Commercial 4-Dimensional Echocardiography for Murine Heart Volumetric Evaluation after Myocardial Infarction

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Research

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Abstract

Background: Traditional preclinical echocardiography (ECHO) modalities, including 1-dimensional motion-mode (M-Mode) and 2-dimensional long axis (2D-US), rely on geometric and temporal assumptions about the heart for volumetric measurements. Surgical animal models, such as the mouse coronary artery ligation (CAL) model of myocardial infarction, result in morphologic changes that do not fit these geometric assumptions. New ECHO technology, including 4-dimensional ultrasound (4D-US), improves on these traditional models. This paper aims to compare commercially available 4D-US to M-mode and 2D-US in a mouse model of CAL.

Methods: 37 mice underwent CAL surgery, of which 32 survived to a 4 week post-operative time point. ECHO was completed at baseline, 1 week, and 4 weeks after CAL. M-mode, 2D-US, and 4D-US were taken at each time point and evaluated by two separate echocardiographers. At 4 weeks a subset of mice underwent cardiac magnetic resonance (CMR) imaging to serve as a reference standard. End systolic volume (ESV), end diastolic volume (EDV), and ejection fraction (EF) were compared between imaging modalities. Hearts were also collected for histologic evaluation of scar size and compared to wall motion severity index (WMSI).

Results: 4D-US provides closer agreement of ESV (Bias: -2.55%) and EF (US Bias: 11.23%) 4 weeks after CAL when compared to CMR, outperforming 2D-US and M-mode estimations. 4D-US is also more reliable than other modalities when estimating EDV ($r=0.88$), ESV ($r=0.86$) and EF ($r=0.74$) at 1 week when compared to 4 week imaging. 4D-US also allows for rapid assessment of WMSI, which correlates strongly with infarct size by histology ($r=0.77$).

Conclusion: 4D-US outperforms M-Mode and 2D-US for volumetric analysis 4 weeks after CAL. 4D-US also provides more reliable measurements at 1 week after CAL and has less inter-user variability than other modalities. 4D-US allows for rapid calculation of WMSI, which correlates well with histologic scar size.

Background

Echocardiography (ECHO) is a highly efficient and reliable tool for clinical and pre-clinical evaluation of cardiac function¹⁻³. Advances in high-frequency and high-resolution image acquisition have allowed for increasing temporal and spatial resolution in small rodent models^{1,4,5}. However, traditional ECHO modalities for evaluating ventricular size and function, including 1-dimensional motion-mode (M-mode) and 2-dimensional (2D-US) long-axis analysis, make geometric assumptions about the heart that limit their accuracy, particularly in the setting of heart disease, where regional ventricular shape abnormalities may exist⁶⁻⁸. For example, mouse models of cardiac pathology, particularly myocardial infarction (MI), frequently result in abnormal ventricular remodeling that deviates from the geometric assumption that the left ventricle has an ellipsoid shape, limiting the utility of conventional echocardiography in such settings and requiring careful consideration of modality choice^{9,10}.

Three-dimensional (3D) ECHO has gained favor recently in clinical and pre-clinical models, allowing full volume visualization of the heart by stacking concentric short-axis images to form a 3D representation at static time points^{4,11,12}. 3D ECHO has been further improved by combining respiratory- and ECG-gated image acquisition to create 3D images throughout the cardiac cycle, referred to as 4-dimensional ultrasound (4D-US)^{13,14}. 4D-US has recently become commercially available for pre-clinical models and has already been validated against cardiac magnetic resonance (CMR) imaging in a mouse model of hypertrophy¹⁵. While CMR imaging remains the gold standard for assessment of cardiac function in mice¹⁶⁻¹⁸, recent studies in 3D ECHO and 4D-US have shown considerable advances in scanning time and reliability¹³⁻¹⁵.

In this study, we aimed to validate commercially available 4D-US in a mouse model of myocardial infarction. We performed coronary artery ligation (CAL) surgery on mice to evaluate traditional M-mode, 2D-US, and 4D-US using the Vevo 3100 preclinical imaging system against CMR imaging. When compared to CMR, 4D-US outperforms M-mode and 2D-US in the evaluation of volumetric parameters 4 weeks after CAL. Additionally, 4D-US allows for reliable prediction of scar size using wall motion severity index (WMSI) compared to histologic analysis. Finally, 4D-US is superior to other modalities when comparing images collected at 1 week after CAL to those collected at 4 weeks, suggesting increased reliability early after CAL.

Methods

Coronary Artery Ligation and Mouse Model

All animal use was performed at the University of Pittsburgh in compliance with the National Institutes of Health Guide for Care and Use of Experimental Animals and was approved by the University of Pittsburgh Animal Care and Use Committee. Previously characterized mice harboring a floxed TFAM gene via lox-stop-lox manipulation were crossed with α -myosin heavy chain Cre transgene (MHC-Cre) to generate mice that were MHC-Cre (+) x LSL-TFAM and MHC-Cre (-) x LSL-TFAM. Both males and females were used in this study. Mice were aged to twelve weeks, at which point they underwent baseline echocardiography followed by coronary artery ligation surgery. Mice were anesthetized and ventilated prior to open thoracotomy through the 4th rib followed by opening of the pericardium and coronary artery ligation (CAL) by suture placement around the proximal coronary artery²⁷. All surviving underwent follow-up echocardiography at 1 week and 4 weeks. A total of 33-mice were evaluated.

Echocardiography

Mice were anesthetized using isoflurane delivered by nose cone at baseline, 1 week following CAL, and 4 weeks following CAL. Depilatory cream was applied to the thorax to remove hair. Animals were maintained at 37° C via heating pad and rectal probe and monitored using surface ECG limb electrodes throughout imaging. Transthoracic echocardiography was performed using the Vevo 3100 imaging

systems (Visual Sonics, Toronto, Canada) with a 40 MHz linear probe attached to a step motor. Heart rate was maintained between 400–500 bpm during imaging by adjusting isoflurane concentration. M-mode and B-mode images of the heart were obtained for at least ten cardiac cycles in the parasternal long axis and mid-papillary muscle level short axis views. For 4D image acquisition, the step motor was positioned just below the apex and the motor aligned to take concentric short axis images in 0.2 mm steps. At each position, a complete cardiac cycle was recorded using automated ECG and respiratory gating. 4D images were constructed using commercially available Vevo 4D image software. Image analysis was performed independently by two blinded sonographers (CR and BM). End-diastolic volume (EDV), End-systolic volume (ESV), Ejection Fraction (EF), and Stroke Volume (SV) were calculated using the following formulas:

M-Mode (short-axis):

$EDV = [7.0 / (2.4 + LVID;d) \times LVID;d^3]$ where LVID;d = Left ventricular internal diameter at end diastole

$ESV = [7.0 / (2.4 + LVID;s) \times LVID;s^3]$ where LVID;s = Left ventricular internal diameter at end systole

$EF = 100 \times ((EDV - ESV)/EDV)$

2D-US analysis of the parasternal long-axis was completed using operator-defined LV trace function of Vevo software and calculations of EDV, ESV, and EF made by Simpson's method. 4D-US measurements were calculated directly from volumetric measurements based on operator-defined edge-tracing using Vevo 4D imaging software.

WMSI

WMSI was calculated using a 16-segment model collected from 3 short axis views collected during 4D-US image acquisition. Short axis views were obtained 1 mm, 3 mm, and 5 mm from the apex to allow for standardization between animals. The most distal image was divided into 4 sections and the remaining sections divided into 6 sections as previously described (Fig. 4)^{24,25}. Individual sections were graded as: 1- normal, 2- hypokinetic, 3- akinetic, 4- dyskinetic, 5- aneurysmal. WMSI was calculated as sum of all motion scores over 16.

CMR

Mice were anesthetized with 4% isoflurane mixed with room air in an induction box for 1 to 3 minutes. The depth of anesthesia was monitored by toe reflex, extension of limbs, and spine positioning. Once anesthetized, the mouse was placed in a neonatal mouse holder and the anesthesia was maintained by 1.5 to 2% isoflurane and 100% oxygen via a nose cone. Respiration waveforms were continuously monitored using a small pneumatic pillow under the animal's diaphragm connected to a magnet-compatible pressure transducer (SA Instruments, Stony Brook, NY). CMR was performed on a Bruker Biospec 7T/30 system (Bruker Biospin MRI, Billerica, MA) with a 35-mm quadrature coil for both transmission and reception. The Bruker Intragate module was used for image-gated cine MRI with

retrospective navigation. Subcutaneous injection of Multi-Hance (Gadobenate dimeglumine, 529 mg/ml, Bracco Diagnostics, Inc, Monroe Twp, NJ 08831) was administered immediately before the CMR acquisition at 0.1 mmol Gd/kg bodyweight. T₁-weighted images to highlight LGE were acquired 15–20 minutes after the subcutaneous administration of Multi Hance. Eight T₁-weighted short-axis imaging planes covering the whole ventricular volume with no gaps were acquired with the following parameters: Field of view (FOV) = 2.5 cm X 2.5 cm, slice thickness = 1 mm, in-plane resolution = 0.97 αm, flip angle (FA) = 10 degrees, echo time (TE) = 3.059 msec, repetition time (TR) = 5.653 msec. White-blood cine movies with 20 cardiac phases were acquired for each mouse with equivalent temporal resolution for the cine loops was about 16.5–21.5 ms per frame. Eight short-axis imaging planes covering the whole ventricular volume with no gaps and one long-axis plane were acquired with the following parameters: Field of view (FOV) = 2.5 cm X 2.5 cm, slice thickness = 1 mm, in-plane resolution = 0.97 αm, flip angle (FA) = 30 degrees, echo time (TE) = 1.872 msec, repetition time (TR) = 38.293 msec.

The extent of myocardial infarction was defined by the percentage of the myocardium displaying hyperintensity 15–20 minutes after Gd administration. To obtain the proportion of myocardial infarction, the area of hyperintensity was manually traced by a blinded operator on the Paravision 5.1 Xtip software (Bruker Biospin MRI, Billerica, MA). The extent of myocardial hemorrhage was defined by dark hypointensity on the cine images. To obtain the proportion of myocardial hemorrhage, the area of hypointensity was manually traced by a blinded operator on the Paravision 5.1 Xtip software (Bruker Biospin MRI, Billerica, MA). The left ventricular endocardium and epicardium boundaries of each imaging slice at the end-systole (ES) and the diastole (ED) were manually traced by a blinded operator on the Paravision 5.1 Xtip software (Bruker Biospin MRI, Billerica, MA) to calculate the following functional parameters: left ventricular blood volume (LVV), left ventricular wall volume (LV wall), LV mass, stroke volume (SV), ejection fraction (EF), heart rate (HR), cardiac output (CO), longitudinal shortening, and radial shortening. LVV is calculated by summation of all the short-axis slices. The ejection fraction (*EF*) was calculated using the following equation:

$$EF = \frac{\sum_i A_i^{es} h_i}{\sum_i A_i^{ed} h_i} \times 100\%,$$

where

$$A_i^{es}$$

is the internal left ventricle area of slice *i* at end systole,

$$A_i^{ed}$$

is the internal left ventricle area of slice *i* at end diastole, and *h_i* is the thickness of each scanned slice.

Tissue Histology

LV tissue was fixed overnight in 10% formaldehyde at 4 °C. Tissues were then washed with PBS and transferred to 70% EtOH and stored at room temperature. After fixation, tissues were brought to the Histology Core at the University of Pittsburgh and sectioned into 10 µm slices at 1 mm intervals throughout the myocardium. Sections were stained with hematoxylin and eosin (H&E) and Trichrome stains and images obtained on a TissueFAXS Histo (TissueGnostics, Vienna, Austria) upright brightfield microscope and images obtained by HistoQuest software. Image analysis was performed by automated red and blue channel separation using Image Measurement 9.0 (Bersoft Imaging, Cologne, Germany).

Statistical Analysis

Differences between imaging modalities in Fig. 1 were evaluated by Bland-Altman analysis and are expressed as % bias and 95% level of agreement (LOA). Bland-Altman percentage bias was calculated as $(100 \times (B-A) / \text{Average vs Average})$. Intraclass correlation was used to evaluate correlation between two users (CR and BM). Regression analysis was performed using Spearman rank correlation to compare scar size based on histology to WMSI and CMR computations (Fig. 4) and by Pearson correlation to compare week 1 to week 4 volumetric measurements (Fig. 5). Correlation was graded as poor (0.0-0.5), moderate (0.5–0.7), strong (0.7 to 0.9), or very strong (0.9-1.0). Supplemental Fig. 2 data are expressed as mean $\pm$ standard error. $p \leq 0.05$ was considered significant for all comparisons. All statistical analysis was completed using Graphpad Prism 7 software (San Diego, CA).

Results

Animal Model of Myocardial Infarction

In order to model myocardial infarction, thirty-seven male mice underwent CAL surgery at 8 weeks of age. Thirty-two of these mice survived to 4 weeks post-CAL (86.4%, Table 1). The mouse background was MHC-CRE (+) x LSL-TFAM or MHC-CRE (-) x LSL-TFAM. Subgroup analysis was performed between MHC (+) (n = 22) and MHC (-) (n = 15) groups and there was no difference noted in survival, scar size, baseline end-diastolic volume (EDV), end-systolic volume (ESV), and ejection fraction (EF), as well as 4 week EDV, ESV, or EF between groups by any ECHO modality, allowing pooled sampling of transgenic mice (Supplemental Fig. 2, Supplemental Table 1).

Table 1
Baseline and 4 week post CAL Animal Data

	Total Mice	Percent
Survival	32/37	86.40%
	Mean	SEM
Baseline Weight (g)	25.26	0.58
Week 4 Weight (g)	25.44	0.537
Baseline HR (bpm)	454.96	7.96
Week 4 HR (bpm)	482.18	15.25
	Mean $\pm$ SEM	Range
Histologic Scar Size	21.54 $\pm$ 4.07%	4.02% – 57.0%
WMSI	1.32 $\pm$ 0.05	1.065–2.0625
CMR Hyperintense Volume	13.53 $\pm$ 2.22%	5.52–28.10%

Comparison of ECHO Modalities to CMR

To compare imaging modalities following infarction, mice were evaluated by ECHO using traditional M-mode, 2D-US, and 4D-US (representative images in Fig. 1, mean values reported in Table 2, n = 32) at baseline, 1 week following CAL, and 4 weeks following CAL. One-day after the 4 week ECHO was performed, a subset of randomly chosen mice underwent CMR to serve as reference standard for volumetric measurements (Fig. 1, n = 12). Bland-Altman analysis of M-mode, 2D-US, and 4D-US was used to compare the percentage difference of ECHO modalities to CMR (Fig. 2). Of the three ECHO modalities, 4D-US demonstrated the lowest bias when comparing ESV (4D-US Bias: 2.55% LOA: –61.55 to 66.66, 2D-US Bias: 29.84% LOA: –43.10 to 102.8, M-Mode Bias: 43.57% LOA: –21.87 to 109.0; Fig. 2) and EF (4D-US Bias: –11.23% LOA: –56.26 to 33.80, 2D-US Bias: –24.42% LOA: –87.99 to 39.16, M-Mode Bias: –12.90% LOA: –53.80 to 28.00) at 4 weeks. 4D-US was outperformed by 2D-US when evaluating EDV (4D-US Bias: –13.21% LOA: –47.71 to 21.28, 2D-US Bias: 5.84% LOA: –24.47 to 36.15, M-Mode Bias: 35.17% LOA: 0.73 to 69.61). Linear regression was also performed between ECHO modalities and CMR and demonstrates strong to very strong correlation between 4D-US and CMR (EDV: r = 0.887, ESV: r = 0.943, EF: r = 0.811; Supplemental Fig. 1). 2D-US has lower correlation values for every modality (EDV: r = 0.806, ESV: r = 0.793, EF: r = 0.406). M-Mode correlation is slightly higher than 4D-US for EDV, but lower for ESV and EF (EDV: r = 0.902, ESV: r = 0.882, EF: r = 0.747). Inter-user variability was evaluated between two users (CR and BM) for each modality by calculating the intraclass correlation (ICC) of measurements. 4D-US demonstrated the highest ICC for estimation of EDV (4D-US: 0.91, 2D-US: 0.72, M-Mode: 0.80) and ESV

(4D-US: 0.93, 2D-US: 0.72, M-Mode: 0.79). M-mode had a higher ICC for evaluation EF (4D-US: 0.64, 2D-US: 0.31, M-Mode: 0.69).

Table 2. Mean Volumetric Data at 1 and 4 weeks by Imaging Modality

1 week	MRI	4D-US	2D-US	M-mode
EF (%)	-	43.94 ± 2.98	38.83 ± 2.28	48.47 ± 3.26
SV (μL)	-	16.37 ± 1.38	22.54 ± 1.31	36.54 ± 2.03
ESV (μL)	-	23.88 ± 3.77	40.12 ± 4.84	42.16 ± 6.97
EDV (μL)	-	40.25 ± 4.30	62.66 ± 5.516	94.45 ± 7.43
4 week	MRI	4D-US	2D-US	M-mode
EF (%)	45.89 ± 5.66	38.89 ± 3.41	34.27 ± 3.14	40.04 ± 4.43
SV (μL)	27.11 ± 1.46	21.41 ± 1.60	21.76 ± 1.12	33.28 ± 3.21
ESV (μL)	41.75 ± 9.57	37.72 ± 6.05	51.44 ± 9.99	57.66 ± 9.45
EDV (μL)	68.86 ± 8.96	59.13 ± 6.66	73.20 ± 10.16	94.34 ± 7.82

Wall Motion Severity Index (WMSI) and Scar Sizing

In order to quantify scar size following CAL, we utilized gadolinium-enhanced CMR, step-wise short-axis images obtained via 4D-US and trichrome staining of tissue sections, which was used as the gold standard (Fig. 3, n = 12). Following sacrifice 4 weeks after CAL, a subset of these mice underwent histologic staining to evaluate scar size. Scar size was estimated histologically by comparing the pixel area of blue stained fibrotic tissue to red stained viable tissue on trichrome-stained cross sections taken every 1 mm (Table 1, Fig. 3, n = 16). The mean scar volume was 21.54 ± 4.07% (Range 4.02-57.00%, n = 16, Table 1). Late gadolinium-enhanced CMR images were used to evaluate hyperintense tissue/LV area, which yielded a mean volume of 13.53 ± 2.22%% (range 5.52–28.10%, Table 2). WMSI was calculated across 16 sections of myocardium, with average WMSI 1.32 ± 0.05 (range 1.07–2.07, Table 2, n = 32). Spearman rank correlation was used to compare both WMSI and CMR hyperintense regions/LV area to histologic staining (Fig. 4). WMSI correlates strongly with histologic scar size (r = 0.77, p = 0.008) and CMR hyperintense volume correlates very strongly with scar size (r = 0.90, p = 0.0003).

Comparison of Week 1 and Week 4 Data

We next evaluated how well traditional M-mode, 2D-US, and 4D-US correlate between week 1 and week 4 following CAL in order evaluate the validity of each modality early in the recovery stage. Image quality tends to be reduced early after CAL on account of residual air in the chest and local inflammation at the

thoracotomy site. 4D-US, 2D-US, and M-mode images were taken at Week 1 and Week 4 following CAL. Week 1 and Week 4 images of the same modality were compared to each other using Pearson correlation. 4D-US demonstrates strong correlation between time points in the evaluation of EDV ($r = 0.88$, $p < 0.0001$, Fig. 5), ESV ($r = 0.86$, $p < 0.00001$), and EF ($r = 0.74$, $p < 0.0001$). 2D-US provided strong correlation in EDV ($r = 0.81$, $p < 0.0001$) and ESV ($r = 0.86$, $p < 0.0001$) and moderate correlation of EF ($r = 0.52$, $p < 0.01$). M-mode demonstrated strong correlation between week 1 and week 4 EDV ($r = 0.75$, $p < 0.001$) and moderate correlation between ESV ($r = 0.63$, $p < 0.001$) and EF ($r = 0.64$, $p < 0.01$).

Discussion

This study evaluated commercially available 4D-US in a mouse model of myocardial infarction 1 week and 4 weeks after CAL and compared 4D-US endpoints to 2D-US, M-mode, CMR, and histologic parameters. The mouse CAL model presents a challenge for evaluation using traditional echocardiography, given that wall motion abnormalities and dyssynchronous contraction can cause abnormal LV geometry. Traditional M-mode and 2D-US assume ellipsoid geometry for volumetric measurements⁶. Following CAL, mice typically have a hyperdynamic base, static apex, and increased sphericity that limit the effectiveness of the geometric assumptions^{17,19-21}. 4D-US allows for real-time image analysis at multiple planes encompassing the LV that is gated by ECG and respiration^{13,14} to minimize motion artifact during image reconstruction. The result is a data-driven volumetric model that eliminates spatial and temporal assumptions about LV morphology. Our data demonstrate that for calculation of EF and ESV, 4D-US provides closer agreement when compared to benchmark CMR than other ECHO modalities 4 weeks after CAL. Additionally, we show that 4D-US has lower inter-user variability when measuring ventricular volumes at 4 weeks. 4D-US has the highest correlation when comparing volumetric measurements taken at 1 week to those taken at 4 week when compared to other ECHO modalities. Finally, 4D-US allows for simplified evaluation of WMSI, which correlates well with scar size by histologic analysis.

We find that 4D-US has lower percentage bias by Bland-Altman analysis than 2D-US and M-mode compared to CMR for the evaluation of ESV and EF at 4 weeks (Fig. 2), suggesting more accurate assessment of volumetric parameters. 4D-US is outperformed by 2D-US in the evaluation of EDV at this time point. However, across the three endpoints evaluated (EDV, ESV, and EF), 4D-US is the most reliable modality in terms of bias and 95% level of agreement. 4D-US has much lower bias than M-mode, which demonstrated $> 40\%$ bias in the estimation of ESV. 2D-US bias was improved compared to M-mode, but is characterized by the highest inter-user variability as demonstrated by lowest ICC for the evaluation of EDV, ESV, and EF (Fig. 2C). Inter-user variability is lowest using 4D-US for evaluation of EDV and ESV, but was mildly outperformed by M-Mode when evaluating EF (Fig. 2C). In total, our data is consistent with other studies that finds that 4D-US provides more accuracy and less inter-user variability when comparing to CMR than 2D-US or M-mode values, making it the optimal modality for surgical animal studies.

4D-US uniformly underestimates EDV and ESV when compared to CMR, though the changes were more pronounced in EDV (Fig. 2B, Table 2). The 4D-US measurements may suffer limitations in surgical mouse

models, as the base of the heart is difficult to clearly visualize given the overlying scar following thoracotomy required in the CAL surgery, and may result in lower total volumes. However, a similar underestimation of volumes was previously noted in evaluation of round “phantoms” using identical software¹⁵, suggesting that a systemic underestimation of volumes may be present in this modality. Regardless of this underestimation, the modality still represents an improvement over other traditional ECHO modes.

Recent work by Russo et al. has shown that step-wise short-axis ECHO imaging performed on CAL mice strongly correlates with CMR¹⁷, providing reproducible volumetric evaluations nearly on par with CMR, but at a fraction of the time and cost required for CMR. Our data support the increased value of this step-wise approach, but incorporate ECG- and respiratory-gating and the higher resolution of the Vevo-3100 for enhanced temporal and spatial resolution. Soepriatna et al. previously demonstrated similar benefits of gated 4D-US in infarcted mice using manual 3-D reconstruction throughout the cardiac cycle¹⁴. While that study excellently demonstrated the value of the hardware and imaging through the cardiac cycle, here we use an easily-accessible commercial system, which utilizes edge-tracing software to simplify volumetric calculation for users, resulting in both rapid analysis and low inter-user variability.

An additional benefit of the 4D-US imaging modality is the ability to quickly and reproducibly quantify scar characteristics using clinical scales of wall-motion. Previous works have quantified scar size using ECHO based on wall-motion abnormalities from 2D-US and 3-d reconstruction, demonstrating good comparison between ECHO estimations and histologic scar size^{22,23}. We evaluated WMSI using a 16-segment model across three short axis views used in the clinical settings as previously described^{24,25}. We found that WMSI correlates strongly with histologic analysis of scar size ($r = 0.77$, Fig. 5), while gadolinium-enhanced CMR correlated very strongly ($r = 0.90$). The 4D-US method allows for easy standardization on the WMSI across animals, as the automated 0.2 mm step function utilized in this paper can be utilized to standardize the height of short-axis image. In this study, we used three short-axis images taken 1 mm, 3 mm, and 5 mm from the apex to quantify WMSI. It should be noted that this model utilizes permanent coronary occlusion. Models of transient occlusion may be complicated by myocardial stunning, which may impair the value of WMSI and its correlation to scar size.

A key point of this study is the comparison of 1 week and 4 weeks with 4D-US, 2D-US, and M-mode to assess the predictive value of early imaging vs 4 week imaging. A major challenge following CAL is that cardiac remodeling is a dynamic process, characterized by progressive LV dilation, scar thinning, and compensatory hypertrophy of viable tissue^{9,19,26}. Early imaging after CAL in the mouse model is limited by free air in the chest cavity and inflammation at the thoracotomy site that reduces the image quality of ECHO images. These post-surgical changes compound the limitations of 2D-US and M-Mode, as slight errors in 1-dimensional or 2-dimensional analysis cause major deviations in volumetric calculations. 4D-US does not rely on these geometric expansions. We find that 4D-US has highest correlation across volumetric measurements when comparing 1 week and 4 week values.

Potential caveats

A potential confounder in the study is the use of multiple genotypes on a single background. Specifically, we utilized transgenic mice for the evaluation of volumetric measurements, pooling data from α -MHC-Cre (+) x TFAM-flox and α -MHC-Cre (-) x TFAM-flox mice. Because we found no difference between survival, scar size, baseline and 4 week EDV, ESV, or EF between groups (Supplemental Fig. 1, Supplemental Table 1), we included both models for this data set. We believe that the transgenic background does not limit the correlations drawn in this paper between volumetric studies and agrees with the NIH's effort to reduce animal suffering by utilizing mice readily available in our lab.

Conclusions

In this paper we demonstrate that commercially available 4D-US provides quick and reliable volumetric measurements of the heart following CAL that compares favorably to CMR values. 4D-US correlates better with CMR than 2D-US and M-Mode for evaluation of volumetric parameters 4 weeks after CAL. 4D-US also allows simple evaluation of WMSI, which is a clinically relevant metric that correlates well with scar size by histologic analysis. Finally, 4D-US provides more reliable volumetric assessment early after CAL compared to 2D-US and M-mode assessments.

Declarations

Ethics approval and consent to participate

All animal use was performed at the University of Pittsburgh in compliance with the National Institutes of Health Guide for Care and Use of Experimental Animals and was approved by the University of Pittsburgh Animal Care and Use Committee

Consent for publication

Not Applicable

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

CR obtained, analyzed and interpreted ECHO data was a major contributor to writing the manuscript. GC and FV performed manuscript review and helped write the discussion portion. BM contributed to ECHO analysis. LG performed all animal surgeries. BK is the primary investigator overseeing the project.

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List Of Abbreviations

2-Dimensional Ultrasound	2D-US
3-Dimensional	3D
4-Dimensional Ultrasound	4D-US
Anterior	A
Anterior Lateral	AL
Anterior Septal	AS
Cardiac Magnetic Resonance	CMR
Cardiac Output	CO
Coronary Artery Ligation	CAL
ECHO	Echocardiography
Echo Time	TE
Ejection Fraction	EF
End Diastole	ED
End Diastolic Volume	EDV
End Systole	ES
End Systolic Volume	ESV
Field of View	FOV

Flip Angle	FA
Hematoxylin and Eosin	H&E
Inferior	I
Inferior Lateral	IL
Inferior Septal	IS
Intraclass Correlation	ICC
Lateral	L
Left Ventricle	LV
Left Ventricular blood volume	LVV
LOA	Level of agreement
Motion-mode	M-Mode
Myocardial Infarction	MI
Repetition Time	TR
Septal	S
Stroke Volume	SV
Wall Motion Severity Index	WMSI

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Figures

Figure 1

Representative Images Using 4D-US and CMR. A) 4D-US cube reconstruction of concentric stacked images in an infarcted heart. B) Representative wire tracing of 4D-US reconstruction in an infarcted heart 4 weeks after CAL. C) Representative short-axis images taken during end-diastole (left column) and end-systole (right column) at 1 mm intervals from the apex (top) to base (bottom) of an infarcted heart 4 weeks after CAL. D) Representative mid-ventricular CMR images of an infarcted heart during end-diastole (top) and end-systole (bottom).

A

B

C

Figure 2

Bland Altman Analysis and Inter-user Variability of ECHO Modalities. A) Representative ECHO modalities for 4D-US, 2D-US, and M-Mode B) Bland-Altman analysis demonstrating the difference between selected measurement modality (4D-US, 2D-US, and M-Mode) and CMR. Data are presented as % bias and dotted lines represent 95% confidence interval. X-axes represent CMR volumetric measurements and Y-axes represent % Difference of ECHO values over CMR values. C) Inter-user variability between two users for each ECHO modality as calculated by Intraclass Correlation.

Figure 3

Representative Concentric Images of a Single Heart by CMR, 4D-US, and Histology. CMR (top row), 4D-US (middle row), and trichrome-stained histologic slides taken from a single infarcted heart at ~1 mm steps from the apex (left) through basal-ventricle (right). 4D-US images (middle row) are divided into 16 sections for wall-motion score index, labeled as anterior (A), lateral (L), inferior (I), septal (S), anterior-lateral (AL), inferior-lateral (IL), inferior-septal (IS), and anterior-septal (AS).

Figure 4

Correlation between WMSI and CMR to Histology. Regression analysis with Spearman rank correlation comparing wall motion score index (WMSI) to histological infarct size (left), and CMR hyperintense tissue to histologic infarct size (right).

Figure 5

4D-US Demonstrates the Highest Correlation between Week 1 and Week 4 Volumetric Measurements. Regression analysis with Pearson correlation comparing individual ECHO modalities taken at 1 week and 4 weeks following CAL.

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