# Improved Arterial Spin Labeling After Myocardial Infarction in Mice Using Respiratory and Cardiac Gated Look-Locker Imaging with Fuzzy C-Means Clustering for T1 Estimation

**M. H. Vandsburger[1], R. L. Janiczek[1], Y. Xu[1], B. A. French[1], C. H. Meyer[1], C. M. Kramer[1], and F. H. Epstein[1]**
[1]University of Virginia, Charlottesville, VA, United States

## Introduction

MRI applied to genetically-engineered mice enables noninvasive serial studies of the roles of individual genes in development, normal physiology, and pathophysiology. Cardiac MRI in particular has previously elucidated important roles of specific genes in myocardial structure and function in the normal heart and during left ventricular (LV) remodeling in a mouse model of experimental myocardial infarction (MI)[1-4]. Although also critical in infarct healing, changes in myocardial perfusion after infarction have not been previously measured in mice. While quantitative Look-Locker arterial spin labeling (ASL) of myocardial perfusion has been demonstrated in mice[5], due to erratic respiratory patterns and increased variability of the ECG early after MI, this technique has not been successfully applied to mouse models of acute MI and post-MI infarct healing. We developed a cardio-respiratory gated (CRG) Look-Locker spiral ASL sequence which has reduced sensitivity to variable respiratory and ECG waveforms[6]. Using this method, we tested the hypothesis that ASL could detect an increase in infarct zone perfusion during the time-course of infarct healing in mice.

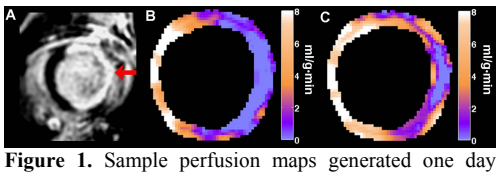


**Figure 1.** Sample perfusion maps generated one day post-MI. (A) Gd-enhanced image displays enhancement of the infarcted region (arrow). (B) Perfusion map generated using CRG-ASL with FCM clustering displays a perfusion defect with close spatial agreement to the infarct region as defined in (A). (C) The perfusion map generated from the same mouse using a conventional ECG-gated acquisition underestimated the region with reduced perfusion.

## Methods

A Look-Locker spiral ASL sequence was developed on a 7T Clinscan MR system (Bruker, Germany) using combined respiratory gating and ECG triggering, where ECG triggers were detected only during the quiescent phase of expiration. Image acquisition and reconstruction have been previously described in detail[6]. Briefly, complete Look-Locker data sets (sequences of images at an array of inversion times (TIs)) are acquired after both non-selective and slice-selective inversion pulses. Due to the use of cardio-respiratory gating, Look-Locker raw data are acquired at inconsistent TIs. To account for the distribution of TIs, a fuzzy C-means (FCM) clustering algorithm[7] is used to sort the CRG data and assign TI values to the series of Look-Locker images. Myocardial $T_1$ is estimated for regions of interest in the heart using Bloch equation simulations which incorporate both variable intervals between RF pulses by using FCM determined TIs, as well as a repeated pattern of CRG triggers that approximate the actual ECG and respiratory waveforms during scanning. Myocardial perfusion (P) is calculated according to Bauer et al[8]. The CRG-ASL method was validated by measuring $T_1$ in a stationary phantom without and with CRG from live mice. The method was then applied to mice at baseline (n=7), with and without ATL313 (vasodilator), and 1, 7, 14, and 28 days following experimental reperfused MI (n=6). Animal preparation and specific ASL imaging parameters have been previously described[6]. Delayed gadolinium(Gd)-enhanced images were also acquired to determine the infarct region.

## Results

Phantom studies performed using CRG from live mice demonstrated the improved accuracy of CRG-ASL. Specifically, using a fixed sampling interval, the $T_1$ of a gel phantom was measured as 1.45s (true value). Using ECG gating from a live post-MI mouse with prospective TI assignment based on the mean cardiac interval, $T_1$ of the phantom was incorrectly measured as 1.32s. In contrast, $T_1$ was measured as 1.45s using live mouse CRG with retrospective TI assignment. For all *in vivo* ASL scans, the mean difference of prospectively assigned TIs compared to retrospectively determined TIs was 53.4 ± 7.0 ms at baseline, 125.0 ± 23.7 ms with ATL313, 102.2 ± 14.9 ms at day 1, 44.8 ± 10.6 ms at day 7, 44.5 ± 8.4 ms at day 14, and 70.4 ± 12.6 ms at day 28 post-MI. These values are highly significant given that a typical cardiac interval (difference between successive TIs) is around 120 ms.

Reduced motion artifact using CRG-ASL, as well as improved $T_1$ estimation using retrospective TI assignment, enabled accurate measurement of myocardial perfusion during the time-course of infarct healing. For the mice studied at baseline, myocardial perfusion by CRG-ASL was 4.9 ± 0.5 (ml/g·min) (mean ± SEM) and increased to 8.15 ± 1.0 (ml/g·min) following vasodilatation with ATL313. Improved measurement of regional myocardial perfusion using CRG-ASL is illustrated by a comparison of perfusion maps generated one day post-MI using both the CRG-ASL method (Fig. 1B) and using a conventional ECG-gated acquisition (Fig. 1C), where the CRG-ASL perfusion defect more closely corresponds to the Gd-enhanced region. In the infarcted zone ($P_{infarct}$), perfusion decreased to 0.9 ± 0.8 (ml/g·min) ($p<0.05$ vs. baseline) at 1 day post-MI. Next, as we hypothesized, CRG-ASL detected a significant increase ($p<0.05$) in $P_{infarct}$ from 1 to 28 days post MI (Fig 2). Perfusion in the non-infarcted zone ($P_{remote}$) was unchanged from baseline throughout infarct healing (Fig. 2). As a low perfusion reference, chest wall perfusion was measured as 0.12 ± 0.19 ml/g·min at baseline, and 0.07 ± 0.23 (ml/g·min) at 1 day post-MI.

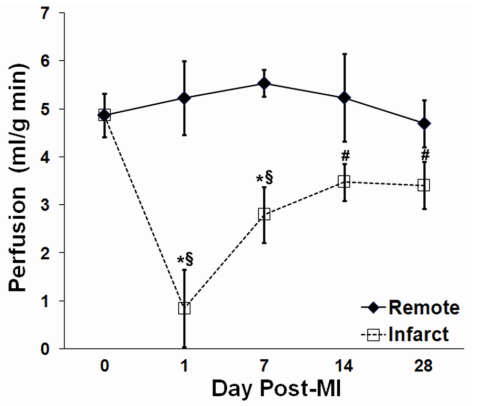


**Figure 2.** Time course of post-infarct myocardial perfusion in infarcted and remote zones. One day after MI, perfusion was very low in the infarct zone and normal in the remote zone. From day 1 to day 28, perfusion in the infarct zone increased and perfusion in the remote zone was unchanged. Symbols designate statistically significant differences ($^{*}P < 0.05$ vs. remote, $^{\S}P < 0.05$ vs. baseline, $^{\#}P < 0.05$ vs. day 1 post-MI).

## Conclusions

Use of CRG during image acquisition and implementation of an FCM clustering algorithm for ASL image reconstruction reduced respiratory artifact and enabled measurement of myocardial perfusion following experimental MI in the mouse heart. Changes in perfusion in the reperfused infarct zone during the time-course of infarct healing parallel *in vitro* findings of neovascularization in reperfused myocardium following MI[9]. This suggests that when applied to genetically-engineered mice, CRG-ASL will enable studies of the roles of individual genes in myocardial perfusion after MI and during infarct healing.

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