Magnetic resonance imaging based assessment of aortic valve area: a methodology proposal and an experimental case study

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Abstract. Abstract: Introduction: Even if Magnetic Resonance Imaging (MRI) is a powerful and wide-spread used tool for non-invasive medical investigation in general, the cardiac heart valves assessment in small animal models is not well standardized given the rodents' particularities. Objective: The present work is focused on developing a real-time MRI based general protocol for measuring aortic valve area (AVA) of healthy Wistar rats. Material and Methods: MRI analysis was performed on Bruker BioSpec 70/16 USR scanner, at 7 Tesla applied magnetic field while using a modified 2D fast imagines protocol (IntraGate-FLASH CINE). The practical utility of the developed protocol was verified in an experimental model of 5 male Wistar rats. Results: AVA determined by planimetry using the modified 2D fast imagines protocol was similar to that obtained by echocardiographic measurements, but in a shorter amount of time and with a better imagine quality. Conclusion: The modified 2D fast imagines protocol could be used for the assessment of AVA in experimental settings.

Key Words: Magnetic Resonance Imaging, Aortic valve area, Wistar rats.

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Introduction

Small animal experimental models, especially rodents are critical for understanding both functional and morphological changes in heart valve diseases. Improved assessment of the heart valves structure and function in rodent models will have diagnostic and therapeutic benefits in humans. These changes could be evaluated and monitored in vivo thanks to imaging technology which provides better spatial and temporal resolution of heart valves (Roosens et al 2013).

Magnetic Resonance Imaging (MRI) is one of the most wide-spread techniques for noninvasive imaging of the cardiovascular system, in some particular cases down to micrometer resolution. The technique has the ability to visualize simultaneously hard, and soft tissues as well fluids, with both high and low signal allowing for noninvasive angiography or vessel wall imaging (Botnar&Nagel 2008).

In experimental settings of the cardiac MRI protocol, and especially that regarding valves assessment is not well established (Vallée et al 2004). There are some important particularities of the rodent hearts that must be taken into account when employing MRI to assess aortic valves. Rodent hearts, have adapted to function at very high cardiac rates where rapid systolic contraction and diastolic filling are necessary to maintain cardiac output. Moreover the successive end-expiratory breath-holding is not compatible with experimental models. Long imaging times

such as 14 hours for a full body scan is another drawback of the method, given the vital risk of animal model and high costs (Bock et al 2003), beside the impossibility of holding in position the animal even when sedated.

Thus, in order to realize the value of MRI based investigation as an effective means of cardiac valve evaluation, different pathways to improve its efficiency must be developed.

The present work is focused on the development of the best MRI setup/protocol to measure aortic valve area (AVA) of healthy Wistar rats. The developed protocol could be used for in-vivo experimental animal models.

Following this introduction, section two describes the proposed methodology. Section three is focused on putting the method into practice in a case study on an animal model. In section four the results are discussed, as in the fifth section the conclusions are drawn and the premises for further work are set.

Methodology proposal

Given the rat's small size and heart rating between 300-400 beats/minute (bpm) tailored imaging protocols and software are essential to achieve appropriate temporal and spatial resolution for MRI in rats. Because the filling factor of an radiofrequency (RF) coil should be maximized in order to the highest signal to noise ratio (SNR), which further allow the highest possible

resolution, the dimensions of the animal to be studied should be carefully considered with coil's active volume.

The aim of MRI based investigation is to determine the morphology of the dynamic cardiac structures such as valves and valves' orifice area. As the heart moves during data acquisition it is important to avoid artifacts. Bearing these in mind, a modified non-invasive IntraGate-FLASH protocol was developed. Ideally, R-wave of ECG should be used for cardiac gating. Unfortunately, ECG gating in rats is very difficult mainly because ECG is an electrical measurement is disturbed by interference with high magnetic field (Pohlmann et al 2011), resulting in image artifacts .

Hardware

MRI was performed on Bruker BioSpec 70/16 USR scanner, operated at 7 Tesla. The superconductor magnet (4.2 Kelvin) has an active diameter of 160 mm, while inside the BG S 9 HP gradient unit 90 mm are available for RF coils and investigated animals. The double-resonance frequency configuration is designed for investigations at 300 MHz for proton (1H) and variable frequency on X channel, respectively. Operating frequency of latest channel can be adjusted in a wide frequency band from 31P frequency (121.44 MHz) down.

According to Rehwald et al. (1997) a male Wistar rat have a weight between 210-370 g, a diameter/length of ribcage of 1,9-2,5 cm/4-5 cm, heart long axis of 2-2,5 cm and a heart rate after anesthesia about 300 bpm, needing a 5 cm long RF coil with a diameter of 5.1 cm. Thus, in our protocol, the RF setup configuration was decided based on animal sizes and weight. We decided to use a surface RF coil. The combination of RF RES 300MHz 1H 089/072 QUAD TO AD and of RF SUC 300 MHz 1H ID=20 LIN RO AD coils were used for all investigated animals in order to avoid size measurements unwished errors.

Imaging protocol and sequence parameters

Employing Bruker's ParaVision® 5.1 for acquisition and reconstruction 5 animals have been investigated. The IntraGate-FLASH protocol was preferred because it allows retrospective gating, provides better SNR and contrast to noise ratio (CNR), reducing the animal preparation time. Moreover, because the specific location of the aortic valve is known, a 2D imaging sequence is preferred to 3D whole imaging sequence, after identification of the right depth inside the magnet. During the examination the respiratory rate was continuously monitored. For the assessment of aortic valve area (AVA) scans the next steps were followed:

Scan 1) The protocol used for geometry was a 2D Tri-pilot with a 6 cm field of view, slice thickness of 1 mm, inter slice thickness 1.5 mm, and a number of 3x5 images in axial, sagittal, coronal position. The used repletion time was set to 80 ms while the echo time was 1.8 ms. The matrix of 256x256 in this field of view offer an resolution by 0.0234 cm/pixel with a repetition time 80 ms and echo time 1.8 ms.

Scan 2) Single slice 2D IntraGate FLASH CINE, scan mode, fast imagines protocol was used for acquisitions with a field of view between 4-6 cm, in axial orientation, 2 mm-2.5 mm slice thickness, under 0 to 8 degree angle, 256x256 matrix that offer a resolution between 0.0164 and 0.0234 cm/pixel. The number of repetition scans was 300 with a repetition time of 8 ms

and the echo time varying between 3 and 8 ms and an acquisition time of 5 minutes 9 s 200ms for 10 cardiac movie frames.

Planning of the standard cardiac views

After acquisition of the tri-pilot the second scan was planned on an axial view in order to obtain the sagittal slice as illustrated in Fig. 1 (upper). Finally, for a better alignment the scan area was positioned under an angle between 0 and 8 degree to the reference scan. AVA quantification and aortic valve movements' observation required the scan of aortic root. The first slice was positioned just below the aortic valve in systole. The last slice showed the aortic lumen. Blood suppression for a better blood/valvular endocardium contrast was achieved by placing a saturation band over the atria parallel to the imaging slice package. The slice thickness of the saturation slice was wide enough to cover the atria and afferent vessels (venae cavae and pulmonary vein) as shown in Fig. 1 (lower).
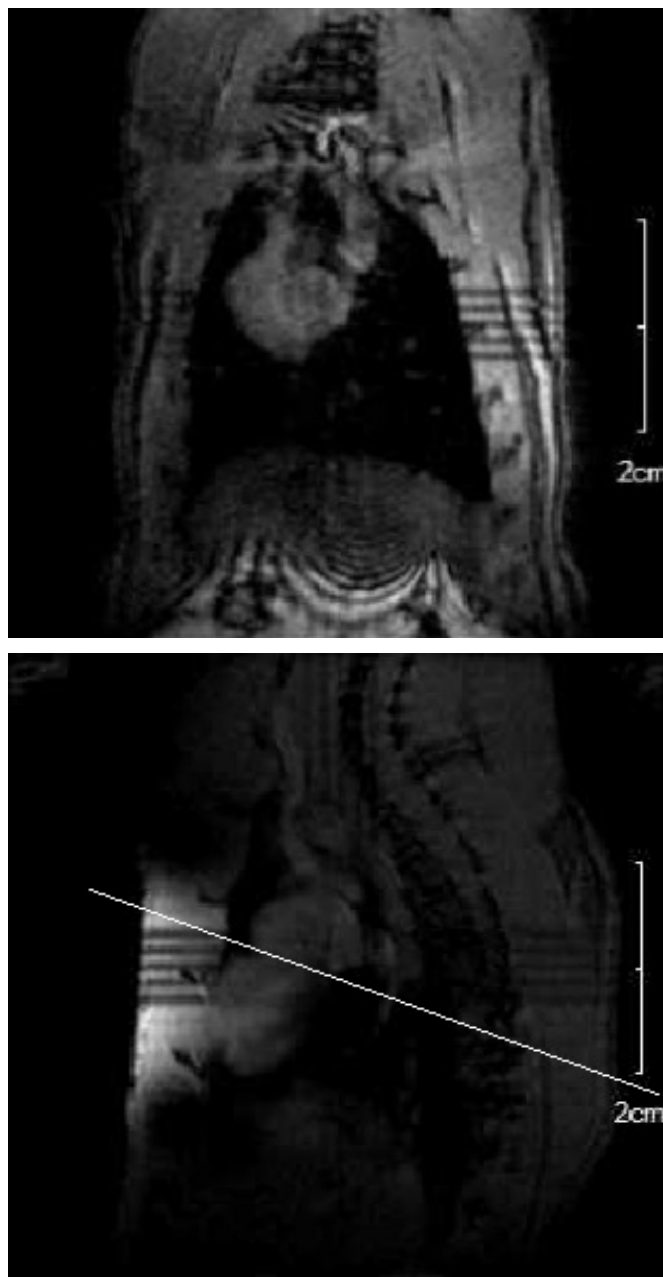

Fig.1. Planning geometry of the slice package to scan de aortic

Image analysis and AVA measurement

The 2D images were post-acquisition reconstructed using ONIS 2.5 software program. Image quality was rated as adequate, impaired or poor in a semi quantitative fashion. Manual planimetry of the area of complete signal loss was performed with the above mentioned free software. The maximal visible opening in early systole in the acquisitions prospectively triggered to the respiration was performed by one observer and the values were averaged. The measurements were repeated after 3 month to appreciate de inter-observer differences (<7%) (Reant et al 2006).
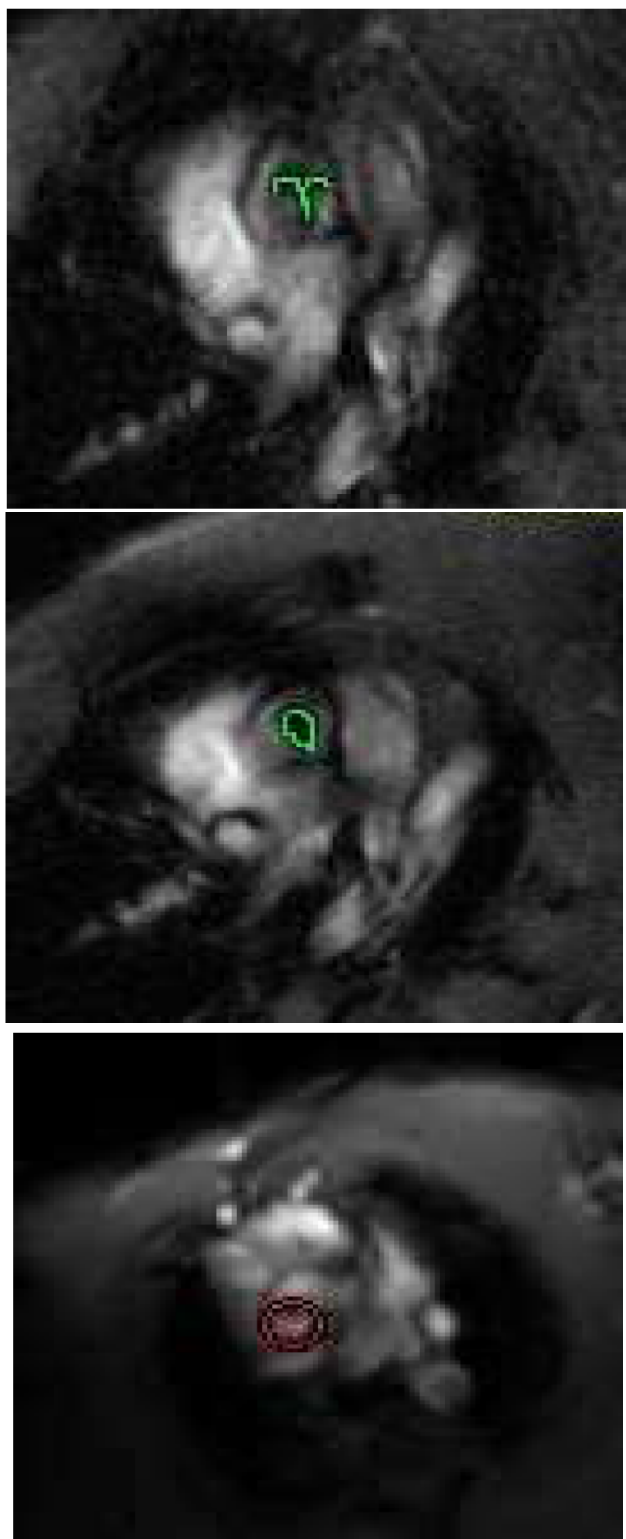

Fig.2. Analysis and manual planimetry of AVA at the maximal visible opening in early systole.

Case study

Objective

The present case-study aimed at verifying the practical utility of the modified 2D IntraGate-FLASH CINE protocol for the assessment of AVA in experimental models (rats). In order to achieve this goal, the AVA measurements obtained by MRI were compared with transthoracic echocardiography (TTE) results.

Material

5 Wistar rats 8-10 weeks old belonging to Physiology Department of the local university were taken under study. The rats were housed in a specific pathogen-free facility on a 12-hour light/dark cycle with free access to food and water. The experimental model was carried out in concordance to the guidelines on accommodations and care of animals formulated by the European Convention for Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. The study protocol was approved by the local Ethic Committee No. 234/6.05.2015.

Echocardiographic assessment of the AVA

We measured aortic velocity and AVA by echocardiogram before MRI was performed. 2D and Doppler transthoracic echocardiography was performed with a VisualsonicsVevo 2100 ultrasound machine under anesthesia with an intraperitoneal administration ketamine (100 mg/kg) and xilazine (50 mg/kg) in a 2:1 proportion. The system uses a transducer MS250 with a 24 MHz frequency with harmonics and 16 MHz with Doppler. The transducer was mounted on a linear positioning dispositive that allows it to transverse the scan plan. Sampling point was set in the center of left ventricular outflow tract and aortic valve. The Doppler incident angle was 51º and angle-correction was performed in individual rat within 60º. The parasternal long axis view was used to measure the diameter of the left ventricular outflow tract. The measurements were done as described before (Slama et al. 2003). Aortic valve area was measured by continuity equation: AVA_{TTE} (mm^2)= SV_{LVOT}/VTI_{Ao} = ($VTI_{LVOT} \times A_{LVOT}$)/$VTI_{Ao}$ where AVA= the aortic valve area calculated by transthoracic echocardiography (TTE), SV_{LVOT}= the stroke volume measured in the left ventricular outflow tract (LVOT), A_{LVOT} = the cross-sectional area of the LVOT (A_{LOVT}=LOVT^2 *0.78540), VTI_{LVOT}= the velocity-time integrals at the LVOT and VTI_{Ao}= the velocity-time integrals at the vena contracted.

MRI images based assessment of the AVA

We manually measured AVA on MRI images obtained using the above described protocol. Based on the cross section shape of aortic valve we decided to use ellipsoidal shape to measure the area of interest.

The animals were anesthetized with an intraperitoneal administration ketamine (100 mg/kg) and xilazine (50 mg/kg) in a 2:1 proportion. After anesthesia each of the 5 Wistar rats was placed in dorsal position on an animal holding bad. As mentioned before, the respiration was monitored throughout the acquisition. After the acquisition that rats were placed in separate cages and received food and water.

Statistical analysis

Results are shown as mean ± SD. The differences in mean value between two groups were analyzed using Student t test.
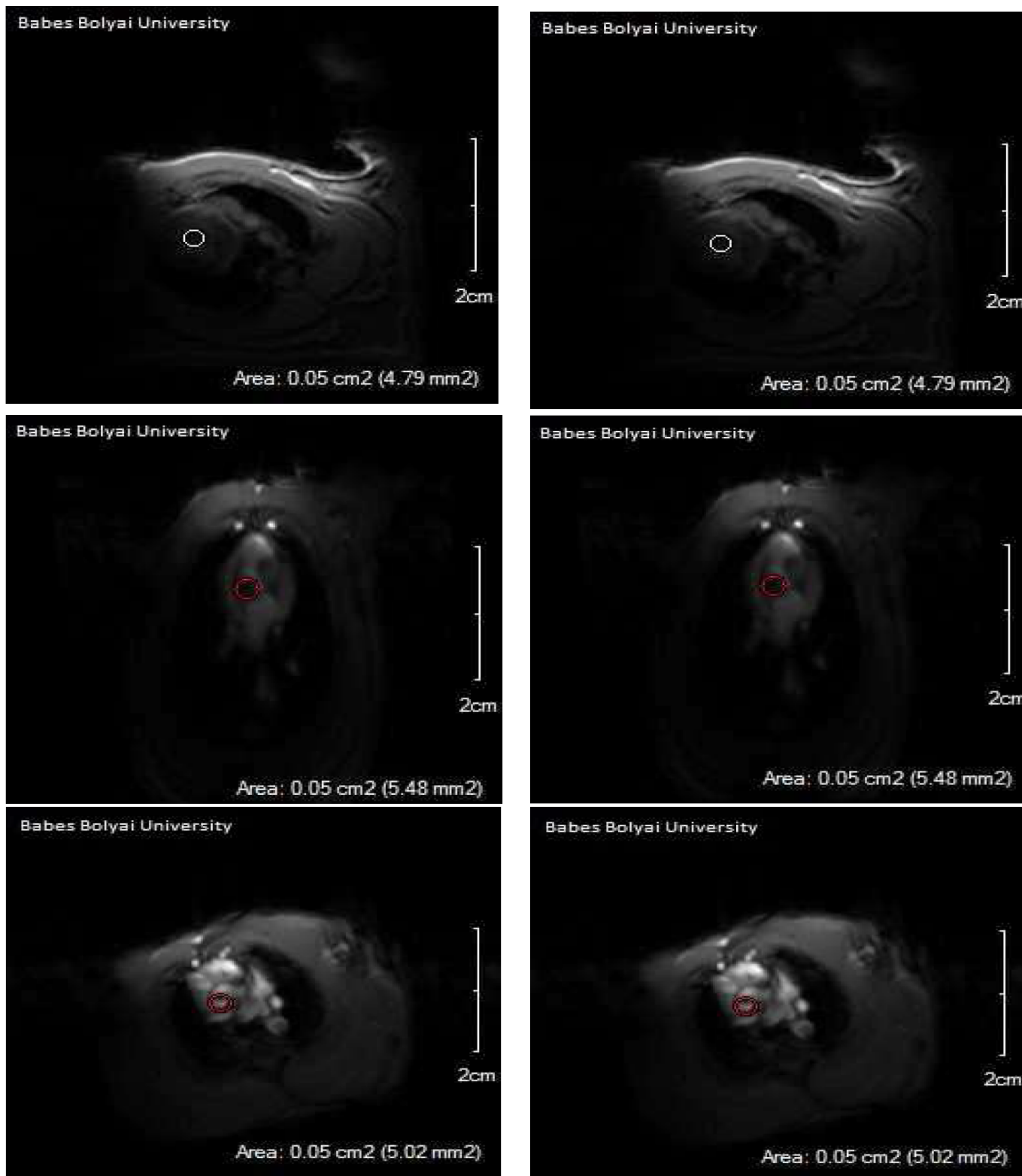
Fig. 3. AVA measured manually by planimetry. In up left corner - coronal section of the heart

The applicability of the test was ascertained by the one-sample Kolmogorov-Smirnov test. The agreement between the 2 methods of quantification of AVA was assessed by univariate regression analysis. A level of significance below <0.05 was defined as statistically significant. Statistical analysis was performed using Statistica 10 (www.statsoft.com).

Results

In Fig. 3 images of AVA obtained by MRI and measured by manual planimetry are shown.

Mean AVA determined by TTE was 5.65 ± 0.85 mm^2. Mean AVA determined by MRI was 5.11 ± 0.24 mm^2. The mean absolute difference between AVA derived by MRI and TTE was 0.54 ± 0.34 mm^2 not statistically different ($p=0.12$). These data are summarized in Fig. 4. AVA by MRI and TTE were closely correlated ($r=0.98$, $p<0.001$) (Fig. 5).

Discussions

MRI scanner technology has evolved to the point at which ventricular function and morphology can be assessed in the matter

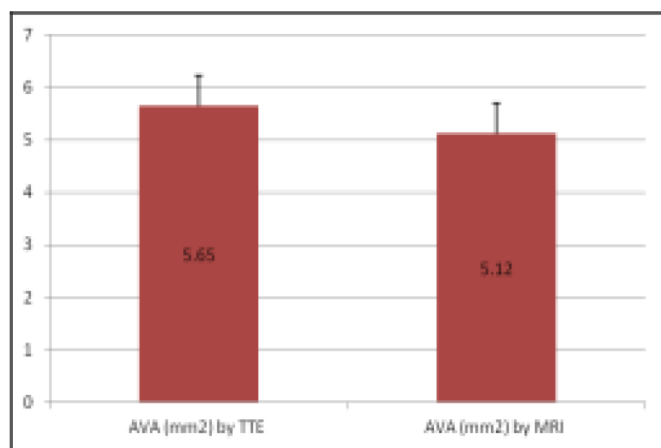Fig. 4. Comparison of TTE and MRI evaluation of AVA in rats (n=5). No statistically significant difference was identified (p>0.05). (TTE: transthoracic echocardiography, MRI: magnetic resonance imaging, AVA: aortic valve area)
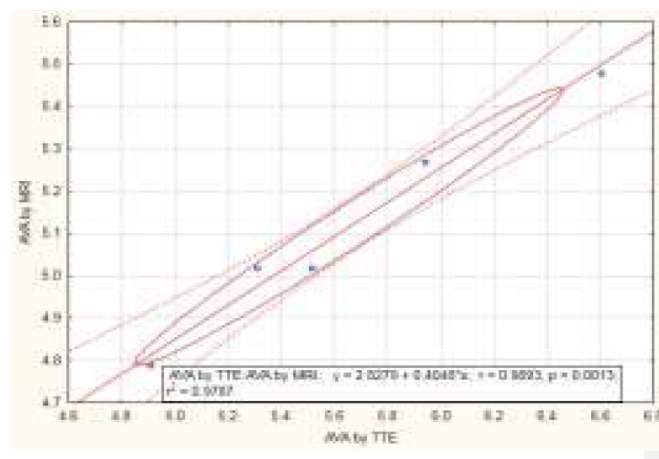

Fig. 5. Scattergram of AVA determined by TTE and MRI (n=5) – univariate analysis. (TTE: transthoracic echocardiography, MRI: magnetic resonance imaging, AVA: aortic valve area).

of minutes (Jerosch-Herold&Kwong 2008). If imaging protocols are efficiently implemented this technology can be efficiently used for valvular assessment, too.

Our study presents a reliable method of recording AVA in rats, using MRI. 2D IntraGate-FLASH CINE protocol has the ability to register real time images of the aortic valve in a shorter time with a very high temporal resolution. Our data demonstrate that visualization and planimetry of the AVA by MRI is possible with a good image quality in rats. A good correlation to AVA measurement by TTE was observed, but image quality was better in MRI than in TTE. The time needed for AVA estimation was shorter in MRI than TTE. The experience needed to correctly obtain aortic valve images and calculate AVA is one week, while for TTE (Doppler technique) assessment few weeks are necessary to learn the technique (Ram et al 2011). The accuracy of AVA calculation by TTE has often been challenged because of potential imprecision introduced by the hemodynamic parameters (mean transvalvular pressure gradient, cardiac output, ejection period) (Slama et al 2003). In our study, using healthy rats, the data obtained are similar to those reported by Slama et al. (2003), regarding the stroke volume in rats. Because only one observer performed all MRI measurements, in order to analyze the inter-observer differences, we decided to repeat them after 3 month. The inter-observer differences for AVA were under <7%, which is considered an acceptable value. The TTE measurements were performed only once by one observer.

Clearly this study is not devoid of limitations. The absence of ECG gating is partly explained by the intention of avoiding image artifacts. There is interaction between the spectrometer's gradients and ECG leads such that false triggers during scanning are problematic or the ECG leads act as antennae that directly introduce stray RF noise from the surroundings to the RF coil, resulting in image artifacts (Rehwald et al 1997) , (Johnson 2008). In future studies either fiber-optic or analog filters could be used to reduce interference from magnetic resonance gradients (Chen 2006). A real-time gating system that used digital low-pass filtering with automatic trigger-level adjustment to compensate for the MRI effects on the ECG signal is another

option described by Sabbah et al. (2007) and Brinegar et al. (2008). Another solution was recently proposed by Krämer (2015), acknowledging that ECG gating remains an issue in experimental models. In his study, self-gated golden ratio based radial acquisition successfully acquired cine images of the rat heart on a clinical MRI system without the need for ECG gating (Krämer et al 2015).

In our study, respiratory gating was of help to avoid interference and to monitor the animal vital signs. Intraperitoneal anesthesia with ketamine and xilazine causes a decrease in heart rate and a depression of cardiac function but is needed to immobilize the rats for data acquisition (Zuurbier et al 2002), (Berry et al 2009). The decrease in heart rate may modify the parameters measured by TTE. The same anesthesia technique was used in both MRI and TTE imaging, with a similar decrease in heart rate, which kept the results in similar limits. The anesthesia with isoflurane might have a milder effect on heart rate and is advisable to be used in further studies, when an inhalator dispositive is available (Zuurbier et al 2002).

Conclusions and further work

In the present paper we proposed and put into practice a real-time MRI method to evaluate AVA in rats. 2D IntraGate-FLASH CINE protocol allowed us to obtain images of aortic valve morphological and functional state and to assess AVA by manual planimetry, well correlated with TTE.

The advantage of this method is that in shorter preparation and examination time, high-resolution, good quality images of aortic valve are obtained. The direct visualization of the valve movements and the estimation of the valve area by planimetry are of utmost importance in experimental in-vivo studies. Our work provides evidence that the evaluation of aortic valve function and morphology, which was the domain of invasive techniques, becomes possible by new MRI techniques.

Further work should focus on evaluating this MRI protocol in pathologic experimental models of aortic valve stenosis or regurgitation, harmonizing the results derived from the basic research scenario with that of clinical imaging.

Acknowledgements

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