Cardiac ASL: optimisation and validation in the mouse heart

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Introduction: The advent of transgenic mice and improvements in surgical procedures for disease models have led to a wider selection of animal models of cardiac diseases. Despite a corresponding increase in the development of cardiac imaging techniques, there is little information on the accuracy and reproducibility of such methods, which are essential requirements for a technique to be suitable for the preclinical environment. Myocardial perfusion describes the rate of blood flow into the capillary bed of a block of tissue in the heart. Perfusion is closely related to the delivery of oxygen and other nutrients to the tissue and is therefore a good indicator of tissue heath. Using arterial spin labelling (ASL), myocardial perfusion can be measured in the mouse non-invasively (eg. [1, 2, 3]). In the mouse heart, a pulsed ASL technique is used in which T_1 is measured with global and slice selective inversion. T_1 maps can be compared pixel-wise to generate a perfusion map.

The aim of this study was to develop a time-efficient ASL sequence for measurement of perfusion in the mouse myocardium, together with improved data acquisition using additional gating and retrospective image analysis. In addition, we present a thorough validation study to analyse the sources of variation and the repeatability of this technique for *in vivo* studies

Methods: Imaging was performed on a Varian 9.4T scanner (Varian Inc. Palo Alto, CA, USA) with 35mm coil (RAPID Biomed, Rimpar, Germany). T_1 was quantified using an optimised ECG-gated Look-Locker approach [1], with 4 lines of k-space acquired every heartbeat during T_1 recovery (TE/TR(inv)/TR(RF)=1.18ms/13.5s/2ms, flip=5°, in-plane resolution=200μm, slice thickness=1.5mm, matrix = 128^2). Using this segmented k-space approach, one ASL measurement requires 15 -20 minutes. A 3-parameter fit was used to generate pixel-wise T_1 estimation.

A data logger (CED) was used to record ECG, RF and respiration events and was programmed to gate on each ECG peak detected as well as to interpolate peaks missed by physiological monitoring software. This reduces uneven spacing of acquisitions due to missed ECG peaks, which can result in inaccuracies in T_1 quantification. Additionally, using the data logger information, lines of k-space were binned as 'acquired during respiration' or not, such that images with greater than 50% of k-space lines corrupted by respiration could be rejected. All image analysis and data logger analysis was done in MATLAB.

Eight male CD-1s were scanned with four repetitions of the ASL sequence in each scanning session and the protocol was repeated one week later. Additional data sets were generated, with reduced respiratory corruption, by taking the "average" of 2 consecutive data sets using only the least corrupted k-space lines. The same region-of-interest in the myocardium was used to calculate mean myocardial perfusion from the perfusion map for all repeats within a session. A two-way repeated-measures ANOVA was performed in SPSS with 'scan within session' and 'across weeks' as independent variables. The coefficient of variation (CV) and the Bland-Altman repeatability coefficient (RC) were calculated (Eqn1 and Eqn 2, respectively) between weeks and between measurements within session. RC is equivalent to the 95% confidence interval of the differences between measures and provides an indication of the magnitude of changes between groups required for detection above the intrinsic variability.

$$CV = \frac{\text{standard deviation}}{\text{mean}} \cdot 100\% \qquad \text{(Eqn1)} \qquad RC = 1.96\sqrt{\frac{\sum(\Delta P^2)}{n-1}} \qquad \text{(Eqn2)}$$

Figure 1: Example ASL data set from one mouse. a) Anatomical image, T_1 map from b) global inversion recovery and c) slice selective inversion recovery, and d) resulting perfusion map with values greater than 20ml/g/min clipped.

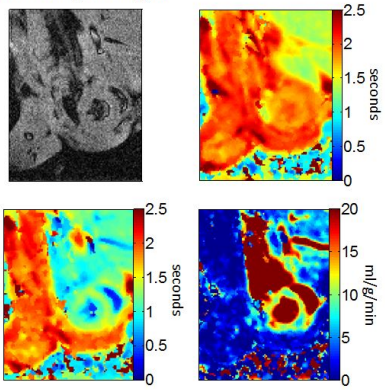
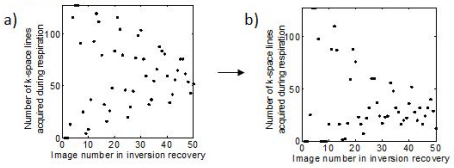


Figure 2: Number of k-space lines acquired during respiration throughout inversion recovery for a) standard data sets and b) averaged data sets.



Results: The segmented k-space ECG-gated Look-Locker T_1 mapping sequence was validated against traditional T_1 mapping in a phantom (data not shown). Figure1 presents an example ASL data set, demonstrating that anatomical image quality is not compromised using this segmented k-space method and T_1 maps appear to have less respiratory corruption. The number of k-space lines acquired during respiration for images throughout an inversion recovery are displayed in Figure 2 for standard and averaged data sets. The de-phasing of respiration and acquisition, with changing respiration rate, manifests as blurring of the final images of the inversion recovery curve. When averaging of un-corrupted k-space lines is performed, the image blurring is reduced, as expected.

Figure 3 and Table 1 present the perfusion estimations generated by the validation study. No significant differences were observed in the measurements across scans (p = 0.61) or across weeks (p=0.32). The CV for the technique was 13% using the standard perfusion data sets and 8.9% using the averaged data sets. Between animals the CV was 22% (standard data sets) and 21% (averaged data sets). This indicates that the greatest source of variability comes from the between animal variation. Figure 4 presents Bland-Altman plots of the between week and between scan variations with their RC values. We demonstrated that with both standard and averaged data sets, an approximately 46% change is needed to detect variations across weeks. From the comparison of scans within session, a 39% change (standard data sets) or a 32% change (averaged data sets) is needed for observation of changes above technique error.

Figure 3: Perfusion estimates from validation study

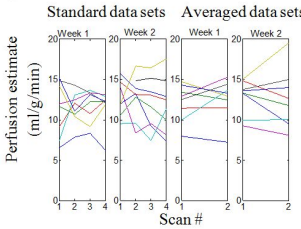
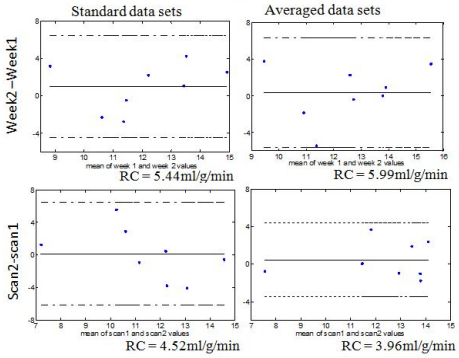


Figure 4: Bland-Altman plots and repeatability coefficients for variation between weeks and between scans in a session.



Discussion: In this study, a time-efficient ASL technique is presented. Data quality was improved using a data logger for gating to eliminate missed ECG triggers and to introduce objective criteria for the rejection of respiration-corrupted images. In addition, averaging images using only uncorrupted lines of k-space was shown to improve respiratory corruption, but required double the scan time. The perfusion values presented here are higher than those presented previously in literature, possibly due to the different strain, age or size of the mice used. The validation study showed that the between animal variability was larger than the technique variability. The repeatability coefficients calculated in this study will be very important when planning future studies involving ASL. The between-scan RC will be important when comparing groups of animals. The week-to-week RC is important to consider in longitudinal studies.

Table 1: Group mean perfusion estimates from ASL validation study

	Standard data sets					Averaged data sets	
	scan1	scan2	scan3	scan4		Avg-scan1	Avg-scan2
week1	11.36 ±3.32	11.47±1.96	11.74±2.08	11.63±2.22		12.14±2.28	12.58±2.47
week2	13.52±5.78	12.80±5.18	11.97±5.17	11.80±5.17		12.86±2.13	12.56±3.65

References: [1] Kober et al, MRM **51**: 62-67 (2004). [2] Streif et al, MRM **53**: 584-592 (2005). [3] Vandsburger et al, MRM **63**: 648-657 (2010).