Usefulness of Two-Dimensional Speckle Tracking Echocardiography and Diffusion Tensor Imaging for Detection of Myocardial Fibrosis in a Rat Model of Right Ventricular Pressure Overload

(**Usefulness of 2DSTE and DTI**)

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Abstract

Background: Two-dimensional speckle tracking echocardiography (2DSTE) has attracted a great deal of attention as an index that more acutely reflects a decrease in ventricular contractility than ejection fraction. Diffusion tensor imaging (DTI) is useful in the diagnosis of myocardial infarction, mainly in the left ventricle. This study aimed to investigate whether 2DSTE and DTI could be used to predict right ventricular (RV) myocardial fibrosis.

Methods: Five-week-old Sprague Dawley (SD) rats were randomly divided into two groups: the pulmonary artery banding (PAB) and sham groups (n = 10 for both). The rats were euthanized after echocardiography and cardiac catheterization at four weeks postoperatively and underwent DTI and histological analysis.

Results: The PAB group showed significant RV fibrosis compared to the sham group, and there was a significant negative correlation between the RV longitudinal strain score and fibrosis (Spearman's $\rho = -0.42$, $P < 0.01$). Moreover, $\lambda 1$ showed a significant negative correlation with RV myocardial fibrosis based on the diffusion eigenvalues ($\lambda 1$, 2, 3) and fractional anisotropy ($\rho = -0.79$, $P = 0.036$). In addition, the number of myocardial fibers depicted on fiber tractography decreased as fibrosis increased in all sagittal, coronal, and lateral sections.

Conclusions: This study demonstrated the potential usefulness of 2DSTE and DTI as noninvasive options for the early diagnosis of RV fibrosis.

Keywords:

Right ventricular fibrosis, two-dimensional Speckle tracking echocardiography, diffusion tensor imaging, fiber tractography

Abbreviations list

BW body weight

CO cardiac output

DTI diffusion tensor imaging

DWI diffusion-weighted images

EF ejection fraction

FOV field of view

FA fractional anisotropy

FAC fractional area change

HR heart rate

IVS interventricular septum

IQR interquartile range

LV left ventricle

LVp left ventricular pressure

MV mitral valve

PAB pulmonary artery banding

PA pulmonary artery

RV right ventricular

RVp RV pressure

RAp right atrial pressure

RVOT right ventricular outflow tract

4Ch. RVFAC right ventricular fractional area in the 4-chamber view

2DSTE two-dimensional Speckle tracking

3D three-dimensional

TV/MV tricuspid to mitral valve ratio

TAPSE tricuspid annular plane systolic excursion

TR time to repetition

TE time to echo

TV tricuspid valve

Background

Right ventricular (RV) pressure overload is a common clinical feature encountered in pediatric cardiology, which results from various congenital conditions and procedures, including pulmonary valve stenosis, pulmonary artery banding (PAB) for pulmonary overcirculation, and pulmonary artery (PA) stenosis after tetralogy of Fallot repair. It is difficult to perform an accurate RV function assessment due to its anatomically and functionally complex morphology. A pressure-loaded RV eventually becomes enlarged, which progresses to fibrosis. The degree of fibrosis affects the long-term prognosis of RV function. Thus, the early diagnosis of RV myocardial fibrosis by noninvasive means and timely pressure load release are vital. However, owing to the complex RV morphology, the structure of normal RV is poorly understood, and the distribution of myocardial fibrosis within the pressure-loaded RV is also unclear. Further, the assessment of the traditional RV function is inadequate owing to the complex RV morphology; therefore, the diagnosis of RV myocardial fibrosis is challenging[1-3].

Two-dimensional Speckle tracking echocardiography (2DSTE) is an echocardiographic technique that could be used to measure strain values in arbitrary regions independent of the angle, and has attracted a great deal of attention as a functional evaluation tool for both the left and right ventricles[4-6]. Since cardiac function at the site of myocardial fibrosis

is impaired, we hypothesized that 2DSTE could be used to locally diagnose RV myocardial fibrosis after PAB.

Furthermore, we focused on diffusion tensor imaging (DTI), which is a noninvasive technique similar to echocardiography. This method expresses the strength and direction diffusion anisotropy information by fitting it to a three-dimensional (3D) elliptical sphere (consisting of three basis vectors) called a tensor[7]. As diffusion anisotropy could be quantified by tensor analysis, we postulated that the extent of RV myocardial fibrosis could also be quantified. Moreover, the anisotropy is weakened when the myocardial alignment is perturbed, such as in the infarcted foci of myocardial infarction, and the fractional anisotropy (FA) value approximates 0[8-10]. We hypothesized that, similar to myocardial infarction, RV myocardial fibrosis would also result in perturbations in the myocardial alignment, thereby leading to reduced anisotropy and diffusion coefficients in three directions (λ1, 2, and 3).

Thus, this study aimed to demonstrate the potential for early diagnosis of RV myocardial fibrosis using two noninvasive measures: 2DSTE and DTI.

Methods

Experimental protocol

Five-week-old Sprague Dawley rats that weighed between 170 and 200 g (Sankyo Labo Service Corporation, Inc.; Tokyo; Japan) were randomly allocated to the PAB or sham groups (n = 10 for both; Fig. 1). Animals were anesthetized by isoflurane inhalation, intubated (18-G Angiocath; Nippon Becton Dickinson, Tokyo, Japan), and maintained on 2% isoflurane using a small animal ventilator (Harvard Apparatus, Holliston, MA, USA). The ventilator was set at 10 μl/g of the full tidal volume and 100 breaths/min. In the sham group, a left-side thoracotomy was made between the fourth and fifth ribs, and the thymus gland was resected. In the PAB group, thoracotomy was performed in a similar way. Subsequently, using a 20-G Angiocath (1.88 mm in external diameter) as a guide, we ligated the main PA twice using a 4-0 silk suture just enough to induce mild RV enlargement. The first main PA ligation was done softly, and the second ligation was done to the extent that the rat presented with mild macroscopic RV hypertrophy, but without any RV dysfunction. We performed echocardiography after the chest was closed chest, confirmed that tricuspid regurgitation (TR) was trivial, and noted no decrease in the RV fractional area (RVFAC). Cardiac function, pneumothorax, and hemorrhage were evaluated in all animals before closure, and they were extubated after the return of

spontaneous respiration.

PAB flow ≤2.5 m/s was not observed on echocardiography in the PAB group at one week postoperatively. After echocardiography and cardiac catheterization at four weeks postoperatively, euthanasia was performed with pentobarbital. The entire heart was removed and the heart weight was measured, followed by histological analysis. Seven of the removed hearts (two and five in the sham and PAB groups, respectively) underwent DTI prior to histological examination.

All animals were kept at 22°C ± 2°C under a 12-h light/12-h dark cycle, and all experiments were performed according to the study protocol. This study was performed after receiving approval from The Jikei University Institutional Animal Care and Use Committee (No. 2016-093C1).

Echocardiography

Echocardiography was performed using Vivid E9 (General Electric Healthcare, Chicago, IL, USA) under inhalation sedation with 1.5% isoflurane. The parameters measured were as follows: estimated RV pressure (RVp), RV fractional area change (FAC) in the four-chamber view, left ventricle ejection fraction (LVEF), tricuspid-to-mitral valve ratio

(TV/MV), tricuspid annular plane systolic excursion (TAPSE), RV Tei index, heart rate (HR), and cardiac output (CO). RVp was calculated using the formula RVp = right atrial pressure (RAp) + RV-PA pressure gradient, where an RAp of 5 mmHg was assumed in all animals, and the simplified Bernoulli equation ($4 \times v^2$) was used for the RV-PA pressure gradient. LVEF was measured in a single plane using the modified Simpson's method.

2DSTE analysis was performed using Vivid E9 and the dedicated software EchoPAC (version 112; GE Healthcare, Chicago, IL, USA). The heart was divided into four segments: global RV, which included the interventricular septum (IVS); RV free wall; global LV, which included the IVS; and LV without the IVS. For each segment, longitudinal, circumferential, and radial strain measurements were recorded. The frame rate was 180–185 fps for all samples, and HR was 352 ± 42 bpm.

Cardiac catheterization

Cardiac catheterization studies were performed with 2% isoflurane sedation and assisted ventilation before euthanasia at four weeks after the procedure. Left ventricular pressure and RVp were measured with Mikro-Tip catheters (SPR-320NR, 2F; Millar Instruments,

Houston, TX, USA) that were inserted into the LV and RV via the left carotid artery and right jugular vein, respectively. Pressure data were analyzed using the Chart program supplied with the PowerLab system (AD Instruments, Colorado Springs, CO, USA), which calculated mean pressures for at least 10 consecutive beats. The correlation between mean RVp and echocardiographically estimated RVp was examined and confirmed.

Histopathology

The whole heart was removed to determine the extent and distribution of fibrosis and fixed in 10% formalin after cardiac gravimetry. After four weeks of fixation, the heart was divided evenly into four sections in a transverse direction from the RV outflow tract to the apex of the heart (Fig. 2a). After DTI, specimens were fixed in 10% formalin for two weeks and histological examination was performed thereafter. Masson's trichrome staining was performed to evaluate the muscle fibers. Using Image-Pro Premier 9.2 (Media Cybernetics, Washington, WA, USA), we determined the fibrotic area-to-total-area ratio for each region to calculate the fibrosis rate (%; Fig. 2b). Slice 1 was used to estimate the fibrosis rate at the RV outflow tract (RVOT) level, while the global RV, RV free wall, global LV, LV without IVS, and IVS fibrosis rates were determined from each

corresponding area on slices 2–4, and the means of three slices were calculated.

Magnetic Resonance Imaging

Sample processing

After cardiac catheterization, the heart, which was fixed for two weeks in diastole, was wrapped in a sponge, soaked in fluorine solution (Sumitomo 3M Ltd., Tokyo, Japan), and placed in a plastic container; it exhibited no signal on magnetic resonance images.

Samples were also degassed under vacuum to reduce air artifacts. DTI was performed in seven cases (two in the sham group without RV fibrosis after echocardiography at four weeks postoperatively, three in the PAB group with a moderate pressure load of $40 \leq RVp \leq 70$ mmHg, and another two in the PAB group with $RVp > 70$ mmHg).

Image acquisition

A 9.4-T preclinical magnetic resonance imaging (MRI) scanner (BioSpec 94/30; Bruker, Billerica, MA, USA) with a shielded gradient system (max gradient strength, 630 mT/m) and a transmit/receive birdcage coil (inner diameter, 23 mm; Bruker) were used.

Conventional spin-echo T2-weighted data were acquired using the following parameters: TR/time to echo (TE) = 3000/21.2 ms; field of view (FOV) = 16 × 16 mm; matrix = 160 × 160; slice thickness = 0.2 mm; and acquisition time = 24 min. For the myocardial anisotropy evaluation, two volumes of pulse-field gradient spin-echo diffusion-weighted images (DWI) were acquired using the following parameters: TR/TE = 3000/21.2 ms; FOV = 16 × 16 mm; matrix = 160 × 160; slice thickness = 0.2 mm; number of DW directions = 30 each, with a b-value of 500 s/mm^2; and b = 0 s/mm^2 to determine the signal-to-noise ratio. The motion probing gradient duration and interval were 4 and 11 ms, respectively, and the acquisition time was 12 h 48 min.

Image processing

For DTI data processing and image reconstruction, Diffusion Toolkit software (TrackVis.org; Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Charlestown, MA, USA) was used to obtain the eigenvalues ($\lambda1$, $\lambda2$, and $\lambda3$) and FA[11]. $\lambda1$ represents the longitudinal diffusivity parallel to muscle fibers, $\lambda2$ the diffusivity perpendicular to $\lambda1$, and $\lambda3$ the diffusivity perpendicular to both $\lambda1$ and $\lambda2$.

TrackVis software (TrackVis.org) was utilized for fiber tracking based on the fiber assignment by a continuous tracking algorithm, which tracked the orientation of λ1 in a pixel-by-pixel manner in a 3D space.

Regions of interest

MRI was performed at the same levels as those in the three histological slices used to determine the fibrosis rate. Regions of interest (ROIs) were drawn to outline the entire RV free wall; the three eigenvalues and FA were measured at each of the three levels, and their mean values were calculated as follows:

$$\text{Mean } \lambda 1 = (\lambda 1 \text{ of slice } 2 + \lambda 1 \text{ of slice } 3 + \lambda 1 \text{ of slice } 4) / 3$$

$$\text{Mean FA} = (\text{FA of slice } 2 + \text{FA of slice } 3 + \text{FA of slice } 4) / 3$$

Fibers with lengths ≤1 mm were excluded from the fiber tractographic analysis. ROIs were drawn to outline the entire sagittal, coronal, and transverse sections of the RV free wall to visualize the fibers that ran within these ROIs. Subsequently, ROIs were drawn around the RV free wall on two coronal and two transverse slices, and muscle fibers that ran within both of these ROIs were visualized.

Statistical analysis

Descriptive statistics were reported using the median and interquartile range. Comparisons among groups were performed using the Mann–Whitney *U* test or the Kruskal–Wallis test for more than two groups. Post-hoc multiple comparisons were conducted using Bonferroni's method. Spearman's correlation coefficients were calculated to assess the correlations between the RV free wall fibrosis percentage and RV free wall longitudinal strain, RV free wall λ value, RV free wall FA value, number of myocardial fibers. Spearman's correlation coefficient was interpreted as follows: <0.25, none; 0.25–0.50, weak; 0.51–0.75, moderate; and >0.75, strong[12]. All statistical analyses were performed using GraphPad Prism version 7.00 (GraphPad Software, La Jolla, CA, USA). In all analyses, $P < 0.05$ indicated statistical significance.

Results

Summary of data obtained at four weeks post-procedure

The data obtained at four weeks post-procedure are summarized in Table 1. The PAB group had a significantly increased whole heart weight-to-body weight ratio compared to the sham group (5.15 vs. 3.15, respectively, $P < 0.01$). Based on the RV systolic pressure-

250 to-LV systolic pressure ratio on cardiac catheterization and echocardiography, the PAB
251 group had a markedly elevated RVp and an adequate RVp load (0.69 and 0.26,
252 respectively, $P < 0.01$). For each echocardiographic parameter, although no reductions in
253 the HR or CO were noted, the PAB group had an increased RVp (78 vs. 21 mmHg,
254 respectively, $P < 0.01$), TV/MV ratio (1.5 vs. 1.0, respectively, $P < 0.01$), and RV Tei
255 index (0.21 vs. 0.10, respectively, $P < 0.01$), as well as a significant decrease in TAPSE
256 (2.0 vs. 3.0, respectively, $P < 0.01$) compared to the sham group.

257

258 ## Fibrosis rates

259 Histologically determined fibrosis rates were significantly higher in the PAB group than
260 in the sham group at the RVOT, for the global RV, and for the RV free wall (15.8% vs.
261 2.2%, $P < 0.01$; 7.3% vs. 1.9%, $P < 0.01$; and 11.4% vs. 2.0%, $P < 0.01$, respectively)
262 (Table 1). LV and IVS fibrosis rates were similar between the groups.

263 To identify areas in the RV that were susceptible to fibrosis, we measured the RVOT and
264 RV free wall fibrosis rates per slice using the method described in Fig. 2. Table 2 and Fig.
265 3 show the right chamber fibrosis distribution in the PAB group. The fibrosis rate was
266 higher in the basal parts (RVOT and slice 2) than in the apex (slice 4) based on post-hoc

multiple comparisons (the asterisk [*] indicates significant difference vs. slice 4 [$P <$ 0.01]). Although no significant difference was noted, the RVOT had the highest fibrosis rate and, within the RV, slices 2, 3, and 4 tended to have higher fibrosis rates, i.e., the basal side of the heart had higher fibrosis rates than the apical side.

2DSTE analysis

The PAB group had significantly lower global RV and RV free wall longitudinal strains than the sham group (global RV: −15.1% vs. −22.2%, respectively, $P < 0.01$; RV free wall: −12.1% vs. −22.0%, respectively, $P < 0.01$); however, no decreases in the RV circumferential or radial strain values were observed (Table 1). The LV circumferential and radial strains decreased significantly in the PAB group when compared to the sham group (−17.5% vs. −20.2%, $P < 0.01$; 20.6% vs. 40.8%, $P = 0.019$, respectively). Figure 4 shows the correlation between the RV free wall fibrosis rate and longitudinal strain. The RV free wall longitudinal strain value decreased significantly as the fibrosis rate increased (Spearman's $\rho = -0.42$, $P < 0.01$). For the LV, the global LV circumferential and radial strains in the PAB group were reduced (Table 1).

DTI data

DTI images of three representative cases with different RV fibrosis rates (one from the sham group with an RV fibrosis rate of 1.5% and two from the PAB group with RV fibrosis rates of 8.9% and 13.8%) are shown in Fig. 5. The RV free wall λ and FA values were measured in all seven cases that underwent MRI imaging, and their correlations with the RV free wall (%) are shown in Fig. 6. Of these parameters, λ1 was significantly negatively correlated with RV free wall fibrosis (%; Spearman's $\rho = -0.79$, $P = 0.036$).

The tractography data and images were obtained. First, the myocardium that only passed through the RV free wall was depicted in sagittal, coronal, and transverse sections; the number of myocardial fibers was reduced in all sections in the PAB group compared to the sham group (Table 3). Three representative tractography images similar to those in Fig. 5 are shown in Fig. 7 (a, b, c). The number of myocardial fibers per voxel is shown because the area of the RV free wall differs from that of RV hypertrophy, and greater RV hypertrophy is associated with a larger RV area. As fibrosis increased, the number of myocardial fibers decreased in the sagittal, coronal, and transverse sections. The numbers of myocardial fibers in both the coronal and transverse sections were reduced in the PAB group compared to the sham group (Table 4, Fig. 8). The distance between two sagittal cross-sections was not determined in this study due to the difficulty in equalizing the

distance between the sham and PAB groups.

Discussion

This study demonstrated that DTI and 2DSTE may predict and locally diagnose RV myocardial fibrosis, which is a compensatory change after chronic pressure loading. Furthermore, DTI allows for successful 3D visualization of RV myocardial fibrosis.

Late gadolinium-enhanced cardiac MRI has been used for evaluating myocardial fibrosis[13]. However, while this method is superior for evaluating myocardial tissue, it cannot detect diffuse fibrosis. Importantly, use of contrast agent is often discouraged in cardiac disease cases, especially in infants. Therefore, we focused on DTI, which is minimally invasive and requires no contrast medium, and fibrosis severity quantification in specific regions that allowed for objective fibrosis staging. We used ex vivo DTI due to difficulties in synchronizing the rats' fast HRs and body movements. Moreover, the ex vivo analysis allowed for the entire RV fibrosis measurement rather than a single section measurement. Few reports have compared in vivo and ex vivo DTI parameters. In a brain MRI study, Guilfoyle et al. and Haga et al. reported that in and ex vivo anisotropy were comparable[14,15]. Thus, we considered our study results comparable to in vivo study

results.

Here, among the DTI indices, λ1 showed a significant negative correlation with RV myocardial fibrosis. This was consistent with the 2DSTE results, where RV longitudinal strain was most closely correlated with RV myocardial fibrosis. RV myocardium is arranged in two layers: longitudinal and ring-shaped muscles on the endocardial and epicardial sides, respectively, with the predominant fibers being longitudinal. RV myocardial fibrosis may disrupt the myocardial alignment of the longitudinal muscle, resulting in decreased RV longitudinal strain and λ1 on echocardiography and MRI, respectively. Additionally, RV fibrosis was stronger on the basal side of the heart than the apical. Weidemann et al. reported strong LV myocardial fibrosis on the basal and endocardial sides of the LV in patients with severe aortic valve stenosis[16]. Here, the PAB group was based on a chronic pressure loading model, in which the degree of PA strangulation gradually increased with weight gain, resulting in a gradual increase in RVp[17]. As in the LVs of severe aortic stenosis patients, fibrosis may have been more severe in the RV on the basal side of the heart due to its proximity to the pressure load source.

We also attempted to visualize myocardial fibrosis using fiber tractography. When evaluating myocardial fibrosis, Sosnovik et al. used a more detailed fiber tractography

technique, with the 515 axis as the diffusion axis[18]. However, myocardial fibrosis has fewer cross-fibers than cerebral nerves and a higher pixel resolution was considered to avoid normal and fibrotic tissue mixing. Thus, here, the 400 μm resolution set by Sosnovik et al. was 100 μm [18]. Moreover, we set 12 diffusion axes, which were applicable in children, and successfully constructed a 3D myocardial arrangement of a fibrotic heart using fiber tractography. Consequently, we successfully constructed a 3D myocardial sequence in any region using red, green, and blue lines. We found that the depicted number of myocardial fibers decreased with fibrosis. Myocardial RV free wall alignment was continuous in the sham group, whereas myocardial alignment continuity in the RV free wall sagittal, coronal, and transverse planes was not observed in the PAB group, and the number of myocardial fibers was significantly reduced. Coronal and transverse sections showed myocardial fibers passing through both planes at the same distance, and, visually, the decrease in the number of myocardial fibers was more pronounced with an increased fibrosis rate. Thus, the number of myocardial fibers may be a useful measure.

Here, a decrease in RV longitudinal strain, which occurred earlier than the decrease in RVFAC (a conventional measure of echocardiographic contractility), was observed on 2DSTE. This was consistent with Coppola et al.'s findings[19] Interestingly, left ventricular circumferential and radial strain also decreased. There are multiple reports of LV STEs in

355 patients with LV lesions[20, 21, 22]. Similarly, studies of RV strain values in patients with RV
356 lesions are numerous[23, 24]. However, to our knowledge, our study is the first to report that
357 LV circumferential and radial strain preceded a decrease in LV longitudinal strain in RV
358 pressure-overloaded hearts. Importantly, in this study, the LV itself did not have any
359 fibrosis or functional problems, possibly due to the RVp load pressurized the IVS, which
360 affected LV function. We believe that the depressurization direction in the IVS is on the
361 short-axis; therefore, LV global longitudinal strain did not decrease, whereas the LV
362 global and circumferential strain did.

363 This study had limitations. First, this was a single center study with a small sample size.
364 Furthermore, we cannot rule out that sedation may have affected the hemodynamic
365 assessments. Therefore, extrapolation of these results onto humans may be difficult.
366 However, since we used an animal model with little inter-individual bias, the tests'
367 internal validity and results' robustness were ensured. We plan to increase the case
368 numbers to examine the reproducibility of these results. Second, the 4-week observation
369 period may not be optimal for early RV dysfunction assessment. However, when the rats
370 were observed for more than four weeks in the PAB model, many died due to decreased
371 overall cardiac contractility, which included the LV. Moreover, because a decrease in the
372 strain on 2DSTE is considered an earlier indicator of reduced cardiac function than in the

EF[17], we believe that the 4-week observation period may reasonably reflect early RV dysfunction. Third, DTI requires a significant amount of time. However, recently there has been extensive research into compressed sensing[25] and multi-band methods[26, 27] that may reduce the imaging time. These innovations should contribute to the development and clinical application of cardiac MRI. When these problems are overcome and application in pediatric patients becomes possible, this study will contribute to DTI parameter interpretation in pediatric patients.

Conclusions

In this study, we suggested that 2DSTE and DTI could predict RV myocardial fibrosis in a rat model of chronic RVp-loaded rats subjected to PAB.

Authors have no conflict of interest.

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Tables

Table 1. Summary of data obtained at four weeks post-procedure

	Sham (*n* = 10)	**PAB (*n* = 10)**	***P*-value**
	Median (IQR)	Median (IQR)	
BW (g)	342 (333–355)	335 (323–346)	0.47
Whole heart weight/BW	3.15 (3.1–3.3)	5.15 (4.4–5.7)	<0.01
Fibrosis (%)			
RVOT	2.2 (2.0–2.3)	15.8 (13.8–18.7)	<0.01
Global RV	1.9 (1.5–2.1)	7.3 (6.5–9.7)	<0.01
RV free wall	2.0 (1.6 – 2.6)	11.4 (9.4–13.4)	<0.01
Global LV	3.0 (2.5–3.4)	3.9 (3.0–5.1)	0.10
LV without IVS	2.6 (2.2–3.2)	3.3 (2.0–3.6)	0.70
IVS	2.4 (2.1–2.6)	3.2 (2.1–3.7)	0.42
Cardiac catheterization			
RV systolic pressure (mmHg)	19 (16–20)	84 (68–100)	<0.01
RV end-diastolic pressure (mmHg)	1.7 (1.4–1.9)	5.0 (3.0–6.8)	<0.01

LV systolic pressure (mmHg)	70 (66–72)	120 (113–130)	<0.01
LV end-diastolic pressure (mmHg)	2.0 (2.0–2.8)	7.4 (5.3–8.2)	<0.01
RV systolic pressure/LV systolic pressure	0.26 (0.25–0.28)	0.69 (0.52–0.85)	<0.01
Echocardiography			
RVp (mmHg)	21 (20–22)	78 (53–95)	<0.01
4Ch. RVFAC (%)	72 (67–73)	65 (57–69)	0.072
LVEF (%)	56 (52–60)	55 (54–63)	0.74
TV/MV	1.0 (1.0–1.1)	1.5 (1.0–1.6)	<0.01
TAPSE (mm)	3.0 (2.6–3.5)	2.0 (1.6–2.3)	<0.01
RV Tei index	0.10 (0.10–0.15)	0.21 (0.19–0.27)	<0.01
HR (bpm)	389 (382–402)	378 (354–392)	0.36
CO (ml/min)	129 (112–156)	119 (99–147)	0.58
2DSTE			
Longitudinal strain (%)			
Global RV	−22.0 (−22.5 to −21.0)	−15.1 (−18.1 to −12.2)	<0.01
RV free wall	−22.0 (−22.9 to −20.0)	−12.1 (−13.9 to −10.5)	<0.01

Global LV	−18.2 (−19.0 to −16.9)	−16.3 (−19.0 to −14.8)	0.31
LV without IVS	−17.7 (−20.4 to −16.1)	−15.4 (−17.7 to −15.7)	0.075
Circumferential strain (%)			
Global RV	−15.6 (−18.5 to −13.2)	−16.8 (−17.7 to −15.7)	0.36
RV free wall	−13.4 (−15.3 to −9.0)	−15.4 (−16.9 to −14.4)	0.12
Global LV	−20.2 (−22.2 to −19.0)	−17.5 (−19.2 to −16.6)	0.010
LV without IVS	−22.2 (−23.7 to −18.0)	−18.3 (−18.9 to −15.1)	0.022
Radial strain (%)			
Global RV	17.0 (13.5–24.3)	19.3 (13.1–29.0)	0.85
RV free wall	14.7 (12.7–20.2)	18.8 (12.2–31.7)	0.49
Global LV	40.8 (27.9–46.6)	20.6 (18.6–33.4)	0.019
LV without IVS	24.9 (21.0–32.6)	36.1 (14.6–39.4)	0.97

476 PAB, pulmonary artery banding; IQR, interquartile range; BW, body weight; RVOT, right
477 ventricular outflow tract; RV, right ventricle; LV, left ventricle; IVS, interventricular
478 septum; RVp, right ventricular pressure; LVp, left ventricular pressure; 4Ch. RVFAC,
479 right ventricular fractional area in the 4-chamber view; LVEF, left ventricular ejection
480 fraction; TV, tricuspid valve; MV, mitral valve; TAPSE, tricuspid annular plane systolic
481 excursion; HR, heart rate; CO cardiac output; 2DSTE, two-dimensional Speckle tracking.

Table 2. Fibrosis rate for each slice

	Sham (*n* = 10)	**PAB (*n* = 10)**
Fibrosis (%)	Median (IQR)	Median (IQR)
RVOT	2.4 (2.1–2.7)	15.9 (13.8–20.2)
Slice 2	2.3 (2.1–2.5)	15.7 (13.2–17.1)
Slice 3	1.7 (1.4–1.7)	11.4 (8.0–14.6)
Slice 4	1.9 (1.5–2.2)	6.4 (3.8–9.4)

PAB, pulmonary artery banding; IQR, interquartile range; RVOT, right ventricular outflow tract.

Table 3. Number of myocardial fibers only passing through the RV free wall

	Sham (*n* = 2)	**PAB (*n* = 5)**
No. of fibers/voxel	Median (IQR)	Median (IQR)
Sagittal	10.2 (9.9–10.5)	7.2 (3.7–8.4)
Coronal	24.8 (23.0–26.5)	15.3 (10.1–15.7)
Transverse	19.3 (18.9–19.8)	8.6 (4.6–12.9)

Sagittal, coronal, and transverse sections all showed fewer myocardial fibers in the PAB

group compared to the sham group. PAB, pulmonary artery banding; IQR, interquartile range.

Table 4. Number of myocardial fibers passing between the two RV free wall levels

	Sham (*n* = 2)	**PAB (*n* = 5)**
No. of fibers	Median (IQR)	Median (IQR)
Coronal	3065 (2857–3481)	2456 (106–2559)
Transverse	3026 (2700–3679)	595 (127–1283)

The numbers of myocardial fibers were lower in the PAB group than in the sham group in both the coronal and transverse sections. PAB, pulmonary artery banding; IQR, interquartile range.

Figure legends

Fig. 1. Study protocol. UCG, ultrasound cardiography.

Fig. 2. Histological evaluation of fibrosis. (a) The image source is an isolated heart. The whole heart was dissected into four slices of equal thickness from the base to the apex. Slice 1 represented the RVOT. Slices 2 and 3 contained the global RV, RV free wall, global LV, LV without IVS, and IVS regions. (b) The image source is a strip preparation. Example of RV free wall region in slice 2 stained with Masson's trichrome (× 100). Fibrosis rate (%) = fibrotic area: blue / (myocardial area: red + fibrotic area) × 100. Scale bar = 1 mm. RVOT, right ventricular outflow tract; RV, right ventricle; LV, left ventricle; IVS, interventricular septum.

Fig. 3. Fibrosis rates (%) in the RV free wall in the PAB rats (*n* = 10).

Box plots showing the RV free wall in the subgroups of the PAB rats (*n* = 10). The lower half of the box plot depicts the 25th percentile; the upper half of the box plot, the 75th percentile; the horizontal line dividing the upper and lower halves of the box plot, the median; the upper whisker, the maximum; and the lower whisker, the minimum.

515 Significant differences ($P < 0.01$) between groups are indicated by arrows and asterisks
516 (*). The fibrosis rate was higher in the basal parts (RVOT and slice 2) than in the apex
517 (slice 4). PAB, pulmonary artery banding; RVOT, right ventricular outflow tract; RV, right
518 ventricle.

519

520 **Fig. 4. Correlation between fibrosis (%) and longitudinal strain in the RV free wall.**
521 The figure shows the correlation between the fibrosis (%) and longitudinal strain in the
522 RV free wall. Examples of trichrome-stained RV free wall regions with different degrees
523 of fibrosis are shown (blue). A significant negative correlation was observed between the
524 RV longitudinal strain score and fibrosis (Spearman's $\rho = -0.42$, $P < 0.01$). Thus, the RV
525 longitudinal strain decreased as the fibrosis rate increased. Scale bar = 1 mm. RV, right
526 ventricle.

527

528 **Fig. 5. Representative DTI images in the transverse plane.**
529 The image source is an MRI. (a, f, k) b0 images (no diffusion encoding). (b, g, l) FA maps.
530 (c–e, h–j, m–o) λ maps of the heart from a rat in the sham group (top panels: this rat had
531 a histologically determined RV fibrosis rate of 1.5%) and from rats in the PAB group
532 (middle and bottom panels: fibrosis rates of 8.9% and 13.8%, respectively). R, right; L,

left. DTI, diffusion tensor imaging; MRI, magnetic resonance imaging; FA, fractional anisotropy; PAB, pulmonary artery banding.

Fig. 6. Correlation between fibrosis of the RV free wall and DTI parameters.

Among the DTI parameters, only λ1 showed a significant negative correlation with fibrosis of the RV free wall. DTI, diffusion tensor imaging; RV, right ventricle.

Fig. 7. Representative fiber tractography images in sagittal (a), coronal (b), and transverse (c) planes.

The source of image is an MRI. Myocardial fibers passing through the RV free wall are visualized using the same three hearts shown in Fig. 5. Compared to the sham rats, PAB rats had fewer myocardial fibers per voxel in all planes. Red, transverse fibers; green, anteroposterior fibers; blue, craniocaudal fibers. MRI, magnetic resonance imaging; RV, right ventricle; PAB, pulmonary artery banding.

Fig. 8. Myocardial fibers running through the two RV free wall levels based on fiber tractography.

The image source is an MRI. (a) Coronal. (b) Transverse. The same specimens as shown

551 in Figs. 5 and 7 were used. Fibers passing through the two levels (in light blue and purple)
552 are visualized. The PAB group had fewer fibers running these distances than the sham
553 group. MRI, magnetic resonance imaging; RV, right ventricle; PAB, pulmonary artery
554 banding.
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