

Video Article

Echocardiographic Measurement of Right Ventricular Diastolic Parameters in Mouse

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Abstract

Diastolic dysfunction is a prominent feature of right ventricular (RV) remodeling associated with conditions of pressure overload. However, the RV diastolic function is rarely quantified in experimental studies. This might be due to technical difficulties in the visualization of the RV in the apical four-chamber view in rodents. Here we describe two positions facilitating the visualization of the apical four-chamber view in mice to assess the RV diastolic function.

The apical four-chamber view is enabled by tilting the mouse fixation platform to the left and caudally (LeCa) or to the right and cranially (RiCr). Both positions provide images of comparable quality. The results of the RV diastolic function obtained from two positions are not significantly different. Both positions are comparably easy to perform. This protocol can be incorporated into published protocols and enables detailed investigations of the RV function.

Introduction

Diastolic dysfunction is a prominent feature of right ventricular (RV) remodeling¹ and is associated with pressure-overload conditions². Echocardiography (EchoCG) can be used for the characterization of RV diastolic dysfunction^{3,4}. Despite recent developments in small animal echocardiography, measurements of diastolic parameters are rarely reported. In contrast, measurements of the systolic function are widely used for the characterization of transgenic mice⁵, as well as for the evaluation of a treatment response⁶.

This can be partly explained by the difficulties in the measurement of diastolic parameters from the apical four-chamber view. Visualization of the heart in this position can be facilitated by tilting the fixation platform LeCa or RiCr. Even if these manipulations are used, echocardiographers do not report them in their manuscripts^{4,7}. Therefore, it remains unclear whether these manipulations provide comparable results. Moreover, this also precludes a development of standardized nomenclature of this position for mice.

The aim of this study was to describe two positions for apical four-chamber view visualization and compare their results. To determine the differences between the two positions, we have utilized the mouse pulmonary artery banding (PAB) model, in which a tantalum clip leads to a partial occlusion of the pulmonary artery. This occlusion results in right ventricle remodeling and dysfunction. Full details of the PAB operation can be found in previously published work³. Sham-operated mice, where the clip was placed next to the pulmonary artery, were used for comparison. EchoCG investigations were performed three weeks post-operation using the imaging system with a 30-MHz scan head (see **Table of Materials** for both). Nomenclature for the description of the positions and orientations between the mouse and the ultrasound beam is used as described by Zhou *et al.*⁷.

Protocol

The study was performed according to national regulations for animal experimentation and EU Directive 2010/63. Prepare equipment as described previously by Brittain *et al.*⁸.

1. Mouse Preparation

1. Obtain 12- to 13-week-old male C57Bl6/J mice and house them with a 12-h light/dark cycle, at a constant room temperature, and with *ad libitum* access to standard laboratory chow and water, until the start of the experiment.
2. Anesthetize the mouse using general anesthesia approved by the Institute and check for the lack of response to the toe pinch. Under mild anesthesia with isoflurane 0.8% - 1.2%, fix the mouse on a heated platform. Apply electrode gel to its extremities for the continuous monitoring of its heart rate and temperature.
3. Depilate the mouse's chest hair using depilation creme. To reduce pressure on its thorax, do not apply the ultrasound coupling gel directly on the thorax; rather, apply a layer of the gel to the tip of the transducer.

2. Image Acquisition

1. Apical four-chamber view with a left and caudal tilt of the platform

1. After the mouse preparation, angulate the platform to the left at 10° - 15° and then caudally at 10° - 15° .
2. Position the transducer above the apex with the imaging plane $\sim 45^{\circ}$ to the coronal plane and the central axis of the ultrasound beam directed cranially, posterior, and to the left to obtain the apical four-chamber view. Press the **B-Mode** button to activate the B-mode/2-D image.
NOTE: The transducer can be held manually or fixed by a stage. The term "B-mode" comes from the imaging system that was used instead of the more familiar term "two-dimensional" (2-D) and is used throughout the protocol.
3. Look for the appearance of the following structures in the acoustic window: the left ventricle (LV), the left atrium (LA), the RV, the right atrium (RA), the mitral valve (MV), and the tricuspid valve (TV).
4. Manipulate the imaging plane in the coronal plane and rotate clock- and counterclockwise around the central axis until both ventricles are visualized at their longest dimension and both atria are visible. This is the four-chamber view (**Figure 1**).
5. Press the **Cine store** button to save the recording.
6. Press the **Scan/Freeze** button to pause the system.

2. Measurement of transtricuspid blood flow velocities

1. Press the **Scan/Freeze** button to activate the system.
2. Press the **Overlay** button several times to activate the sample volume for PW (pulsed wave) mode.
3. While keeping the obtained four-chamber view, use the trackball to position the sample volume at the opening of the tricuspid valves for the measurement of inflow velocities (E and A peak velocities).
4. Press the **PW** mode button for the measurement of inflow velocities (E and A peak velocities).
NOTE: Because tricuspid valves are difficult to visualize in this position, performing several measurements helps to align correctly the sample volume with the blood flow. Perform the Doppler sampling with the smallest incidence angle between the Doppler beam and the blood flow direction. The obtained blood flow profile should correspond to the following criteria: 1) an inflow profile similar to an M-shape with the first peak lower than the second; 2) a respiratory modulation with an increased amplitude at inspiration; 3) a maximal amplitude of velocities in several measurements (**Figure 2**).
5. Press the **Cine store** button to save the optimized recording.
6. Press the **Scan/Freeze** button to pause the system.

3. Measurement of the tricuspid annular plane systolic excursion (TAPSE)

1. Press the **Scan/Freeze** button to activate the system.
2. Switch to B-mode by pressing the **B-Mode** button. Some manipulations on the image might be necessary to regain the correct four-chamber view.
3. Press the **Overlay** button several times to activate the sample volume of the M-mode. Using the trackball, align the sample volume with the lateral part of the tricuspid annulus. By pulling the edges of the sample volume using the trackball, align the length of the sample volume to cover the entire amplitude of the cardiac movement during the cardiac cycle.
4. Press the **M-Mode** button to activate M-mode. Tricuspid annulus' movements should appear as a wave (**Figure 2**).
5. Press the **Cine store** button to save the recording.
6. Press the **Scan/Freeze** button to pause the system.

4. Measurement of tissue Doppler parameters

1. Press the **Scan/Freeze** button to activate the system.
2. Press the **B-Mode** button to activate B-mode.
NOTE: Some manipulations by angulation in the coronal plane and rotation clock- and counterclockwise around the central axis of the image might be necessary to regain the correct four-chamber view.
3. Press the **Overlay** button several times to activate the sample volume for TDI (tissue Doppler imaging). Using the trackball, align the sample volume with the lateral part of the tricuspid annulus, where the RV free wall creates an angle with the tricuspid valve. By pulling the edges of the sample volume using the trackball, adjust the sample volume to include both the systolic and the diastolic extreme positions of the annulus.
4. Press the **Tissue** button to activate TDI mode.
NOTE: Yellow tracing of the TDI recording appears corresponding to the following criteria: 1) a recording similar to an inverted M-shape; 2) clearly distinguishable E' and A' peaks during diastole and S' peak during systole; 3) a maximal amplitude of velocities in several measurements (**Figure 2**).
5. Press the **Cine store** button to record an optimized image.
6. Press the **Scan/Freeze** button to pause the system.

5. Apical four-chamber view with right and cranial tilt of the platform

1. Angulate the platform to the right at 10° - 15° and then cranial at 10° - 15° . Perform the measurements as described in the previous sections for the LeCa steps (steps 2.1, 2.2, 2.3, and 2.4).
NOTE: During the investigation, isoflurane should be titrated between 0.8 - 1.2 to keep the mouse's heart rate at 400 - 440 bpm. In this range, separate peaks of transtricuspid blood flow and tissue Doppler (DTI) velocities are measurable. To avoid the effects of the heat loss on hemodynamics, the data are recorded, and the analysis is performed off-line. Only signals obtained at the end-expiration are used for analysis. Measurements of 3 - 5 heartbeats are averaged.

Representative Results

The apical four-chamber view is difficult to obtain in mice. Therefore, manipulations of the platform position can help to visualize the heart by changing its position in the thorax. The tilting of the platform to the left and to the right improved the acoustic window and provided images of comparable quality in B-mode (**Figure 1**). After obtaining the correct positions, measurements in PW-, M-, and TDI-modes provided images of comparable quality (**Figure 2**). The measurement of diastolic parameters was performed on sham- and PAB-operated mice (**Table 1**). Both positions (RiCr and LeCa) gave similar results in the diastolic parameters (**Table 2**). Furthermore, the EchoCG investigations in both positions revealed similar differences between the sham and PAB groups (**Table 2**, Dunnet's test). Correlation analysis revealed a good agreement between values obtained from these two facilitated positions (**Figure 3**). As small groups of animals were used for this study, non-parametric tests have been applied^{9,10}. Intra-observer variability for some analyzed parameters has been published previously³.

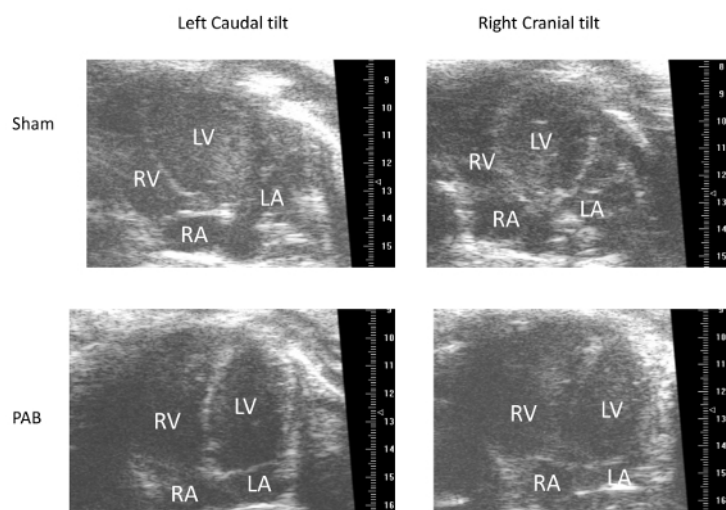


Figure 1: Representative images of the apical four-chamber view. The apical four-chamber view is enabled by tilting the mouse fixation platform to the left and caudally (LeCa) or to the right and cranially (RiCr). LA = left atrium; LV = left ventricle; RA = right atrium; RV = right ventricle. [Please click here to view a larger version of this figure.](#)

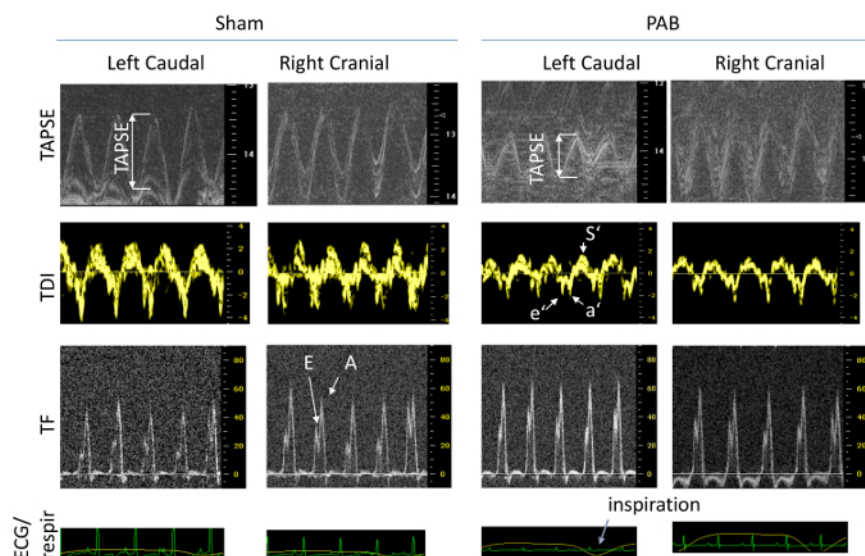


Figure 2: Representative images of the TAPSE, TDI, and transtricuspid flow measurements obtained from two facilitated apical four-chamber view positions. TAPSE = tricuspid annulus plane systolic excursion; E' = early peak of right ventricular relaxation velocity; A' = late peak of right ventricular relaxation velocity; S' = velocity of the right ventricular contraction; E = early peak of diastolic tricuspid inflow; A = late peak of diastolic tricuspid inflow. Note the change in the transtricuspid blood flow profile at the inspiration (Insp). [Please click here to view a larger version of this figure.](#)

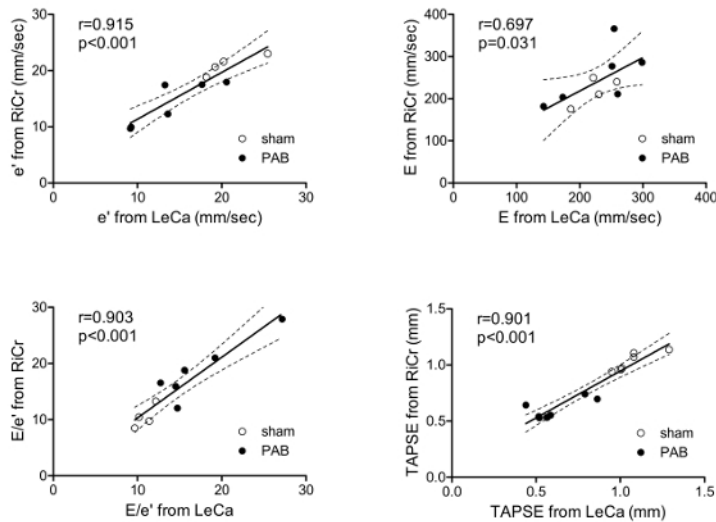


Figure 3: Correlation analysis of data obtained from two facilitated apical positions. Correlation analysis was performed using non-parametric Spearman's test. [Please click here to view a larger version of this figure.](#)

Parameters	Sham	PAB	Mann Whitney test
	(mean ± SD)	(mean ± SD)	(p value)
Gradient velocity (mm/sec)		2.88 ± 0.15	
RVFW (mm)	0.19 ± 0.05	0.41 ± 0.1	0.006
Heart rate for VTI (beats per minute)	406 ± 39	413 ± 38	0.267
Stroke Volume (mL)	38.9 ± 5.0	29.1 ± 8.6	0.016
Cardiac Output (mL/min)	15.7 ± 0.8	11.9 ± 3.4	0.043

Table 1: Characterization of the operated groups three weeks after the operation. RVFW = right ventricular free wall thickness; VTI = velocity-time interval.

Parameter	Sham		PAB		Dunnet's post-hoc test	
	RiCr (mean ± SD)	LeCa (mean ± SD)	RiCr (mean ± SD)	LeCa (mean ± SD)	RiCr (Sham vs PAB)	LeCa (Sham vs PAB)
E (mm/sec)	222 ± 27	231 ± 30 [§]	251 ± 64	230 ± 59 [§]	n.s.	n.s.
A (mm/sec)	423 ± 50	404 ± 42 [§]	517 ± 74	491 ± 53 [§]	n.s.	n.s.
TAPSE (mm)	1.03 ± 0.08	1.08 ± 0.11 [§]	0.60 ± 0.08	0.61 ± 0.14 [§]	**	**
e' (mm/sec)	22.2 ± 2.4	20.1 ± 3.2 [§]	13.8 ± 3.7	13.9 ± 4.5 [§]	*	*
a' (mm/sec)	28.4 ± 4.4	26.9 ± 7.0 [§]	24.8 ± 4.9	26.2 ± 6.7 [§]	n.s.	n.s.
S (mm/sec)	23.8 ± 1.8	26.0 ± 3.8 [§]	18.4 ± 3.6	18.7 ± 6.3 [§]	n.s.	n.s.
E/e'	10.1 ± 1.7	11.7 ± 2.1 [§]	18.9 ± 5.0	17.3 ± 5.3 [§]	**	*
HR (bpm)	400 ± 33	401 ± 15 [§]	408 ± 41	409 ± 43 [§]	n.s.	n.s.

Table 2: Comparison of the results obtained from the apical four-chamber view facilitated by the left caudal or right cranial platform tilt. EchoCG-derived RV functional parameters are shown. As every mouse was investigated in both positions, the signed rank Wilcoxon test was used for intra-group comparisons. [§] $p > 0.05$ between RiCr and LeCa. The Kruskal-Wallis test, followed by Dunnet's *post hoc* test, was used for multiple group comparisons. The results of two selected intergroup comparisons are presented in the table. * $p < 0.05$, ** $p < 0.01$. PAB = pulmonary artery banding; LeCa = left caudal tilt; RiCr = right cranial tilt; E = early peak of diastolic tricuspid inflow; A = late peak of diastolic tricuspid inflow; TAPSE = tricuspid annulus plane systolic excursion; e' = early peak of right ventricular relaxation velocity; a' = late peak of right ventricular relaxation velocity; S' = velocity of the right ventricular contraction; HR = heart rate; bpm = beats per minute.

Discussion

The echocardiographic RV function and dimension assessment from parasternal positions have been well described. In contrast, the apical position in mouse echocardiography has been neglected partly due to technical difficulties. Using a horizontal platform position, it is difficult to obtain a sufficient acoustic window for four-chamber view imaging. To facilitate the imaging of this position, the platform can be tilted to the left, a manipulation similar to the left-sided positioning of patients. This should result in a leftward and more superior positioning of the heart, thereby improving the acoustic window. Therefore, LeCa is our standardized position for apical visualization. However, in approximately 30% - 35% of mice, the image quality in this position can be insufficient. Here, imaging in the RiCr position can be helpful.

From these positions, transtricuspid blood flow velocities (E and A) and tissue Doppler velocities (E' and A') can be measured, providing information about the RV diastolic function. We observed a good correlation between TDI parameters obtained from the two positions. Less satisfactory was the correlation of E. In general, the visualization of the transtricuspidal blood flow profile was the most challenging part of the protocol presented here and exhibited the highest variability. The measurement of TAPSE and S' by tissue Doppler provided an estimate of the RV systolic function. However, in the light of recent findings, the physiological meaning of TAPSE is not clear¹¹. We do not routinely measure the RV fractional area of contraction from the apical position because, in the conditions of pressure overload, the lateral part of the enlarged RV is partly covered by the sternum and not completely visible from this position³. Thus, the visualization of the apical position in mice enables the measurement of the parameters routinely used in the clinic and, thereby, delivers more information, which allows a more complete functional characterization.

Strain, strain rate analysis, and speckle tracking echocardiography are novel modalities of cardiac ultrasound¹². Its high sensitivity can detect cardiac dysfunction at initial stages¹³ and has the power to predict mortality¹⁴; therefore, its application is also warranted in experimental studies. Unfortunately, in mice, the RV free wall is partly hidden behind the sternum's shadow, which might hinder the analysis of strain. Furthermore, strain analysis requires good image quality and visualization of the entire free wall.

The cardiovascular system responds quickly to changes in the posture by activating baroreceptor mechanisms¹⁵. Therefore, it could be expected that the cranial tilt of the platform would cause reflexory changes in the measured cardiac parameters. Indeed, both the head-up and the head-down tilt position caused a transient change in the heart rate and cardiac electric axis in mice¹⁶. While a 90° head-up tilt causes an increased heart rate, a 90° head-down tilt caused transient and statistically insignificant bradycardia. In contrast, we recommend tilting the mouse only by 10° - 15° in either direction. These mild changes in posture did not cause any measurable hemodynamic perturbances.

LV diastolic function in mice is another understudied area. Although not tested in this study, the protocol presented here should be able to be used for the quantification of the LV diastolic function.

Theoretical and practical limitations of the small animal EchoCG have been described in detail elsewhere⁸. In this protocol, measurements are performed at heart rates of 400 - 440 bpm. At this range of heart rate, measurements of the E and A velocity peaks, as well as of TDI indexes, are feasible. At higher heart rates, peaks merge, making quantification impossible. Since the physiological heart rate for mice is 500 - 600 bpm, the heart rate used in this protocol is rather low. Nevertheless, the measurements at this heart rate range appear reliable and enable distinguishing between physiologic and dysfunctional phenotypes³.

We described a protocol for two positions facilitating the assessment of RV functional parameters from four-chamber views in mice. The positions provide comparable results and can be used interchangeably.

Disclosures

The authors have nothing to disclose.

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