Video Article

High-resolution Echocardiography in Mice: Left Ventricular Function Evaluation

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Abstract

Small animal models such as rats or mice represent the principal resource in cardiovascular research, mainly for their low cost and short reproductive cycle. Genetically and/or surgically altered animal models, created to mimic human cardiac disease such as myocardial infarction or heart failure, offer a great potential for the investigation and treatment of various cardiac disorders. Quantification of myocardial dysfunction represents a valuable tool for monitoring experimental results. Echocardiography represents a useful non-invasive technique in assessing cardiac chamber morphology and function [1-2] in experimental animal models. An ultra high-frequency transducer (30-40 MHz probe), providing high-resolution echocardiography for small and rapid beating hearts, such those of mice and rats, recently became available [3]. Because of the simplicity and speed of acquisition, our method is applicable as an accurate and reproducible technique. We describe the basic protocol for performing high-resolution echocardiography in mice, with particular emphasis on the images view and measurements assessment of left ventricular function.

Video Link

The video component of this article can be found at http://www.jove.com/video/2289/

Protocol

Echocardiographic System

Echocardiographic study is performed by using a commercially available high-resolution echocardiographic system ("Visualsonics VeVo 770®, Toronto, Canada) equipped with a single-element mechanical transducer with a center frequency of 30 MHz.

Animal Preparation

- Mouse is anesthetized by intraperitoneal injection of tribromoethanol (7 µl/g of 2.5% solution) and placed in a supine decubitus on a heated platform, designed with micrometer precision rotation and translation capabilities
- ECG and respiratory gating are obtained by using electrodes adapted with fine needles and secured on the mouse limbs
- Chest is shaved and further cleaned with alcohol or water to minimize ultrasound attenuation
- A specific gel preheated, from which air bubbles have been expelled, is applied to the thorax surface to optimize visibility of the cardiac chambers. Particular attention is paid in avoiding excessive pressure of the probe on the chest, which may induce hypotension and bradycardia.

Setting for Image Acquisition

The setting of image acquisition is maximally standardized.

- Frame-rate in B-mode imaging is set at 100 Hz with the focal length of 12,5 to 12,7 mm
- Pulse Doppler imaging is performed with the smallest sample volume (0,5 mm) at a paper speed of 200 mm/s
- Heart rate and respiratory rate are constantly monitored by ECG leads

Before starting the exam time is required (about 15 minutes) to restore a heart rate of about 500 bpm, initially depressed by the anesthetic agent [4]

Image Acquisition

Here as follows are explained in detail the steps of echocardiographic examination:

1-Parasternal long-axis view

- B-Mode (2D imaging)

The "notch" of the scanhead should be pointed towards the head of the animal and rotated approximately 30-45 degrees counter-clockwise.

The left atrium, left ventricle, aortic valve, left ventricular outflow tract (LVOT), mitral valve, interventricular septum and posterior left ventricular wall are visualized.

- M-Mode

Same scanhead position. Cursor is positioned in the middle of the image. This view allows left ventricular diameters and wall thickness measurements and visual assessment of systolic function.

2-Parasternal short-axis view

- B-Mode

From the parasternal long axis view scanhead is rotated 90 degrees clockwise to the short axis: we move the scanhead notch from the position towards the head of the mouse to the left side of the mouse. A completely round view of the left ventricle should appear. This image is optimized by angling the probe.

The basal, mid and apical level of left ventricle are visualized by tilting the probe towards the legs of the mouse.

- M-Mode

Scanhead position is the same. Cursor is positioned in the middle of the image

When we get the short axis view at the level of the valves, the aortic valve will appear in the middle. The tricuspid valve is on the right (left side of the screen) and the pulmonary valve will be on the left (right side of the screen). Scanning down to the apex, ad up to the base of the heart allows to analyze all aspects of the left ventricle.

3-Apical four chamber view

An apical four chamber view is obtained from the lower left side of the animal thorax: we are trying to look up from the apex, towards the base of the heart. We point the scanhead in a transverse position with the notch towards the left side of the mouse, and angle the mouse (by moving the platform) slightly with the head down. If necessary, the scanhead may be angled by 60-70 degrees. Then we move the scanhead to the lateral wall of the thorax. From this position, some fine tuning may be required to obtain the apical view. Right and left ventricles are visualized at the top and the atria at the bottom of the screen. Further assessment of left ventricular function is achieved by this view. Pulsed wave Doppler of mitral inflow, tricuspid inflow and LVOT outflow can be performed.

Quantitative Analysis of Left Ventricle

Acquired images are reviewed off -line and measurements are taken. Left ventricular end-diastolic and end-systolic diameters, and left ventricular wall thickness (anterior septum and posterior wall) are measured from M-mode parasternal short-axis view at the midpapillary level (figure 1). LVOT diameter can be obtained by placing markers from leading edge to leading edge , immediately below the level of the aortic valve leaflets in B-mode parasternal long axis view (figure 2). Left ventricular base-apex length is obtained in B-mode parasternal long axix view (figure 3). Endocardial border trace of the left ventricle is performed in B-mode parasternal short axis view at basal, papillary and apex level (figure 4). From these measurements we derive left ventricular ejection fraction (LVEF), left ventricular fractional shortening (LVFS), left ventricular (LV) mass and volume (table 1).

In addition, pulsed wave Doppler of mitral inflow (figure 5), for evaluating LV diastolic function, and LVOT outflow (figure 6), for calculating LV stroke volume, is performed from a four chamber view (figure 7), isovolumic contraction time (IVCT), isovolumic relaxation time (IVRT) and ejection time (ET) are measured for myocardial performance index (MPI) calculation (table 1).

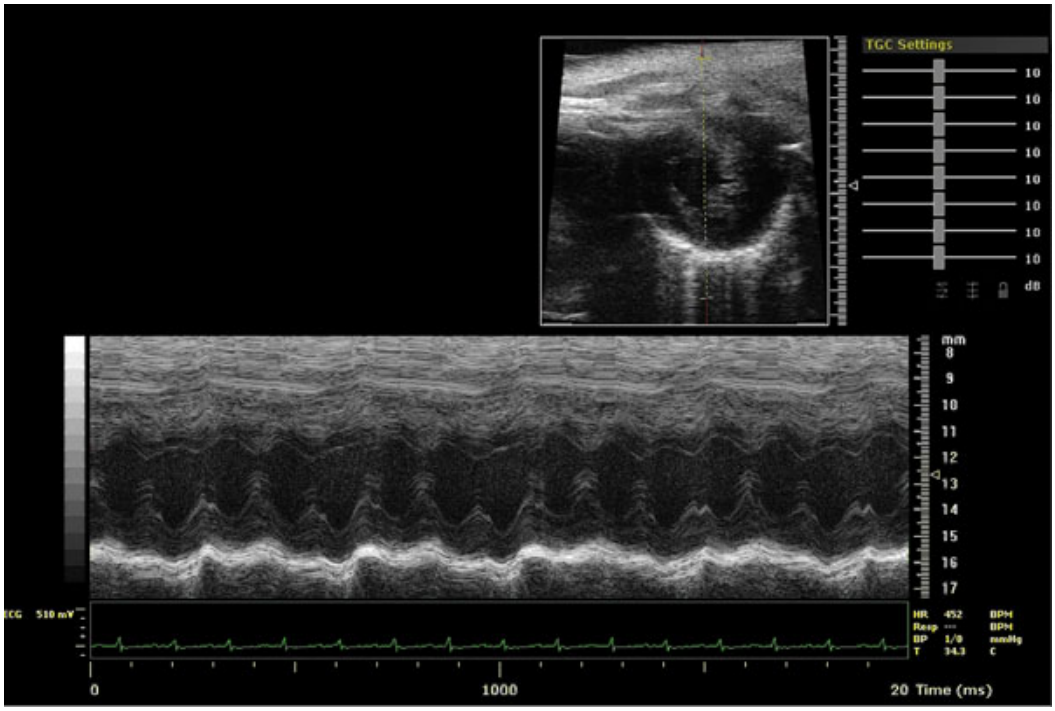

Figure 1. Parasternal short axis view M-Mode: linear measurements of left ventricular diameters are taken at the midpapillary level. IVSd, interventricular septum, diastole; LVIDd, left ventricular internal diameter, diastole; LVPWd, left ventricular posterior wall, diastole; LVIDs, left ventricular internal diameter, systole.

Figure 2. Parasternal long axis view B-Mode: LVOT diameter.

Figure 3. Parasternal long axis view B-Mode: left ventricular base-apex length.

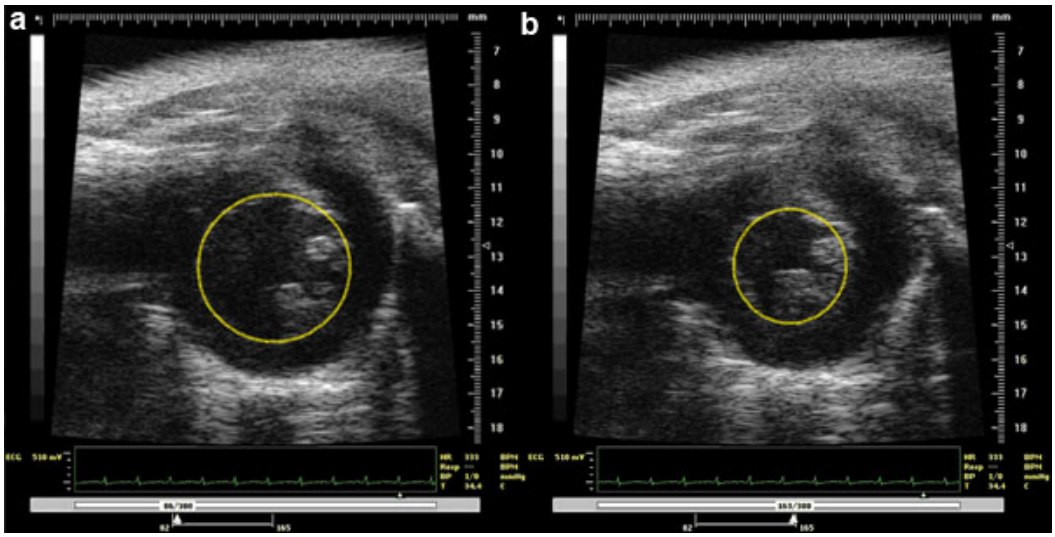

Figure 4. Parasternal short axis view B-Mode: endocardial border trace of the left ventricle at papillary level in a) diastole and b) systole.

Figure 5. Apical four chamber view. Pulsed Doppler of mitral flow: the sample volume is positioned and correctly angulated at the level of the mitral valve; the mitral flow velocity is about 80 cm/s.

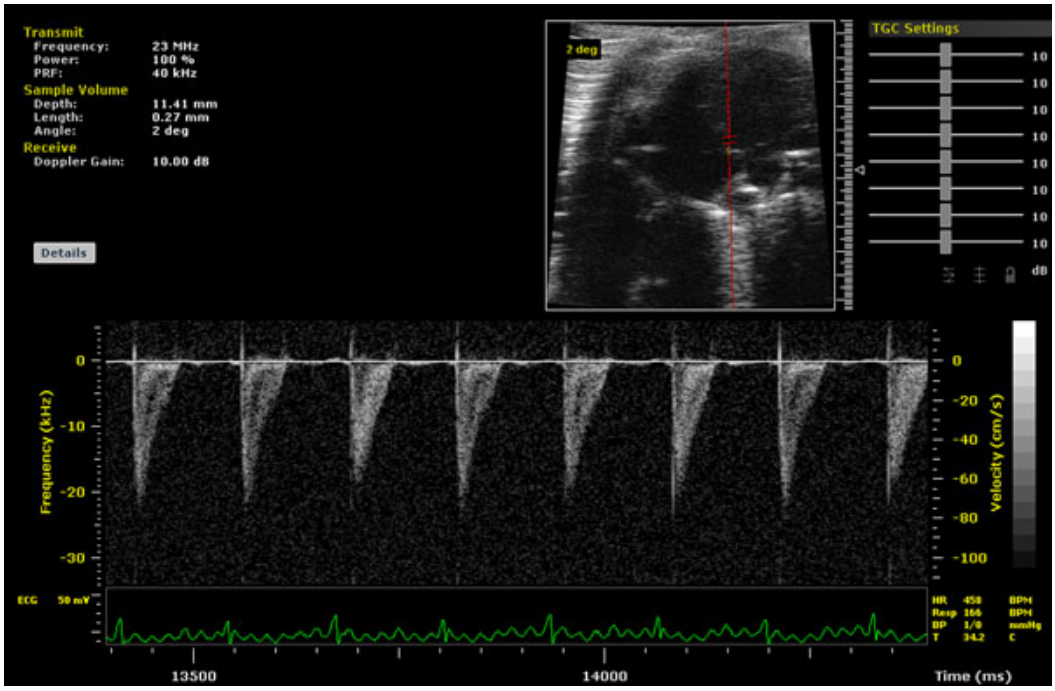

Figure 6. Apical four chamber view. Pulsed Doppler of LVOT outflow : the sample volume is positioned and correctly angulated just below the aortic valve; the aortic flow velocity is about 1 m/s.

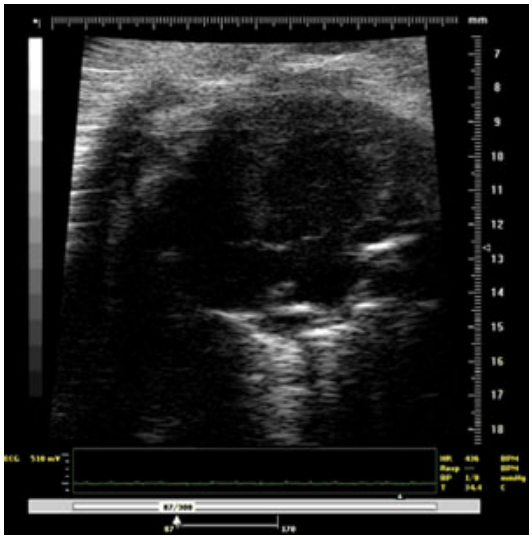

Figure 7. Apical four chamber view: the sample volume for Doppler imaging is positioned between transmitral flow and LVOT flow for measuring IVCT, IVRT, ET.
LVOT, left ventricular outflow tract; IVCT, isovolumic contraction time; IVRT, isovolumic relaxation time; ET, ejection time.

Table 1.

Derived Parametert	Formula	Linear /Doppler Measurements Used	Echocardiographic view
FS%	LVIDd - LVISd / LVIDd x 100	LVIDd, LVISd	LAX view and/or SAX view M-Mode papillary level
LVEDV, LVESV	LVEDV= (Ad1+Ad2)hd +A3hd/2+#/6hd^3 LVESV= (As1+As2)hs +A3hs/2+#/6hs^3	Ad1-3, hd As1-3, hs	A: SAX view B-Mode at basal, mid and apical level; h: LAX view B-Mode
LVEF%	LVEDV-LVESV/LVEDV x 100	See box	See box

		LVEDV/LVESV	LVEDV/LVESV
LV mass	(0.8 X {1.04[(LVIDd + PWTd + SWTd)3 - (LVIDd)3]} + 0.6 g)	LVIDd, PWTd , SWTd,	LAX view and/or SAX M-Mode papillary level
SV	LVOT area x LVOT TVI	LVOT diameter, LVOT VTI (PW Doppler)	diameter: LAX B-mode TVI: apical four chamber
CO	SV x HR	See box SV	See box SV
MPI	IVCT+IVRT/ET	IVCT, IVRT, ET (PW Doppler)	Apical four chamber

FS: fractional shortening; **LVIDd:** left ventricular internal diameter, diastole; **LVIDs:** left ventricular internal diameter, systole; **LVEDV:** left ventricular end-diastolic volume; **LVESV:** left ventricular end-systolic volume; **Ad:** area, diastole; **hd:** left ventricular base-apex length, diastole; **As:** area, syastole; **hs:** left ventricular base-apex length, systole **LVEF:** left ventricular ejection fraction; **LVEDV:** left ventricular end-diastolic volume; **LVESV:** left ventricular end-systolic volume; LV mass: left ventricular mass; **PWTd:** posterior wall thickness, diastole; **SWTd:** septal wall thickness, diastole; **SV:** stroke volume; **LVOT:** left ventricular outflow tract; **LVOT VTI:** left ventricular outflow tract, time velocity integral; **CO:** cardiac output; **HR:** heart rate; **MPI:** myocardial performance index; **IVCT:** isovolumic contraction time; **IVRT:** isovolumic relaxation time; **ET:** ejection time.

Discussion

Small animal models such as rats or mice represent the principal resource in cardiovascular research, mainly for their low cost and short reproductive cycle. Animal models of cardiac dysfunction are obtained by mean of various manipulations, transgenic and surgical. Quantification of myocardial dysfunction represents a valuable tool for monitoring experimental results. For this reason there is an increasing requirement of non-invasive imaging modalities for an accurate assessing of mice cardiac mechanics and wall motion abnormalities. Echocardiography, from clinical practice to experimental setting, is considered the more reliable non-invasive technique for assessing cardiac chamber morphology and function [1-2].

Unfortunately, current available systems are still limited by relatively low-frequency probes and offer inadequate visualization of small size and fast beating hearts.

Recently, high-resolution echocardiography with an ultra high-frequency transducer (30-40 MHz probes) became available [3], and this manuscript helps in providing the best visualization of the small size and fast beating hearts of the mice using this technique. Because of the simplicity and speed of acquisition (20 minutes for a complete study), our method is applicable as an accurate and reproducible technique for assessing LV morphology and function in different experimental scenarios.

Nevertheless, some technical issues are to be considered: the probes currently available have still a bigger size than the mice chest, and they imply low spatial resolution; futhermore, any change in technical setting, such as air bubbles in the gel and mild chest pressure, could reduce the scanning capacity. Therefore we consider the opportunity of a short period of training, alongside an experienced operator, before starting the experiments.

A last consideration regards the well known limitation of two-dimensional echocardiography in depicting three-dimensional structures as cardiac chambers. In the near future we intend to improve this methodic by integrating three-dimensional echocardiographic modality [5].

In conclusion, the technique described in this manuscript represents a safe, rapid, reproducible and non-invasive method for qualitative and quantitative analysis of regional and global left ventricular function in experimental small animal settings.

Disclosures

No conflicts of interest declared.

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References

1. Keith A. Collins, Claudia E. Korcarz, and Roberto M. Lang "Use of echocardiography for the phenotypic assessment of genetically altered mice " . Physiol Genomics 13: 227-239, 2003.
2. " Xiao-Ming Gao, Anthony M. Dart, Elizabeth Dewar, Garry Jennings, Xiao-Jun Du "Serial echocardiographic assessment of left ventricular dimension and function after myocardial infarction in mice" .Cardiovascular Research 45 (2000) 330-338.
3. Kazue Okajima, MD, Yukio Abe, MD, Kohei Fujimoto, MD, Kana Fujikura, MD, Erin E. Girard, MS, Tomohiro Asai, MD, Susan H.Kwon, MD, Zhezhen Jin, PhD, Yasuhiro Nakamura, MD, Minoru Yoshiyama, MD, and Shunichi Homma, MD -"Comparative study of high-resolution microimaging with 30-MHz scanner for evaluating cardiac function in mice" .J Am Soc Echocardiogr 2007; 20:1203-1210.
4. Kiatchoosakun S, Kirkpatrick D, Hoit BD,"Effect of tribromoethanol anesthesia on echocardiographic assessment of left ventricular function in mice" Compar Med 51:26-29, 2001.

5. Marielle Scherrer-Crosbie, MD, Wolfgang Steudel, MD, Patrick R. Hunziker, MD, Noah Liel-Cohen, MD, Roman Ullrich, MD, Warren M.Zapol, MD, Michael H. Picard, MD "Three-Dimensional Echocardiographic Assessment of Left Ventricular Wall Motion abnormalities in mouse myocardial infarction" J Am Soc Echocardiogr- Volume 12, Issue 10 (October 1999)