Evaluation of cardiac stress perfusion and functional MRI biomarkers in healthy nonhuman primates: Reproducibility and repeatability study

Sarayu Parimal[1,2], Smita Sampath[1,2], Michael Klimas[2], Dai Feng[3], Richard Baumgartner[3], Elaine Manigbas[4], Willy GSell[4], Jeffrey L. Evelhoch[2], and Chin Chih-Liang[1,2]

[1]Imaging, MSD, Singapore, [2]Imaging, Merck & Co. Inc., WestPoint, Philadelphia, United States, [3]Biometric Research, Biostatistics and Research Decision Sciences, Merck & Co. Inc., Rahway, New Jersey, United States, [4]MRI department, Maccine Pte Ltd, Singapore

Target Audience: Researchers involved in nonhuman primate (NHP) animal model development and drug research related to cardio-metabolic diseases.

Purpose: Drug discovery studies using NHPs are of importance due to their phylogenetic closeness to humans, thus allowing disease animal models with better translatability. Imaging biomarkers play a critical role in the non-invasive phenotyping of NHP disease models, especially identifying robust biomarkers that afford predicting disease progression or detecting early responses to treatments. It has been shown that the assessment of cardiac function and perfusion during stress tests is a powerful tool that can reveal early onset of cardiac disease; for example, early detection of ischemic myocardial regions [1] and early onset of myocardial fibrosis can both be uncovered through stress testing [2]. Herein, to develop translational imaging biomarkers in NHPs, we evaluated the robustness of cardiac regional function and perfusion biomarkers both at rest and subjecting to higher metabolic demands through inotropic stress testing. Further, intra- and inter-observer reproducibility analyses were performed on all quantified biomarkers. These imaging biomarkers may help design and guide future longitudinal drug safety and efficacy studies of experimental compounds in NHP cardiac disease models as well as in translational studies in the clinic.

Methods: ***Animal Preparation and MRI:*** Healthy Vietnamese cynomologus macaques (n=7, 5.8±0.2 kg, 9±2 years, resting heart rate: 123±13 bpm) were anesthetized (ketamine 10 mg/kg IM induction and isoflurane 2-3% maintenance) and imaged using a 3T Trio MRI scanner at Maccine Pte Singapore. Each animal underwent 2 imaging sessions (test and retest) conducted 1-2 weeks apart, while within each session imaging data were acquired at rest and stress (~20 minutes apart). Dobutamine, as inotrope, was infused i.v. with step-wise increases in drug concentration (3-min intervals at 5,10,20,30, and 40 µg/kg/ml) [4]. The increment was stopped when the heart rate at stress reached 150% of the resting heart rate and then a constant infusion at that concentration level was maintained for the rest of the scan. ***Imaging Protocols:*** For tagging MRI, SPAMM [3] tagging (FOV: 200×200 mm^2, imaging matrix: 208×208, slice thickness: 5 mm, segments: 2, echo spacing: 6.4 ms, TR/TE: 12.72 ms/2.97 ms, FA: 12°, tag separation: 4 mm) was employed to encode 2-D displacement in 3 short-axis (SA) slices. For perfusion MRI, T_1 weighted images (for the matched 3 SA slices) were acquired using saturation prepared segmented gradient echo-planar sequence (FOV: 150 ×135 mm^2, imaging matrix: 128×96, slice thickness: 5 mm, segments: 56, echo spacing: 2.2 ms, TR/TE/TI: 259 ms/1.15 ms/200 ms, FA: 10°, GRAPPA with acceleration factor: 2). A dual bolus first pass protocol using contrast agent gadolinium-DTPA (Magnevist) was used [5]. The concentration for the first and second boluses were 0.005 and 0.1 mmol/kg respectively each followed by 2 ml saline flush.

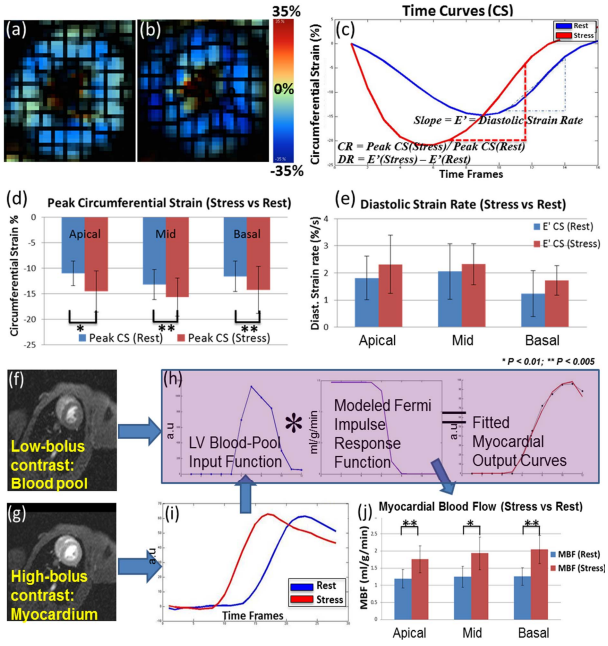

Figure 1: Representative end-systolic CS map at (a) rest, and (b) stress. (c) Time-courses of LV CS are used for computing peak CS and early diastolic strain rate (E'). Bar graphs depicting quantified (d) peak CS and (e) E' at rest and stress. Gadolinium infused (f) low-bolus image, (g) high-bolus image, (h) model-based convolution, (i) myocardial tissue response function and (j) bar graph depicting quantified MBF at rest and stress.

Reproducibility and Repeatability CC (Mixed-effects model)									
	Slices	Peak CS (Rest)	Peak CS (Stress)	CRv	Log (CRv)	E′ (Rest)	E′ (Stress)	DRv	Log (DRv)
Intra-Observer	Apical	0.81	0.55	0.41	0.55	0.81	0.67	0.68	0.44
	Mid	0.86	0.75	0.46	0.62	0.81	0.39	0.84	0.83
	Basal	0.82	0.54	0.48	0.52	0.80	0.43	0.72	0.60
Inter-Observer	Apical	0.81	0.53	0.28	0.56	0.81	0.67	0.68	0.45
	Mid	0.86	0.74	0.46	0.61	0.78	0.45	0.86	0.69
	Basal	0.83	0.55	0.48	0.67	0.81	0.51	0.71	0.61
Test-Retest	Apical	0.57	0.53	0.24	0.43	0.24	0.63	0.52	0.63
	Mid	0.67	0.39	0.40	0.52	0.31	0.44	0.29	0.19
	Basal	0.75	0.53	0.48	0.49	0.40	0.51	0.30	0.21

Table 1: Intra-observer, inter-observer reproducibility and test-retest repeatability for function biomarkers.

Data Analysis: HARP [6] analyses were done on tagged images to quantify myocardial displacement where averaged peak circumferential strain (CS, in %) and early diastolic strain-rate (E′, in %/s) for the three slices was quantified (see Fig. 1 a-c). Contractile reserve (CRv) was then computed as the ratio of peak CS at stress over rest. DRv was computed as the difference of E′ at stress from rest. For quantitative perfusion analysis, a Levenberg-Marquardt optimization algorithm was used to solve a deconvolution equation to derive the parameters of a modeled Fermi impulse response function that results in the best-fit estimate of the myocardial tissue response (see Fig. 1 f-i). Slice-wise myocardial blood flow (MBF, in ml/min/g) was then computed from the Fermi function. Myocardial perfusion reserve (MPR) was computed as a ratio of MBF at stress over rest. All analyses were repeated twice by two independent observers. ***Statistics:*** To evaluate the repeatability/reproducibility, we used a linear mixed-effects model as follows: $y_{ijkl} = \mu + \alpha_i + a_j + b_k + c_l + \varepsilon_{ijkl}$; where μ is the overall mean, y_{ijkl} is the observation from subject i, observer j, repetition k, and test/retest l; α_i is used to capture the animal to animal variability; aj, b_k, and c_l are used to model the difference between two observers, within each observer and between test and retest, respectively. Correlation coefficients (CC) were calculated to determine the intra-observer, inter-observer reproducibility and test-retest repeatability. Student t-test was also performed between rest and stress for CS, E′ and MBF.

Results and Discussion: The correlation coefficients for all biomarkers are shown in Tables 1&2. For the functional biomarkers, we found good repeatability for peak CS and E′ at rest and overall moderate repeatability/reproducibility during stress. The log(CRv) shows moderate intra- and inter- observer reproducibility, while the DRv shows better performance. However, both parameters demonstrate lower test-retest repeatability, and this finding could be reconciled with higher sensitivity to the variability in experimental conditions during stress on the two imaged days. For the perfusion biomarkers, it was observed that overall good reproducibility is obtained for MBF at rest and stress. Log(MPR) also performed well with good repeatability. Comparison between stress and rest yields significant differences in peak CS and MBF found for all slices. Although E′ is typically higher during stress, we do not observe significant differences (See Fig. 1 d,e,j). **Conclusion**: We have successfully performed test-retest cardiac regional function and perfusion imaging studies in NHPs during inotropic stress testing and evaluated the intra-and inter-observer reproducibility of key potential biomarkers. Our results highlight that perfusion biomarkers such as MBF and log(MPR) are more robust under all conditions. Functional biomarkers are repeatable at rest, but demonstrate lower test-retest repeatability under stress.

References:[1] Thomas DS et. al., JCMR, 10:59, 2008. [2] White JAA et. al., JCMR, 11: P55, 2009. [3] L. Axel et. al., Radiology, 172: 349-350, 1989. [4] Nagel E., Medica Mund, 43 (2): 31-38, 1999. [5] Hsu L et. al., JMRI, 23: 315-322, 2006. [6] Osman et. al., PMB, 45:1665-1682, 2000.

Reproducibility and Repeatability CC (Mixed-effects)					
	Slices	Rest (MBF)	Stress (MBF)	MPR	Log MPR
Intra-Observer	Apical	0.78	0.81	0.47	0.45
	Mid	0.76	0.61	0.48	0.46
	Basal	0.78	0.58	0.49	0.70
Inter-Observer	Apical	0.70	0.75	0.46	0.49
	Mid	0.53	0.58	0.38	0.46
	Basal	0.53	0.58	0.51	0.72
Test-Retest	Apical	0.62	0.71	0.49	0.74
	Mid	0.71	0.60	0.50	0.72
	Basal	0.78	0.49	0.49	0.68

Table 2: (right) Intra-observer, inter-observer reproducibility and test-retest repeatability for perfusion biomarkers.