Non-invasive assessment of experimental autoimmune myocarditis in rats using a 3 T clinical MRI scanner

Shunit Rinkevich-Shop1,2,3, Eli Konen4, Tammar Kushnir4, Frederick H. Epstein5,6, Natalie Landa-Rouben1,2,3, Orly Goitein4, Tamar Ben Mordechai1,2,3, Micha S. Feinberg1,2,3, Arnon Afek7, and Jonathan Leor1,2,3*

1Tamman Cardiovascular Research Institute, Leviev Heart Center, Sheba Medical Center, Tel-Hashomer, Israel; 2Regenerative Medicine Stem Cells and Tissue Engineering Center, Sheba Medical Center, Tel-Hashomer, Israel; 3Neufeld Cardiac Research Institute, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel; 4Diagnostic Imaging Department, Sheba Medical Center, Tel-Hashomer, Israel; 5Department of Radiology, University of Virginia, Charlottesville, VA, USA; 6Department of Biomedical Engineering, University of Virginia, Charlottesville, VA, USA; and 7Department of Pathology, Sheba Medical Center, Tel-Hashomer, Israel

Received 29 October 2012; accepted after revision 5 March 2013; online publish-ahead-of-print 3 May 2013

Aims The aim of this study was to assess the use of a 3 T clinical cardiac magnetic resonance (CMR) scanner to detect injury to the heart in experimental autoimmune myocarditis (EAM).

Methods and results The use of 3 T CMR for the detection of cardiac injury was assessed in EAM (n = 55) and control (n = 10) male Lewis rats. Animals were evaluated with serial CMR imaging studies, using a 3 T scanner, and with 2D echocardiography before, and at 2 and 5 weeks after EAM induction. By CMR, regional wall motion abnormalities were noted in seven out of eight rats with myocarditis 5 weeks after induction. Subsequently, the rats developed significant left ventricular (LV) dilatation, wall thickening, and pericardial effusion. Average LV systolic and diastolic volumes increased from 131 ± 10 to 257 ± 20 µL (P = 0.0008), and from 309 ± 14 to 412 ± 24 µL (P < 0.0001), and ejection fraction markedly deteriorated (from 58 ± 2 to 37 ± 5%; P = 0.0003). Areas of fibrosis were located by late gadolinium enhancement (LGE) CMR at the subepicardium, mainly within the anterior, lateral, and inferior walls. The extent and location of LGE were highly correlated (r = 0.94; P < 0.0001) with areas of myocardial fibrosis by histopathology, with 85% sensitivity and 86% specificity.

Conclusion A clinical 3 T CMR scanner enables accurate detection, quantification, and monitoring of experimental myocarditis in rats, and could be used for translational research to study the pathophysiology of the disease and evaluate novel therapies.

Keywords Cardiac MRI • Myocarditis • Remodelling

Introduction Myocarditis is a life-threatening, inflammatory heart disease characterized by myocardial inflammation, necrosis, and chronic fibrosis.1,2 This disease is a common but under-diagnosed cause of acute heart failure, sudden death, and chronic dilated cardiomyopathy.3 To date, no specific therapy has been proved beneficial for either acute or chronic myocarditis.

Rodent models of myocarditis have facilitated much of our understanding of the disorder and have emerged as a potential tool to test and develop new therapies.1–5 Non-invasive imaging of cardiovascular indices is crucial for evaluating animals with myocarditis. Currently, cardiac magnetic resonance (CMR) imaging is the preferred non-invasive imaging tool in the workup of myocarditis in humans.6–8 CMR is more accurate than echocardiography in providing measurements of cardiac chamber volumes, regional and global function, perfusion, and tissue characterization, with high reproducibility.7,9 However, CMR studies in small rodents have been usually done on special high-field MR scanners,10,11 which are expensive and not readily available at most research institutes.
The ability to use clinical 1.5 or 3 T CMR scanners to study small animals with myocarditis could open up new options in the research of myocarditis, particularly, since such scanners are available in many medical centres. Korkusuz et al. have already taken important steps in this direction by using late gadolinium-enhanced (LGE) MRI in a rat model of myocarditis using a 1.5 T scanner. We sought to extend this approach, where, in addition to LGE MRI at a single time point, we investigated cine MRI of LV volumes and ejection fraction and performed serial measurements of disease progression. Furthermore, our study was performed using a clinical MR system, specifically a 3 T system. The use of a clinical MR scanner, currently the most accurate non-invasive modality to assess myocarditis, could significantly boost translational research in this area, and promote the development of new targeted therapies against this serious disorder.

**Methods**

Sixty-five male Lewis rats (Harlan, Israel), aged 6–8 weeks, weighing ~250 g were included in this study and allocated to a control (n = 10) or experimental autoimmune myocarditis (EAM) group (n = 55). Among the EAM group, 45 rats developed myocarditis, whereas 10 rats did not develop the disease and were excluded from the study.

To optimize and validate the induction of a myocarditis model in rat, we evaluated 25 rats by weekly echocardiography. The echocardiography studies assessed the development and severity of LV remodelling and dysfunction, characteristics of myocarditis development and progression. Concomitantly, we euthanized randomly selected rats at certain time points for histopathological evaluation. LV remodelling and function were studied in 12 rats, at baseline, and at 2 and 5 weeks after immunization. Then, to determine the feasibility and accuracy of a CMR scanner to detect myocarditis, we studied the remaining 20 rats. Of them, 5 rats died from myocarditis prior to the first CMR exam, and 7 rats died after the first study. Consequently, a CMR study was done in 15 and 8 rats, at 2 and 5 weeks after immunization.

**Rat model of autoimmune myocarditis**

To induce EAM, we anaesthetized rats with a combination of ketamine–xylazine (40 mg/10 mg/kg), and subjected them to porcine cardiac myosin (PCM; M0531, Sigma-Aldrich, Israel) immunization, as previously described. In brief, we emulsified PCM with an equal volume of complete Freund’s adjuvant (CFA; Difco, USA) mixed with desiccated Mycobacterium tuberculosis H37Rv (Difco, USA). On Days 0 and 7, 0.2 mL of PCM-CFA emulsion (containing 1 mg PCM) was subcutaneously injected into the footpads of experiment animals, whereas only CFA was injected into control animals.

**CMR protocols**

Animals were anaesthetized with a combination of ketamine–xylazine (40 mg/10 mg/kg). The fur surrounding the hind paws was removed by a depilatory cream, four electrode stickers were positioned on fore and hind paws and were attached firmly using adhesive tape. To record ECGs, surface ECG electrodes were placed on four limbs. ECG gating was used in all scans. CMR measurements were not taken if the position of the electrodes did not enable stable gating based on reliable ECG signals without interference from pulse sequences. Signal averaging was effective for reducing breathing artefacts.

CMR examinations were performed in 15 rats under isoflurane anaesthesia (1.5%, Abbott Laboratories Ltd, Berkshire, UK), at a constant temperature of 37°C, at 2 and 5 weeks after immunization. We used a whole-body clinical 3 T MRI system (3 T HDxt Ver.15 M4A, GE Healthcare Systems, Milwaukee, WI, USA) and a custom-built quadrature cylindrical radiofrequency volume coil, ID 77 mm × 178 mm length (Doty Litzcage Coil-Doty Scientific, Inc., Columbia, SC, USA). To assess LV anatomy and function as well as tissue fibrosis, cine CMR and LGE protocols were acquired in long- and short-axis views. For cine CMR, sequence parameters were as follows: ECG-gated multiphase gradient-echo pulse sequence with TR = 11.8 ms, TE = 4.3 ms, flip angle = 20°, field of view (FOV) = 8 cm, matrix = 384 × 192 (in plane resolution of 208 μm), receiver bandwidth (BW) = 31.25 kHz, slice thickness = 1.5–2 mm, two averages, and one view per segment. The total scan time for one slice was ~2 min (depending on heart rate), and the entire heart was covered typically using eight slices in ~15 min. These parameters were empirically optimized to balance spatial resolution, temporal resolution, signal-to-noise ratio (SNR), signal dropout due to dephasing, and total scan time.

LGE imaging of the fibrotic/necrotic region was carried out 30 min following i.p. injection of magnetol-gadolinium (Gd)-DTPA (1 mmol/kg, Soreq Radiopharmaceuticals, Israel). To determine the optimal time of LGE imaging after i.p. Gd administration, we used another group of rats 4 days after myocardial infarction. We performed serial imaging, starting with a pre-injection scan, then every 10 min for ~45 min (see Supplementary data online, Figure S1). Based on these experiments, LGE images were acquired 30 min after i.p. injection of Gd in rats with EAM. An ECG-gated inversion recovery gradient-echo sequence was used with TR = 7.8 ms, TE = 2.7 ms, flip angle = 20°, FOV = 8 cm, matrix = 256 × 128 (in plane resolution of 313 μ), BW = 31.25 kHz, slice thickness = 1.5 mm, 4 averages, #RR intervals = 4, TI = 300 ms, and views per segment = 4. The total scan time for one slice was ~2.5 min (depending on the heart rate), and the entire heart was covered using around eight slices in ~20 min. A similar empirical optimization of parameters was performed for this protocol, with the constraint that the maximum #RR interval allowed by the scanner software was 4. This ultimately constrained the time between inversion pulses, and limited the achievable contrast-to-noise ratio.

We analysed CMR images using a dedicated post-processing CMR Workstation (Medis Medical Imaging Systems BV, QMass MR 7.4, Leiden, The Netherlands). Cine images acquired in the short-axis plane with multiple slices were used for functional analysis. Epicardial and endocardial LV borders at end-diastolic and systolic phases were manually contoured. We then evaluated end-diastolic and systolic volume, ejection fraction, LV wall thickness, wall thickening, and wall motion.

To assess LGE images, we used manual planimetry of enhanced regions, and regions of LGE were expressed as percent of the LV mass. CMR data were transformed into polar maps and subdivided as follows: six basal, six mid-, and four apical regions based on the clinical LV segmentation model suggested by the American Heart Association. This mapping was then used to compare regional wall thickness, wall thickening, and segmental distribution of LGE at 2 and 5 weeks after immunization.

Sensitivity and specificity of LGE were evaluated by comparison of LGE–CMR polar maps with polar maps of equivalent heart sections stained with Masson’s Trichrome. Segments were considered positive or negative for fibrosis by the cut-off ≤10% of the LV segment area. Sensitivity of CMR to detect fibrosis was defined as the proportion of true positive segments by LGE. Specificity was defined as the proportion of true negative segments by LGE. Balanced accuracy was calculated by the sum of sensitivity and specificity divided by 2.
Echocardiography

Echocardiography of both long- and short-axis views were obtained in all animals, using a commercially available echocardiography system (Sonos 5500, Philips, Andover, MA, USA) equipped with a 12 MHz phased-array transducer. Examinations were performed 1 day prior to and weekly throughout the 5 weeks after the induction of myocarditis. All measurements were done blindly by an experienced technician, averaged for three consecutive cardiac cycles, and blindly reviewed by an echocardiography expert (M.S.F.).

Histological and morphometric analysis

We randomly euthanized animals by an overdose of phenobarbital and with 15% KCl, at certain time points during the follow-up. The hearts were perfused with 4% formaldehyde (15 mm Hg) for 20 min, after which they were sectioned into three to four transverse slices parallel to the atrioventricular ring. We then fixed each slice with 4% formalin, embedded them in paraffin, and sectioned them into 5 µm slices. Serial sections were stained with haematoxylin–eosin or Masson’s Trichrome (Sigma, St Louis, MO, USA) to assess fibrosis. All slides were digitally photographed and used for morphometric analysis (Sigma Scan Pro ver.5; SPSS, Inc., Chicago, IL, USA). The fibrotic area was calculated as a percentage of the whole myocardial area and averaged for each heart independently. To map the distribution of fibrosis, all sections were subdivided according to the 16-segment model.

Statistical analysis

Statistical analysis was carried out with the GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA). All variables were expressed as mean ± SD. The difference between means of groups was compared by a two-tail unpaired t-test, paired t-test, or ANOVA, when appropriate. A Kruskal–Wallis test was performed if data were not normally distributed. To test the hypothesis that changes in measures of LV function over time varied among the experimental groups, a general linear model two-way repeated measures ANOVA was used. The Pearson correlation test was used to estimate the relationship between CMR and echocardiography or histology measures.

Results

Immunized rats developed fulminant myocarditis

Rats subjected to EAM developed a marked enlargement of the heart, with diffuse patchy scars spread throughout the left and right ventricles (Figure 1). Two weeks after immunization, microscopic examinations revealed typical findings of myocarditis: intense inflammation, foci of mononuclear cell infiltration, occasional appearance of giant cells, and foci of tissue necrosis (Figure 2). At 3 weeks, we noted an increase in the extent of cellular infiltration of up to ~70% of the myocardium (Figure 2). In addition, we found mononuclear cell infiltrate, granulation tissue formation, and significant myocardial oedema. After 3 weeks, myocardial infiltrate declined, and a significant part of the myocardium was replaced with massive fibrosis (Figure 2). Notably, myocardial lesions were located pre-dominantly in the epicardial region and the mid-wall portion of the LV wall.

CMR imaging of LV remodelling and dysfunction

Complete CMR data sets were successfully obtained in 15 rats 2 weeks after immunization and in 8 rats 5 weeks after immunization. The average heart rate of animals at 2 and 5 weeks after immunization increased from 360 ± 12 to 415 ± 7 bpm (P = 0.003). By CMR, rats developed significant LV remodelling and dysfunction 5 weeks after the induction of myocarditis (Figure 3). Five weeks after immunization, regional wall motion abnormalities were noted in seven out of eight rats, with hypokinesis of the lateral and infero-lateral regions being the most frequent (see Supplementary data online, Video S2). Akinesis or paradoxical motion of the interventricular septum was noted in four rats. Average LV systolic and diastolic volumes increased significantly from 131 ± 10 µL to 257 ± 20 µL (P = 0.0008), and from 309 ± 14 µL to 412 ± 24 µL (P < 0.0001). Consequently, ejection fraction deteriorated significantly (from 58 ± 2 to 37 ± 5%; P = 0.0003). Notably, pericardial effusion was detected in five of eight rats.

CMR data were transformed into polar maps and subdivided using a standard 16-segment model. A representative polar map of the rat myocardium displays an increase in LV wall thickness (Figure 4). Five weeks after immunization, we found a significant increase in end-diastolic wall thickness in all segments, probably due to tissue oedema and inflammation, with the exception of the basal and apical septum (S2, S3 and S13, S14; Figure 4). Notably, from 2 to 5 weeks after immunization, we found a trend towards a decrease in LV wall thickening as a measure of contractility, in basal segments (S4 and S5), mid-segments (S7 and S10–S12), and all apical segments (S13–S16; Figure 5).

Myocardial tissue characterization with CMR

LGE images acquired 30 min after i.p. injection of Gd showed a hyper-intense signal in areas of necrosis and fibrosis. Two weeks
after immunization, LGE was detected in 3 out of 15 rats. Of them, two hearts were processed for histological analysis (one rat had died during the previous night), and fibrosis was detected in both. Five weeks after immunization, LGE was detected in five of eight rats, and all were positive for fibrosis by histology. One rat was negative for both LGE and fibrosis by histology, and two hearts were neither processed for histology nor LGE imaging due to technical difficulties. Thus, in all animals negative for LGE, Masson’s Trichrome staining was also negative.

Regions of enhancement were seen in a patchy-distributed fashion, originating either from the epicardial or sub-epicardial quartile of the LV wall and located pre-dominantly in the lateral and infero-lateral free LV wall (Figure 6). The relative LGE of the LV area was 24.7 ± 4.2 and 21.4 ± 1.8%, at 2 and 5 weeks after immunization.

A 16-segment model was used to quantify and localize LGE and fibrosis.13 The highest percentage of scar tissue was observed in the infero- and anterolateral segments of the basal wall (S5 and S6), in the anterior and lateral mid-wall (S7 and S11–S12), and in the lateral apical segment (S16; Figure 7). This pattern of LGE distribution was seen at 2 and 5 weeks after immunization (Figure 7).

We found a high correlation between the location of fibrosis by CMR–LGE and histopathology (Figure 7A–D). The sensitivity and specificity of LGE for fibrosis detection were evaluated by comparison of LGE–CMR polar maps with equivalent maps of heart sections stained with Masson’s Trichrome (Figure 7A–C). 2 (n = 2) and 5 (n = 5) weeks after immunization. The sensitivity of LGE for the detection of myocardial fibrosis was 91.2% and specificity was 87.3%, with a balanced accuracy of 89.2%. LGE located in the infero-lateral basal, mid-, and apical segments (S5, S11, and S16) had the highest sensitivity (100%).

Finally, when analysing the relative areas of myocardial injury, we compared areas of enhancement in CMR–LGE imaging with areas of fibrosis in histopathology. Significantly, the areas of fibrosis by CMR–LGE imaging were highly correlated with histopathology (r = 0.94, P = 0.0001, n = 8; Figure 7E).

**Correlation between CMR and echocardiography measurements**

Similar to the CMR findings, serial echocardiography evaluations of EAM rats revealed significant increases in anterior and posterior end-diastolic wall thickness 5 weeks after immunization: from 1.3 ± 0.1 to 1.6 ± 0.3 mm (P = 0.008), and from 1.4 ± 0.2 to 1.65 ± 0.4 mm (P = 0.004; Figure 8). Furthermore, EAM rats developed continuous and progressive increases in systolic and diastolic LV areas (from 11.3 ± 6 to 23.8 ± 8 mm²; P = 0.0009, and from 31.8 ± 9 to 48.2 ± 6 mm²; P = 0.0002), indicating substantial LV dilatation, compared with baseline and control (Figure 8). Notably, echocardiography measurements were correlated with
Those of CMR ($r = 0.78$ and $r = 0.92; P < 0.0001$). However, while MRI clearly demonstrated significant functional impairment, as indicated by deterioration in ejection fraction, echocardiography did not.

**Discussion**

The major new finding of the present study suggests that experimental myocarditis in a rat model can be accurately detected and monitored by LGE and cine MRI using a clinical whole-body 3 T CMR scanner with a custom-built RF coil. Significantly, the present study expands upon previous studies by providing new findings on myocarditis in a rat model. First, in addition to LGE imaging at a single time point, we analysed serial CMR measurements, thereby monitoring changes in LGE, LV volume, structure, and global and regional LV function. Secondly, we used a clinical LV segmentation model to map function and fibrosis of the injured myocardial regions, with better sensitivity, specificity, and predictive accuracy (87–91%), compared with previous reports. Thirdly, we imaged rats without slowing their heart rate, thereby avoiding potential influence on cardiac function. Finally, our study also included detailed comparisons with serial 2D echocardiography examinations and histopathology. This comparison clearly demonstrated the superiority of CMR over echocardiography in this animal model, particularly in detecting changes in LV volumes, segmental and global LV function, and fibrosis. Thus, we have provided a comprehensive, in-depth CMR analysis into myocarditis development, progression, cardiac remodelling, and dysfunction in a small animal model.

Previous reports have described the use of clinical scanners for normal small animals, after myocardial infarction, and for rats with myocarditis. For example, Korkusuz et al. examined rats on Day 21 after the induction of EAM, mapped the distribution of LGE using a seven-segment model of the LV, and reported that LGE was located mainly in the anterior and lateral LV walls, and was correlated with histopathology findings.

In the present study, CMR accurately identified the location and the extent of myocardial damage, which corresponded with the histopathological picture of myocarditis. These findings also correlate with typical findings in humans with myocarditis, showing a subepicardial distribution of LGE mainly in basal and mid-infero-lateral segments. In addition, CMR in rat detects regional and global LV dysfunction and an increase in wall thickness that most likely reflects inflammation and oedema. Therefore, a significant outcome of our study is the development of CMR protocols for quantifying and monitoring experimental myocarditis using a clinical CMR system.

**Methodological considerations**

Cardiac imaging of small animals continues to be technically challenging due to animal size and fast heart and breathing rates. While clinical CMR systems are more available than high-field...
pre-clinical scanners, the latter (typically 4.7–11.7 T) have advantages over clinical scanners for imaging small animals. The higher field strengths provide higher SNR, and their more powerful gradient systems provide faster imaging through shorter echo and repetition times. While imaging at lower-field strengths has the advantage of less B0 inhomogeneity and associated artefacts, this is only a minor advantage. Because suitable RF coils contribute substantially to SNR, our experience is that adequate SNR efficiency can be obtained using a 3 T scanner with a custom-built RF coil.

We systematically and empirically optimized a cine protocol for rat imaging on a clinical 3 T system, where, notably, the gradient system has a much lower slew rate, full strength and lower B0 field strength, when compared with small animal systems. The relatively poor gradient performance of the clinical system lead to relatively long TR and TE values for a given spatial resolution and BW. Also, due to the lower main magnetic field strength, SNR is lower. Given these disadvantages, the challenge was to find parameters that achieve a balance between high temporal resolution (requiring short TR), adequate SNR (benefiting from short TE, but also benefiting from low BW, which increases TE and TR), high spatial resolution (requiring a small FOV and large matrix, which tend to increase TE and TR), minimal signal dropout due to spin dephasing (requiring short TE), and minimal flow artefact (requiring low or moderate flip angle). Balancing these many competing factors, our empirically determined optimal protocol used an ECG-gated multiphase gradient-echo sequence with TR = 11.8 ms, TE = 4.3 ms, flip angle = 20°, FOV = 8 cm, matrix = 384 × 192, receiver BW = 31.25 kHz, slice thickness = 1.5–2 mm, two averages, and

![Figure 4](image_url) Increase in LV wall thickness in rats with myocarditis by CMR. Representative 16-segment CMR data show increase in diastolic wall thickness from 2 to 5 weeks after the induction of myocarditis (upper panel). Significant increases in wall thickness were noted in basal, mid, and apical segments 5 weeks after the induction of myocarditis (lower panel). LV, left ventricular; CMR, cardiac magnetic resonance. *P < 0.05; **P < 0.01; ***P < 0.001.
Figure 5  Segmental wall thickening in rats with myocarditis by CMR. Representative 16-segment CMR data of the rat myocardium displaying LV wall thickening 2 and 5 weeks after the induction of myocarditis (upper panel). A trend towards a decrease in wall thickening was noted in basal segments (S4 and S5), mid-segments (S7 and S10–S12), and all apical segments (S13–S16) 5 weeks after the induction of myocarditis (lower panel). CMR, cardiac magnetic resonance; LV, left ventricular. *P < 0.05.

Figure 6  LGE imaging of rat heart with myocarditis. LGE imaging of a rat heart in short-axis (left) and four-chamber (right) views, shows typical epicardial LGE (hyper-intense strip, yellow arrows), while sparing the sub-endocardium 5 weeks after the induction of myocarditis. Pericardial effusion is marked by red arrows. LGE, late gadolinium enhancement; LV, left ventricle; and RV, right ventricle.
Figure 7 LGE distribution in the LV of rats with myocarditis. (A) Polar map of percentage of mean LGE in five rats, 5 weeks after the induction of myocarditis. (B) The short-axis view of representative LGE images of a rat heart with typical epicardial LGE (white strip, yellow arrows). (C) Equivalent heart section shows a high correlation in the location of fibrosis between histopathology (blue Masson’s Trichrome staining) and CMR. (D) Temporal changes in the percentage of LGE by polar map of 16 cardiac segments. (E) Combined data from both the 2- and the 5-week LGE measurements show high correlation in the amount of LV fibrosis, as estimated by CMR and histopathology. CMR, cardiac magnetic resonance; LGE, late gadolinium enhancement; LV, left ventricle; and RV, right ventricle. *P < 0.05.
Figure 8 Temporal changes in LV variables in rats with myocarditis by 2D echocardiography, before, at 2 and 5 weeks after the induction of myocarditis. (A) A representative short-axis view of normal rat heart at diastole. (B) A representative short-axis view of rat heart 5 weeks after immunization showing significant LV dilatation. (C) Anterior wall diastolic thickness increased in rats 5 weeks after the induction of myocarditis. (D) Posterior wall diastolic thickness. (E) The LV diastolic area increased in rats with myocarditis, compared with baseline and control. (F) The LV systolic area increased in rats with myocarditis, compared with baseline and control. (G) LV contractility reflected by fractional shortening, deteriorated in rats with myocarditis. (H) The LV fractional area change, slightly deteriorated in rats with myocarditis. LV fractional shortening was calculated as a percentage of \((\text{LV diastolic dimension} - \text{LV systolic dimension})\) divided by \(\text{LV diastolic dimension}\). The LV fractional area change was calculated as a percentage of \((\text{LV diastolic area} - \text{LV systolic area})\) divided by the LV diastolic area. LV, left ventricular. *P for interaction, †group factor.
one view per segment. Not surprisingly, the resulting protocol is similar to cine gradient-echo protocols for humans; however, the spatial resolution is much higher and TE is somewhat longer. Using one view per segment enabled us to achieve sufficient temporal resolution. Using the stated FOV, BW, matrix, and TE, we achieved a good balance between spatial resolution, SNR, and minimal dephasing artefact in the lateral wall. By using two averages and the above-stated parameters, we achieved good SNR with a reasonable scan time of ~2 min per slice, depending on the heart rate.

Another methodological point is the mode of Gd administration. We favoured i.p. over i.v. Gd administration, because the former is simpler. The use and efficacy of LGE imaging by IP injection has been demonstrated in several reports. For example, a prior work in mice has shown good LGE imaging in infarcted mice at 30 min post-i.p. Gd administration.16,17

Finally, the potential influence of field strength on MR contrast agents should be considered. The relaxivity, R1, of Gd-DTPA has been shown to decrease from 4.8 to 4.0 (mM x s)–1 as magnetic field strength increases from 1.0 to 4.7 T.18 In the same study, essentially no change in R1 was found between field strengths of 4.7 and 8.5 T. In addition, T1 generally increases as field strength increases, with values for the myocardium of ~1000, 1300, and 1500 at 3, 4.7, and 7 T, respectively.19–21 In our experience, 0.1–0.2 mmol/kg of Gd i.v. at 3, 4.7, and 7 T, provides enhancement in LGE images of small animals, which is comparable with the same dose in humans scanned at 1.5 T.

Limitations
First, we did not perform T2-weighted MRI, which has been used to detect oedema in patients with acute myocardial infarction and differentiate acute from chronic infarction.22 However, while performing T2-weighted imaging in humans and large animals is straightforward, it is more complex in small animals, where longer echo times represent nearly half of the cardiac cycle. In this setting, cardiac motion causes substantial artefacts in T2-weighted spin-echo imaging, and images are of poor quality. Furthermore, while pulse sequences utilizing T2 preparation methods are remarkably insensitive to motion and have recently been developed to enable T2-weighted imaging of the mouse and rat heart,23 they are not generally available on clinical scanners. Methods such as T2-prepared steady-state free precession,22 which are becoming available on clinical systems, may work well for small animals. Unfortunately, we did not have the opportunity to evaluate this sequence on our system.

Secondly, the detection of fibrosis was performed by analysing regions of enhancement in LGE images. Alternatively, a more quantitative approach could be used by combining contrast enhanced imaging with T1 mapping of the myocardium, enabling the assessment of the extracellular volume, which is increased in fibrotic tissue.11,24,25 Unfortunately, a T1 mapping sequence was not available when these studies were performed. Finally, we did not perform ex vivo quantification of the contrast agent in order to confirm its presence.

Summary and implications
Our study suggests that a clinical whole-body 3 T CMR scanner enables accurate detection, quantification, and monitoring of experimental myocarditis in the rat. Based on the availability of such systems in many medical centres, translational myocarditis research in small animals could progress significantly. The unique ability of clinical CMR to locate and monitor myocarditis-related tissue injuries non-invasively, promises to facilitate the understanding of the mechanism of disease progression and evaluation of novel, targeted therapies for myocarditis.

Funding
This work was supported by the US-Israel Binational Science Foundation (F.H.E., J.L.) and the Schlezak Foundation (J.L.), Tel Aviv University, Israel. This work was performed in partial fulfillment of the requirements for a PhD degree of Shunit Rinkevich-Shop, Sackler Faculty of Medicine, Tel Aviv University, Tel-Aviv, Israel.

Conflict of interest: none declared.

References
5. Gilson WD, Kraitchman DL. Cardiac magnetic resonance imaging in small rodents using clinical 1.5T and 3.0T scanners. Methods 2007;43:35–45.
20. Pechnik S, Ferreira VM, Dall’Armellina E, Cochin LE, Greaser A, Neubauer S et al. Shortened Modified Look-Locker Inversion recovery (SiMOLLI) for clinical
Benefits of multimodality imaging for pulmonary venous anatomy evaluation

Ferande Peters1, Bijoy K. Khandheria2*, Jaco Botha3, and Mohammed R. Essop1

1Department of Cardiology, Chris Hani Baragwanath Hospital, University of the Witwatersrand, Diepsloot 319-Iq, Soweto 1862, Johannesburg, South Africa; 2Aurora Cardiovascular Services, Aurora Sinai/Aurora St. Luke’s Medical Centers, University of Wisconsin School of Medicine and Public Health, 2801 W. Kinnickinnic River Parkway, #845, Milwaukee WI 53215, USA; and 3Division of Cardiothoracic Surgery, Flora Clinic, Little Falls Road, Roodepoort 1709, South Africa

* Corresponding author. Tel: +1 414 649 3909; fax: +1 414 649 3551. Email: publishing22@aurora.org

A 41-year-old man evaluated for recurrent blackouts was found to have dilated right heart chambers with signs of right ventricular volume overload. There were no valvular abnormalities, intracardiac shunts, or left-sided heart disease noted with transthoracic echocardiography. Transoesophageal echocardiography (TEE) confirmed a sinus venosus atrial septal defect (ASD) (Panel A) associated with four pulmonary veins draining into the left atrium (LA). The right superior pulmonary vein was visualized entering the LA adjacent to the sinus venosus ASD (Panel B). An abnormal vessel was draining blood into the superior vena cava (SVC) just above its entry into the right atrium (see Supplementary material online, Video S1). A 128-slice computed tomography scan confirmed four pulmonary veins draining into the LA (Panel C, arrows) as well as an anomalous right upper pulmonary vein (ARUPV) (Panels C–E, arrow) draining into the posterolateral aspect of the SVC. Computed tomography depicted the entire right middle lobe of the lung draining into what appeared to be the right superior pulmonary vein on TEE (Panel F). These findings were confirmed during surgery where the ARUPV was redirected via a baffle through the ASD.

This case highlights the importance of using complementary multimodality imaging to identify variations in pulmonary venous drainage that cannot always be accurately evaluated using TEE. In addition, it allows for accurate preoperative planning. LLPV, left lower pulmonary vein; LUPV, left upper pulmonary vein; RLPV, right lower pulmonary vein; RMPV, right middle lobe pulmonary vein.

Supplementary data are available at European Heart Journal – Cardiovascular Imaging online.