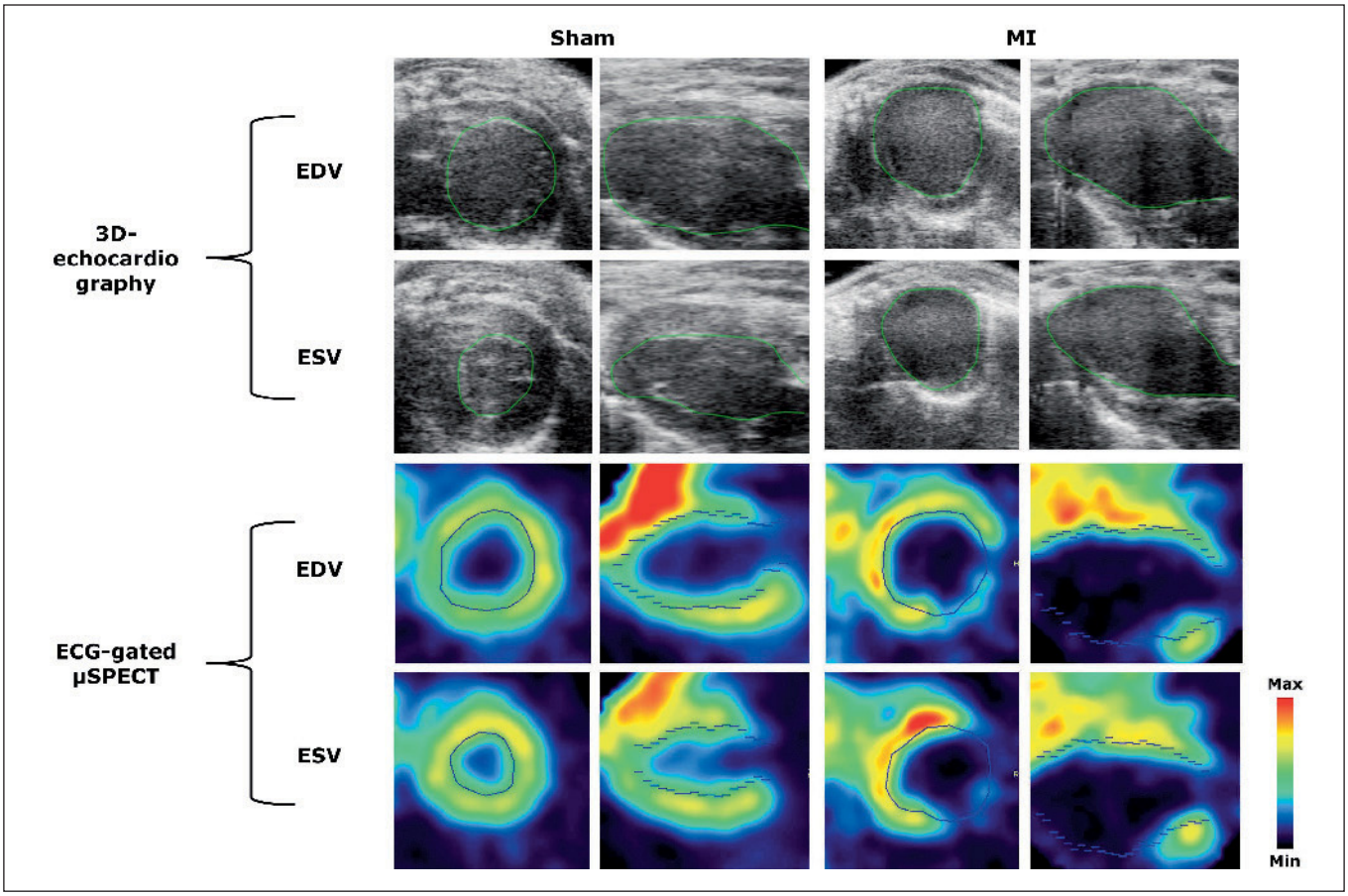

Fig. 2 Left ventricular short and long axis 3D-echocardiography and ECG-gated µSPECT images of EDV and ESV dimensions at day 35 post MI or sham surgery: Representative short (column 1 and 3) and long axis (column 2 and 4) slices of the left ventricle of a sham operated (column 1 and 2) and an infarcted mice (column 3 and 4). In 3D-echocardiography slices, EDV and ESV dimensions are encircled in green. In ECG-gated µSPECT slices, EDV and ESV dimensions are encircled in bright orange. ^{99m}Tc-sestamibi signal intensity is color coded and relative to the injected dose per animal.

chest was shaved. Subsequently, the mice were placed in supine position on a heating pad maintaining the body temperature at 37°C via a rectally inserted feedback thermoprobe. Aquasonic ultrasound gel (Parker Laboratories, Fairfield, USA) was applied to the chest and the cardiac dimensions were measured with the Vevo 2100 echocardiography system (Visual Sonics, Amsterdam, The Netherlands). The endocardial left ventricular dimensions were contoured in a slice by slice fashion in short axis view in Vevo 2100 software (version 1.5.0). The volume of each contoured short axis slice was automatically calculated by the software and summed to determine the total volume. To improve reproducibility end diastolic volume (EDV) and end systolic volume (ESV) were assessed twice by the same observer. The results of both assessments were averaged. Stroke volume (SV) and ejection fractions (EF) were subsequently calculated according to equation 1 and 2.

$$SV = EDV - ESV \quad (1)$$

$$EF = (SV : EDV) \times 100\% \quad (2)$$

ECG-gated µSPECT imaging

ECG-gated µSPECT imaging was performed at baseline and 7 and 35 days after LAD ligation. Mice were anesthetized with isoflurane (induction 2,5%; maintenance 1,5%), a tail vein catheter was placed and the mice were transferred to the VECTor system (MILabs, Utrecht, The Netherlands) fitted with a 0.35 mm multipinhole collimator. The animals were positioned in supine position on a heated bed with integrated ECG monitoring. Throughout µSPECT acquisitions the body temperature was maintained at 37°C.

Prior to image acquisition, the field of view was centered to the heart and ^{99m}Tc-sestamibi (30–240 MBq) was injected via the tail vein catheter in a maximum volume of 200 µl. The large range of injected ^{99m}Tc-sestamibi activity did not influence image quality as non-interpretable scans were evenly distributed over high and low injected dose scans. Immediately after injection 4 consecutive time frames of 15 minutes each were acquired. The acquired list mode data was reconstructed with MILabs reconstruction software (version 2.51) using the POS-EM algorithm (6 iterations and 16 subsets, reconstructed at a voxel size of 0.4 mm) and retrospective cardiac gating (9 time-bins per cardiac cycle). After image reconstruction the time frames were summed and voxel values were averaged. Subsequent image reorientation and left ventricular volume measurements were performed in the PMOD view tool (PMOD technologies, Zürich, Switzerland). Left ventricular volumes were determined through slice by slice delineation and summation in short axis.

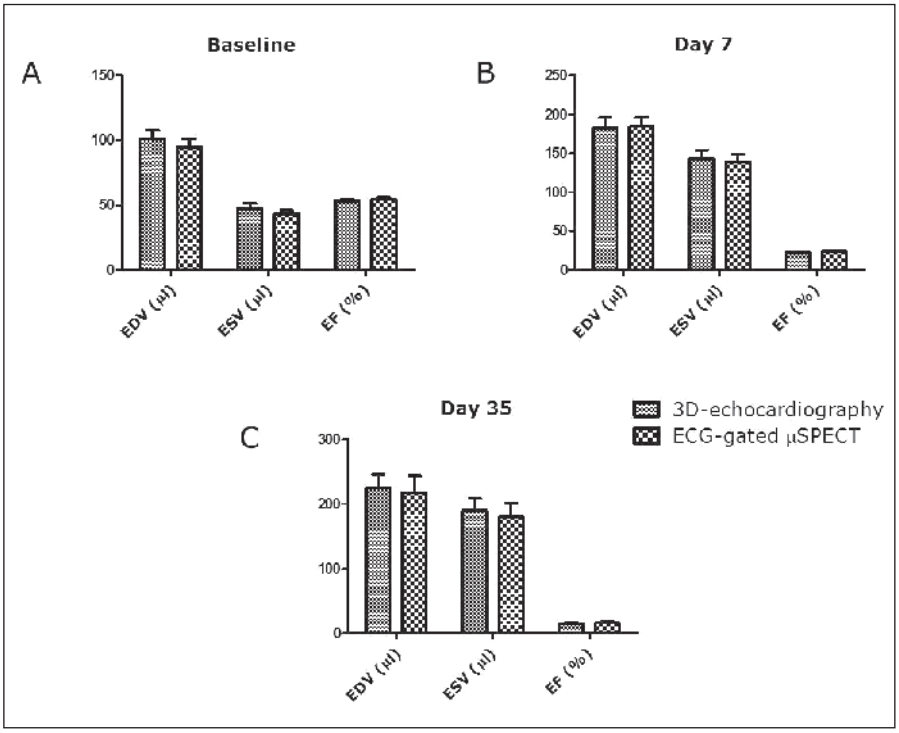

Fig. 3 Comparison of left ventricular parameters over time in infarcted mice as measured using 3D-echocardiography and ECG-gated µSPECT: End-diastolic volume (EDV), endo-systolic volume (ESV) and ejection fraction (EF), were measured at baseline (A), day 7 (B) and day 35 (C) after MI surgery. Mechanical function of the heart after MI continued to decline over the course of 5 weeks. Compared to 3D-echocardiography (n ≥ 8 at every time point) no significantly different values were found for EDV, ESV and EF values measured with µSPECT (n ≥ 8 at every time point). Data are represented as mean ± S.E.M.

2,3,5-Triphenyl-2H-tetrazolium chloride (TTC) staining

Hearts were excised at day 35 post LAD ligation (n = 13) and day 35 post sham surgery (n = 8), rinsed in PBS solution and sectioned in 1 mm sections using a mouse heart slicer matrix (Zivic instruments, Pittsburgh, USA). Subsequently, the slices were stained with 2 % TTC (Sigma-Aldrich, Zwijndrecht, The Netherlands) in PBS for 10 minutes at 37°C, rinsed in cold PBS and digital pictures were acquired for quantification of infarct size. Myocardial borders of the left ventricle were traced manually in Leica Qwin Pro software (Leica Microsystems, Zürich, Switzerland) and expressed as a percentage of the total left ventricular area. The infarct size was subsequently calculated as the sum of the infarcted segments in each individual slice and expressed as the percentage of total left ventricular size.

Autoradiography

After TTC staining, heart sections were placed on a glass slide and exposed to a storage phosphor screen. For subsequent phosphor imaging we used the phosphor imaging settings of the Typhoon FLA 7000 laser scanner (GE Healthcare Bio-sciences, Uppsala, Sweden). Myocardial borders of

the left ventricle were traced manually in Leica Qwin Pro software (Leica Microsystems, Zürich, Switzerland) and expressed as a percentage of the total left ventricular area. The infarct size was subsequently calculated as the sum of the infarcted segments in each individual slice and expressed as the percentage of total left ventricular size.

Statistics

All data were represented as mean ± S.E.M. Functional measurements by 3D-echocardiography and ECG-gated SPECT in the same animal were compared using a two-tailed paired t-test and a Bland-Altman plot. Linear regression analysis was performed to compare infarct size measurements by TTC to autoradiography measurements. The value $p < 0.05$ was considered statistically significant. Data analysis was performed in Graphpad Prism 5 (GraphPad, La Jolla, USA).

Tab. 2 Results of left ventricular function measurements in infarcted and sham operated mice

mice	parameter		mean value ± S.E.M.	
			3D-echocardiography	ECG-gated µSPECT
infarcted	baseline	EDV (µl)	101,2 ± 6,6	95,0 ± 6,0
		ESV (µl)	47,5 ± 3,6	43,2 ± 2,9
		EF (%)	53,3 ± 1,4	54,5 ± 1,4
	day 7	EDV (µl)	183,3 ± 12,8	184,2 ± 12,1
		ESV (µl)	142,7 ± 11,8	139,5 ± 9,8
		EF (%)	22,6 ± 1,1	24,5 ± 1,2
	day 35	EDV (µl)	224,8 ± 18,4	218,0 ± 25,4
		ESV (µl)	190,8 ± 16,5	180,9 ± 20,4
		EF (%)	15,7 ± 1,1	16,9 ± 1,0
sham operated	baseline	EDV (µl)	106,9 ± 5,2	96,5 ± 4,2
		ESV (µl)	47,9 ± 3,2	45,3 ± 2,5
		EF (%)	55,3 ± 1,7	53,0 ± 2,2
	day 7	EDV (µl)	110,1 ± 5,7	100,6 ± 5,0
		ESV (µl)	52,0 ± 3,1	48,5 ± 3,6
		EF (%)	52,8 ± 1,3	51,9 ± 1,7
	day 35	EDV (µl)	116,3 ± 4,3	107,1 ± 5,1
		ESV (µl)	51,8 ± 2,7	47,7 ± 4,1
		EF (%)	55,6 ± 1,3	55,8 ± 2,2

Results

Left ventricular function measurements

ECG-gated 3D-echocardiography and µSPECT datasets were acquired in infarcted and sham-operated mice. Left ventricular short and long axis 3D-echocardiography and µSPECT images acquired in the same animal at day 35 post sham (Fig. 2: column 1 and 2) and MI surgery (Fig. 2: column 3 and 4). Representative 3D-echocardiography images show myocardial dilation and decreased contraction at day 35 post MI while this is not the case in the sham operated animal.

Representative µSPECT images of the uptake of ^{99m}Tc-sestamibi in viable myocardium at day 35 days post MI are displayed (▶Fig. 1) as seen in PMOD software. While in the sham operated animal the uptake is visible in the entire myocardium, the uptake is strongly decreased in the antero-lateral part of the myocardium 35 days after MI surgery. Moreover, enlarged EDV and ESV dimensions were observed 35 days post MI.

Images from 3D-echocardiography and ECG-gated µSPECT were quantified to as-

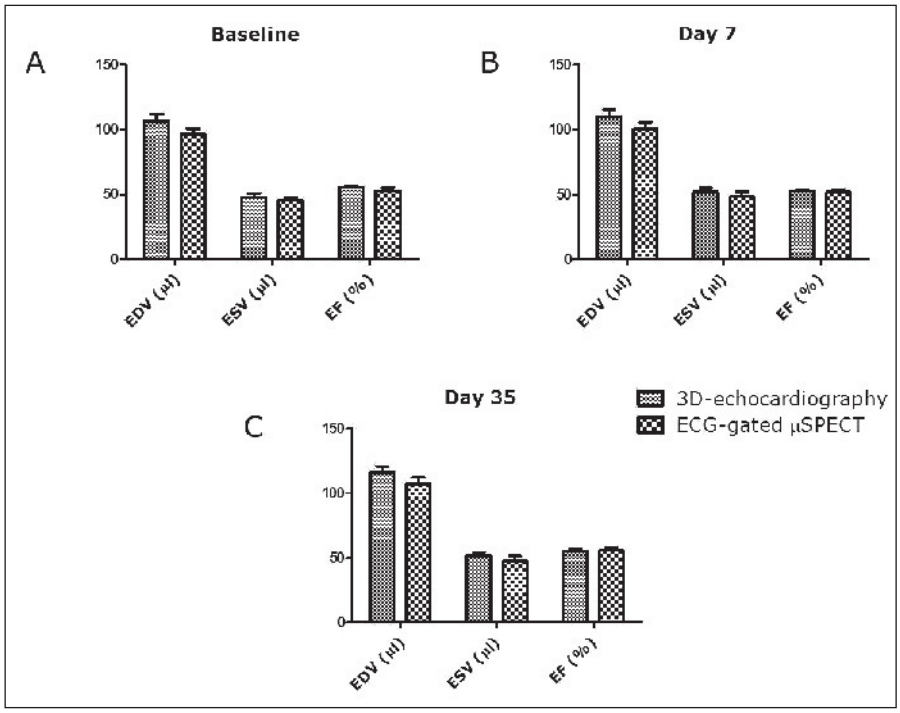

Fig. 4 Left ventricular parameters over time in sham operated mice: End-diastolic volume (EDV), endo-systolic volume (ESV) and ejection fraction (EF), were measured at baseline (A), day 7 (B) and day 35 (C) after sham surgery. Mechanical function of the heart after sham surgery was stable over the course of 5 weeks. Compared to 3D-echocardiography ($n \geq 6$ at every time point) no significantly different values were found for EDV, ESV and EF values measured with µSPECT ($n \geq 6$ at every time point). Data are represented as mean ± S.E.M.

sess the mechanical function of the heart (►Fig. 3A-C, ►Tab. 2). As expected in MI, cardiac function continued to decline while left ventricular EDV and ESV dimensions continued to increase during the 35 days post MI. Both imaging modalities showed similar EDV, ESV and resulting EF values over time (►Tab. 2, ►Fig. 3). No statistical differences were found between measurements from both modalities.

In sham operated mice, cardiac function was stable over time. Again EDV, ESV and EF values measured by both modalities showed similar results (►Tab. 2, ►Fig. 4). No statistical differences were found between measurements from both modalities.

Additionally we observed strong linear relationships for EDV, ESV and EF values measured by both imaging modalities (r = 0.9532, r = 0.9693 and r = 0.9581 respectively) (►Fig. 4A, C, E). Bland-Altman plots further underlined the good agreement between measurements of both modalities (►Fig. 5 B, D, F).

Infarct size measurements using TTC staining and autoradiography

Two techniques were used to determine the left ventricular infarct size immediately after the day 35 post MI SPECT scan. Comparable infarct size values were found at day 35 post MI for both techniques (27.71 ± 1.80 and 29.20 ± 1.18 with p = 0.43 for TTC staining and autoradiography, respectively) (►Fig. 6).

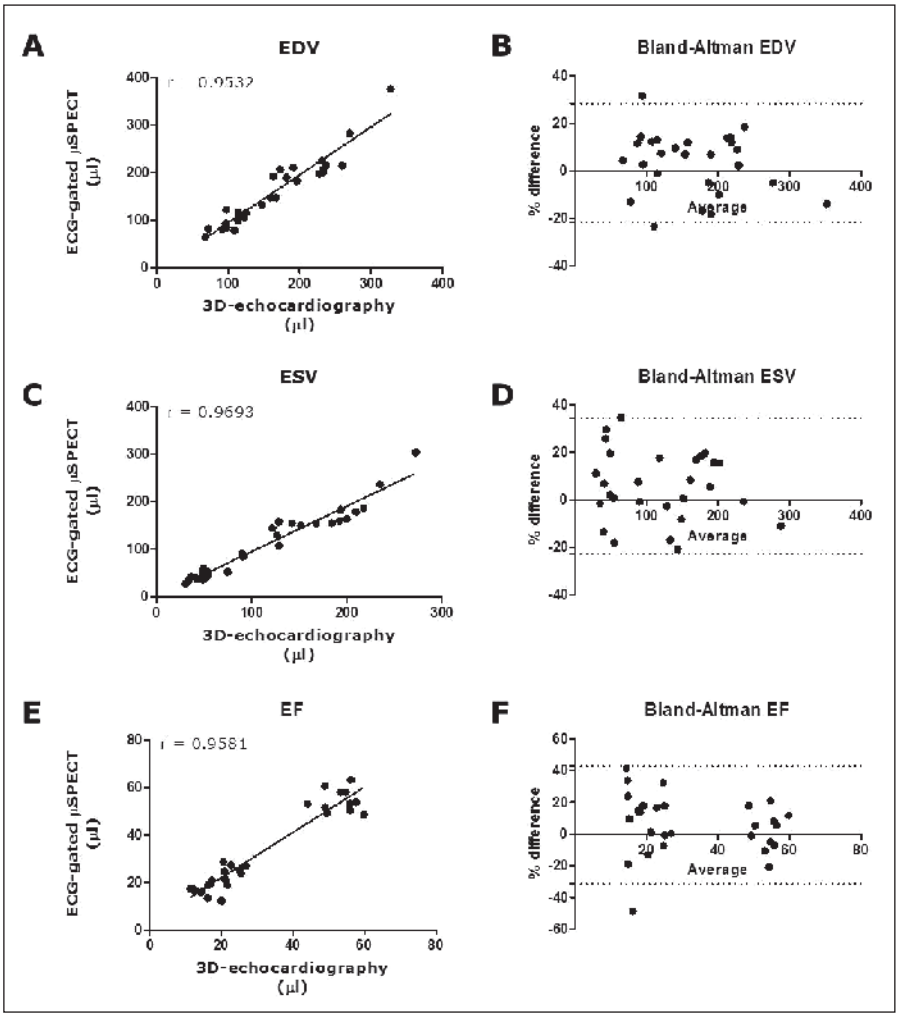

Fig. 5 Correlations between left ventricular parameters measured by 3D-echocardiography and ECG-gated µSPECT: Plots of all EDV (A), ESV (C) and EF (E) values measured at three time points in the MI group by 3D-echocardiography and ECG-gated µSPECT. Bland-Altman plots of all EDV (B), ESV (D) and EF (F) values with limits of agreement shown as dotted lines.

Discussion

With the introduction of 3D-echocardiography more than a decade ago a highly accurate technique for noninvasive, rapid phenotyping of the mouse heart was developed, replacing conventional 2D-echocardiography which relied on geometric assumptions for calculation of left ventricular mass and volume and fell short in case of calculations on asymmetrically shaped infarcted hearts (10, 14). Currently, 3D-echocardiography is a widely available high-throughput technique, known to approach the accuracy of pre-clinical MRI (10). In this study, we monitored left ventricular function at different time points in a mouse model for MI using 3D-echocardiography. Additionally, on the same day and in the same mice we performed ECG-gated µSPECT using the perfusion tracer ^{99m}Tc-sestamibi, assuring optimal comparability of the performance of both techniques.

Most studies measuring left ventricular function in the mouse used C57Bl6 mice which are known to have better post MI preservation of cardiac function (21). We opted for Swiss mice in this study as they are known to have better post MI survival rates than C57Bl6 mice in spite of severe cardiac function deterioration (21). The latter was important as we wanted to compare the accuracy of ECG-gated µSPECT compared to the widely available and highly accurate 3D-echocardiography in a progressively deteriorating infarction model.

Earlier studies, comparing µSPECT with µCT (6) and 3D-echocardiography with MRI (10) were conducted in a similar mouse model for MI, albeit in a different mouse strain (C57Bl6). Whereas Dawson et al. (10) reported EF values of approximately 63% at baseline for MRI and 3D-echocardiography, Befera et al. (6) reported EF values of approximately 70% for both ^{99m}Tc-tetrofosmin ECG-gated µSPECT and iodinated liposomal blood pool agent µCT at baseline. Other studies

using noninvasive imaging to determine left ventricular function also reported baseline EF values that were distinctly higher than the EF values we obtained using 3D-echocardiography and ECG-gated μSPECT (7–10, 12, 17, 18, 22), although some studies reported values that were within a more comparable range of our baseline EF values obtained by both imaging modalities (1–3, 5, 11, 16, 23). Strain differences may account for the variability in reported EF values post MI as was shown in an earlier study (21).

Baseline EF values we found using ECG-gated μSPECT were comparable to 3D-echocardiography values in both the MI and sham group (54.47 ± 1.44 versus 53.26 ± 1.43 and 52.99 ± 2.23 versus 55.27 ± 1,68, respectively) (▶Tab. 2). Comparability of EF values, obtained with both modalities, continued to exist 7 and 35 days after MI and sham surgery. Moreover, the EDV and ESV values determined by both modalities were comparable at any time point in the MI and sham group (▶Tab. 2, ▶Fig. 2, ▶Fig. 3). The results we show here illustrate the reliability of functional cardiac μSPECT measurements in healthy and infarcted mouse hearts. This reliability is further emphasized by the strong correlations between all EDV, ESV and EF values obtained by both modalities (▶Fig. 4). Considering the fact that functional 3D-echocardiography measurements approach the gold-standard for functional cardiac imaging in the mouse (i.e. MRI) (10), current generation μSPECT scanners have to be regarded as a serious option for functional cardiac imaging in small laboratory animals. It has to be noted that ECG-gated μSPECT had a higher number of non-interpretable scans compared to 3D-echocardiography in our study. Non-interpretability of ECG-gated μSPECT images was usually a result of a distorted ECG-signal hampering image reconstruction at true EDV and ESV dimensions. Assuring a stable connection of the ECG leads to the animal throughout the entire scan is important for obtaining a clear ECG signal. Moreover, accurate endocardial contouring in the presence of scar formation (signified here by low uptake of ^{99m}Tc-sestamibi) and high liver uptake requires a trained observer as enlarged left ventricular dimensions

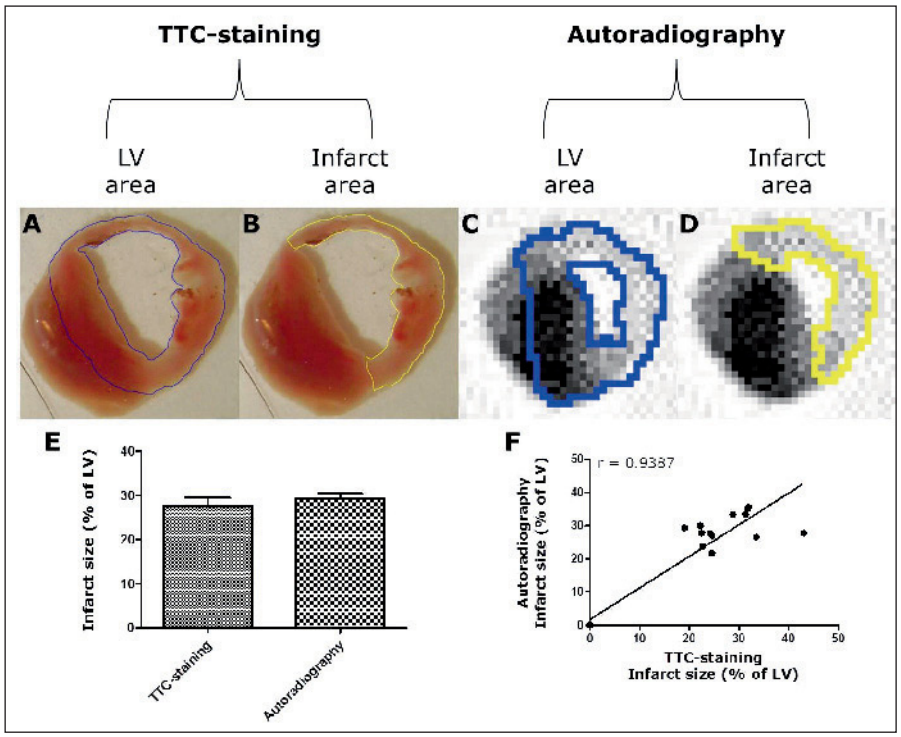

Fig. 6 Comparison of TTC-staining and autoradiography infarct size measurements: Similar slices with manually traced left ventricular (LV) area (A,C) and infarct area (B,D) in TTC staining (A, B) and autoradiography (C, D). Average infarct size ± S.E.M. at day 35 post MI as calculated by both techniques (E). Correlation between infarct size measurements at day 35 post MI and sham surgery by TTC-staining and autoradiography (F).

in combination with low scar uptake and high liver uptake might easily lead to under- or overestimation of the volume. For functional left ventricular measurements solely 3D-echocardiography might remain the preferred option due to its relatively low costs and its image acquisition being independent of the injection of a radioactive tracer. However, ECG-gated μSPECT offers the opportunity to simultaneously monitor multiple biochemical processes through multi-isotope imaging. This enables the assessment of myocardial viability, perfusion and ongoing neovascularization in combination with reliable functional cardiac measurements during a single scan. Especially in therapy studies (both pre-clinical and clinical) directed at improving post infarct cardiac performance, the one-stop shop principle of SPECT imaging will be the better option as it limits the amount of procedures study subjects have to undergo to a single scan while all important data can still be obtained.

The gold standard to measure infarct size is TTC staining (20). At day 35 post MI we compared infarct size measured with TTC staining to autoradiography (i.e. the absence of ^{99m}Tc-sestamibi). Our results demonstrated a good correlation between infarct size values at day 35 post MI and sham surgery measured by both methods (▶Fig. 5). The infarct sizes values were in the same range as reported earlier (24). Infarct size measurements are operator and strain dependent and may vary per laboratory. Our results, however, indicate that autoradiography on heart sections with ^{99m}Tc-sestamibi is an easy and reliable method to determine the infarct size. Therefore, in studies where ^{99m}Tc-sestamibi is used for perfusion imaging, additional TTC-staining based infarct measurements are redundant.

Conclusion

We demonstrated that functional cardiac measurements using ECG-gated μSPECT imaging agreed well with values measured using the highly accurate 3D-echocardiography.

> The one-stop shop principle of μSPECT imaging makes it a serious option for studying cardiovascular disease in small animals.

In the near future it might even prove to be the preferred option in both pre-clinical and clinical studies directed at unraveling the complex and multi-factorial nature of cardiovascular disease.

> Additionally, we showed that autoradiography imaging of ^{99m}Tc-sestamibi heart slices is a reliable alternative to gold standard TTC staining for infarct size measurements, making additional TTC-staining redundant in studies where ^{99m}Tc-sestamibi is used as a perfusion tracer.

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Conflict of interest

FMM received speakers fee from and is consultant of Bayer Healthcare. The other authors declare that they have no conflict of interest.

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