

Establishing a Novel Animal Model of Tricuspid Regurgitation by Self-expanding Stent

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AIM: To establish a simple, safe, and reproducible animal model of tricuspid regurgitation (TR).

METHODS: A self-expanding stent made of nickel-titanium shape memory metal alloy was developed. Ten white pigs were randomized into an experimental group ($n = 7$) and a control group ($n = 3$). TR was induced by an interventional procedure by implanting a self-expanding stent via the internal jugular vein (IJV) to open the tricuspid valve in the experimental group. The pigs in the control group underwent the same procedure without stent implantation. Echocardiography was performed preoperatively and at 1 month and 3 months postoperatively to assess cardiac function and structure modification.

RESULTS: Echocardiography displayed enlargement of the right atrium (RA) and tricuspid annulus, as well as reduced right heart function in the experimental group. Additionally, the TR area ($p < 0.01$), TR/RA ($p < 0.01$), RA ($p < 0.05$), and tricuspid annulus ($p < 0.01$) were significantly higher at 1 month or 3 months postoperatively compared with preoperative values. Likewise, right ventricular Tei index (RVTei) ($p < 0.05$) was significantly increased. However, right ventricular fractional area change (RVFAC) ($p < 0.05$), and tricuspid annular plane systolic excursion (TAPSE) ($p < 0.05$) were decreased, indicating impaired right heart function. Moreover, no significant changes were noted in the body weight of white pigs. All pigs survived, validating the successful establishment of the TR model. Notably, both the success rate and the survival rate were 100% in the experimental group. However, right heart remodeling was observed in the experimental group. Finally, no significant changes were detected in the control group before and after the operation.

CONCLUSIONS: Self-expanding stent implantation via IJV is a simple and easy-to-manipulate approach for constructing an animal model of TR, which is suitable for exploring right ventricular remodeling.

Keywords: tricuspid regurgitation; animal model; self-expanding stent; right ventricular remodeling

Introduction

Tricuspid regurgitation (TR) is defined as a multifaceted pathophysiological condition characterized by right ventricular blood refluxing into the right atrium (RA) during systole [1,2]. It poses considerable health risks and is prevalent in adult patients with cardiovascular diseases. Noteworthy, it is associated with increased mortality and incidence rates, as well as reduced functional ability and quality of life [3,4]. As is well documented, persistent TR promotes right atrial and right ventricular enlargement, myocardial fibrosis, etc., eventually culminating in life-threatening conditions such as right heart failure, liver and kidney congestion, and lower limb edema [5,6,7]. Besides, TR has been identified as an independent risk factor for atrial fibrillation [8].

At present, research on TR is scarce, highlighting the pressing need to establish reliable TR animal models [9,10,11].

While several methods for establishing TR models have emerged in the literature, they are limited by several shortcomings. For example, the model established by Buffington and Nystrom [12] fails to simulate the pathology of functional tricuspid regurgitation (FTR) in patients. Earlier studies have induced FTR by lacerating the valve leaflets, avulsing the papillary muscles, or a combination of both, which completely destroys the tricuspid valve and increases the risk of death [13,14]. While other models, although playing a role in elucidating the pathophysiology of FTR, are costly and linked to high mortality rates [15,16]. In the present study, white pigs were used to establish TR animal models by implanting self-expanding stents via catheterization. Echocardiography was regularly performed over three months to detect changes in the structure and function of the right heart.

Materials and Methods

Animal Preparation

A total of 10 white pigs, each weighing between 55 and 65 kg, were selected and randomly divided into an experimental group ($n = 7$) and a control group ($n = 3$). Anesthesia was performed via an intramuscular injection of atropine sulfate (0.02 mg/kg) and sustained (6 mg/kg) based on the weight

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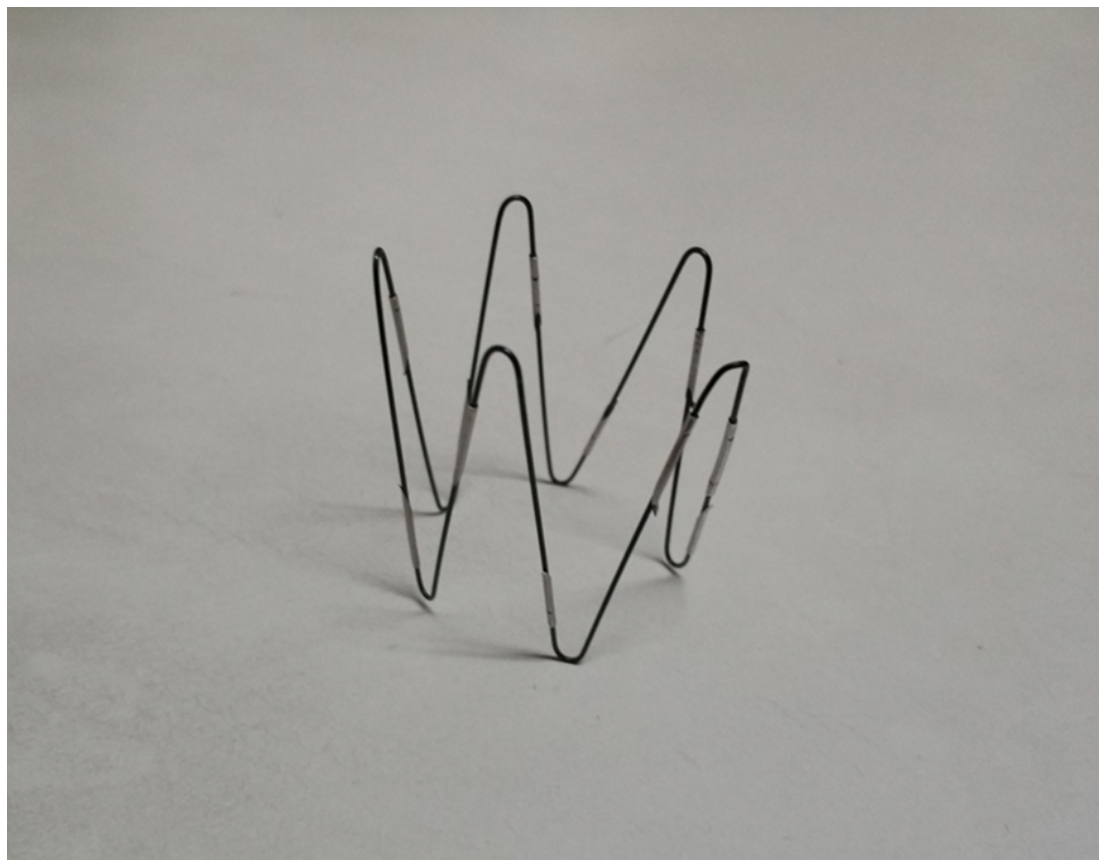


Fig. 1. The diameter of the 0.6 mm nickel-titanium shape memory metal alloy wire was made into a round bracket with a diameter of 3 cm and a side length of 2.5 cm.

of pigs. Next, anesthesia was maintained through inhalation of 2% isoflurane and intravenous injection of propofol following venipuncture and tracheal intubation. Ceftriaxone (1.0 g) was intravenously administered 0.5 h before surgery to prevent infection. Hemodynamic parameters such as electrocardiogram, heart rate, and oxygen saturation were continuously monitored intraoperatively.

Baseline metrics were measured using transthoracic echocardiography (EPIQ 7C, Philips, Amsterdam, Netherlands), and the presence of TR and other heart diseases were assessed (if present, the pigs were excluded from this study) [17,18].

Animal euthanasia was achieved via rapid intravenous injection of 10% potassium chloride, approximately 1 mL/kg, after anesthesia until the animal died. The method and measurement of anesthesia are described above.

Surgical Procedure

The entire surgery process was completed by qualified surgical experts. First, the pig was positioned in a supine position. Next, a 6F sheath of Avanti+ was introduced into the internal jugular vein (IJV), from which a pigtail catheter was inserted into the right ventricle under guidewire guidance. Angiography was performed to confirm the position of the catheter and assess the condition of the tricuspid

valve. Then, a 10F adjustable curved sheath of Lifetech was exchanged into the right ventricle. A circular self-expanding stent with a diameter of 3 cm and a side length of 2.5 cm was made using a nickel-titanium shape memory metal alloy wire with a diameter of 0.6 mm. To prevent displacement of the self-expanding stent, it was wrapped around a barb-like structure (Fig. 1). The 10F adjustable curved sheath served as the support and delivery system. The position of the stent was determined according to the angiographic image. Afterward, the self-expanding stent was compressed into the sheath, advanced with a push rod, and released based on the previously obtained contrast image. Following this, the presence of significant TR was confirmed post-stent implantation by angiography (Fig. 2). Thereafter, the sheath was removed, the IJV was ligated, and the wound was closed in layers. In the control group, no self-expanding stent was implanted following the insertion of the adjustable curved sheath, and only a minimal amount of heparin water was injected. The remaining steps were identical to those in the experimental group. Lastly, the white pigs were transferred to the breeding room after waking up, and the keepers were blinded to group assignments.

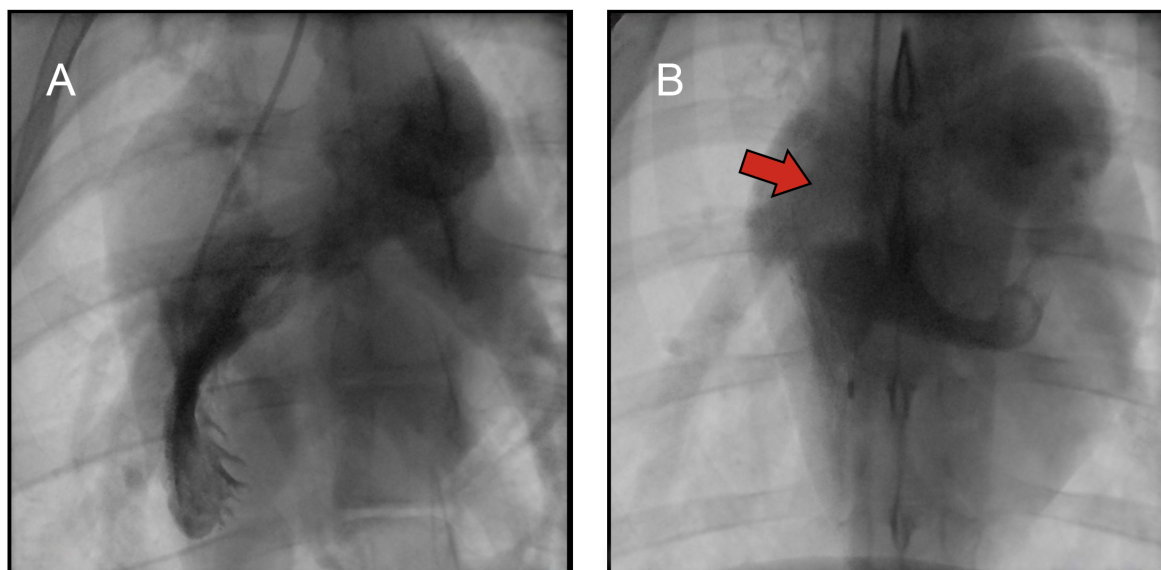


Fig. 2. Angiography images of a pig from the experimental group: (A) preoperative, and (B) within 1 hour postoperatively. The arrow points to the site of tricuspid regurgitation.

Echocardiographic Evaluation and Follow-up

After anesthesia, transthoracic echocardiography (EPIQ 7C, Philips, Amsterdam, Netherlands) was performed by qualified ultrasound professionals to assess cardiac structure and function before surgery (Fig. 3). The primary parameters collected from ultrasound included: (1) degree of TR (TR/RA); (2) right ventricular fractional area change (RVFAC); (3) tricuspid annular plane systolic excursion (TAPSE); (4) right ventricular Tei index (RVTei); and (5) tricuspid ring diameter (Annulus). Follow-up cardiac ultrasound was carried out at 1 and 3 months post-surgery, using the same anesthesia protocol as described in the previous section. At each follow-up, the pigs' appetite, mobility, and the presence of edema in the abdomen and limbs were observed and recorded [19,20].

Statistical Analysis

SPSS statistical software (SPSS19.0, IBM Corp., Chicago, IL, USA) was used for data analysis. Continuous data are expressed as mean $\pm$ standard deviation (SD). Repeated measures analysis of variance (ANOVA) was employed for intra-group comparisons.

Results

General Information

All 10 white pigs in this experiment survived, among which seven white pigs in the experimental group developed severe TR, indicating an operation success rate and a survival rate of 100%. Furthermore, the body weight of white pigs did not significantly change at 1 month and 3 months postoperatively (Table 1, $p > 0.05$).

Echocardiographic Examination

The results are detailed in Table 1. In the experimental group, the tricuspid ring progressively enlarged (Fig. 4). The average area of the RA was $7.310 \pm 2.085 \text{ cm}^2$ preoperatively in the experimental group, $10.550 \pm 2.277 \text{ cm}^2$ 1 month after surgery, and $10.817 \pm 1.360 \text{ cm}^2$ 3 months postoperatively. At the same time, the average regurgitation area before surgery in the experimental group was $0.863 \pm 0.260 \text{ cm}^2$, $3.727 \pm 0.293 \text{ cm}^2$ 1 month after surgery, and $4.168 \pm 0.495 \text{ cm}^2$ 3 months after surgery. Besides, the TR/RA ratio was significantly higher after operation than before surgery in the experimental group (Fig. 5, $p < 0.01$); however, the changes were not significant at 1 month and 3 months post-surgery. In the experimental group, the average of RVTei was 0.894 ± 0.074 before surgery, 1.200 ± 0.098 1 month after surgery, and 1.327 ± 0.040 3 months after surgery (Fig. 5, $p < 0.05$). The average RVFAC was 0.410 ± 0.085 before surgery in the experimental group and significantly decreased to 0.321 ± 0.09 1 month after surgery and 0.235 ± 0.061 3 months after surgery (Fig. 5, $p < 0.05$). Likewise, the mean TAPSE was $2.127 \pm 0.284 \text{ cm}$ before surgery in the experimental group and significantly decreased to $1.543 \pm 0.494 \text{ cm}$ and $1.084 \pm 0.284 \text{ cm}$ at 1 and 3 months after surgery (Fig. 5, $p < 0.05$). No edema was observed in the abdomen and limbs in the experimental group.

There was no significant change in body weight in the experimental group, and TR, Annulus, and RVTei were higher postoperatively compared to their pre-operative levels. On the other hand, both RVFAC and TAPSE significantly decreased postoperatively. Finally, no significant

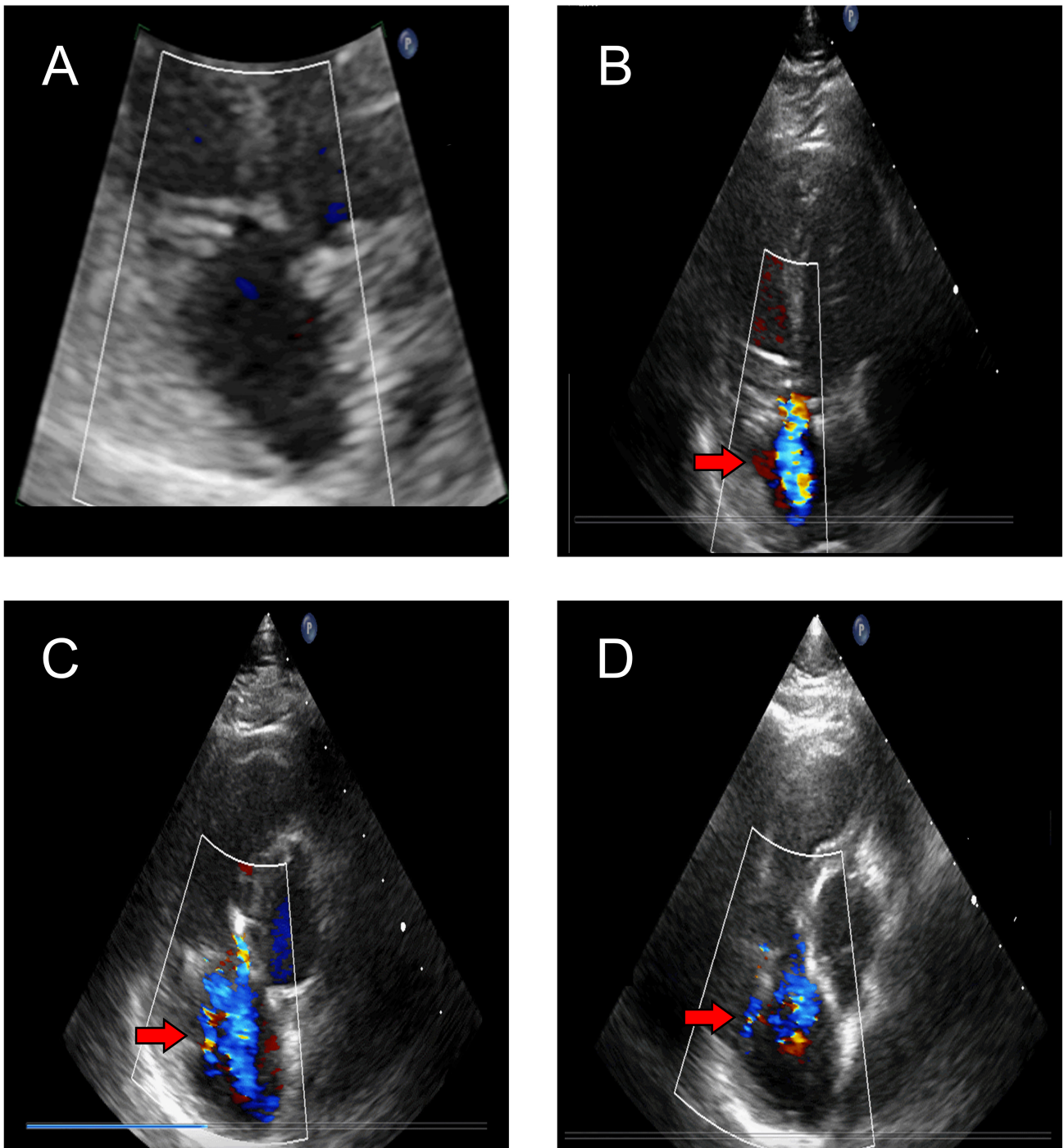


Fig. 3. Echocardiography images of pigs: (A) preoperative, (B) within 1 hour postoperatively, (C) 1 month postoperatively, and (D) 3 months postoperatively. The blue area indicated by the arrow represents tricuspid regurgitation.

differences were observed between preoperative and post-operative body weight, RA, TR, TR/RA, Annulus, RVTei, RVFAC, and TAPSE in the control group.

Position of Self-expanding Stents and Tricuspid Valve Morphological Changes

The white pigs with severe TR were euthanized, and heart tissues were harvested. The RA, right ventricle, and tricuspid ring were significantly enlarged. All seven self-

expanding stents were located at the tricuspid ring, attached to the valves and tendinous cords (Fig. 6), while some stents were embedded in the myocardium. One self-expanding stent was in a transverse position across the valve ring. The self-expanding stent was closely attached to the tendinous cord, and contracture of the tendinous cord was observed. In the control group, no significant differences were noted in the RA and right ventricle, tricuspid valve, and chordae tendinae.

Table 1. Results of cardiac ultrasonography at 1 and 3 months after operation.

Group	Wt (kg)	RA (cm ²)	TR (cm ²)	TR/RA	Annulus (cm)	RVTei	RVFAC	TAPSE (cm)
Control group								
Preoperative	63.250 ±	5.015 ±	0.449 ±	0.090 ±	2.000 ±	0.963 ±	0.354 ±	1.495 ±
	1.768	0.205	0.105	0.025	0.028	0.015	0.001	0.120
1 month after surgery	64.500 ±	5.220 ±	0.421 ±	0.081 ±	2.000 ±	1.025 ±	0.380 ±	1.450 ±
	0.707	0.906	0.081	0.000	0.014	0.007	0.007	0.071
3 months after surgery	64.500 ±	6.090 ±	0.521 ±	0.097 ±	2.040 ±	1.015 ±	0.413 ±	1.645 ±
	1.414	1.047	0.024	0.000	0.014	0.007	0.016	0.007
Experimental group								
Preoperative	62.083 ±	7.310 ±	0.863 ±	0.117 ±	2.283 ±	0.894 ±	0.410 ±	2.127 ±
	1.320	2.085	0.260	0.010	0.317	0.074	0.085	0.284
1 month after surgery	61.133 ±	10.550 ±	3.727 ±	0.338 ±	3.290 ±	1.200 ±	0.321 ±	1.543 ±
	1.925	2.277 ^a	0.293 ^b	0.127 ^b	0.208 ^b	0.098 ^a	0.090 ^a	0.494 ^a
3 months after surgery	61.000 ±	10.817 ±	4.168 ±	0.394 ±	3.560 ±	1.327 ±	0.235 ±	1.084 ±
	2.627	1.360 ^a	0.495 ^b	0.095 ^b	0.287 ^b	0.040 ^b	0.061 ^b	0.284 ^b

Notes: Values are presented as mean ± standard deviation. ^a $p < 0.05$, ^b $p < 0.01$ compared to preoperative values in the experimental group.

Wt, weight; RA, right atrium; TR, tricuspid regurgitation; RVTei, right ventricular Tei index; RVFAC, right ventricular fractional area change;

TAPSE, tricuspid annular plane systolic excursion.

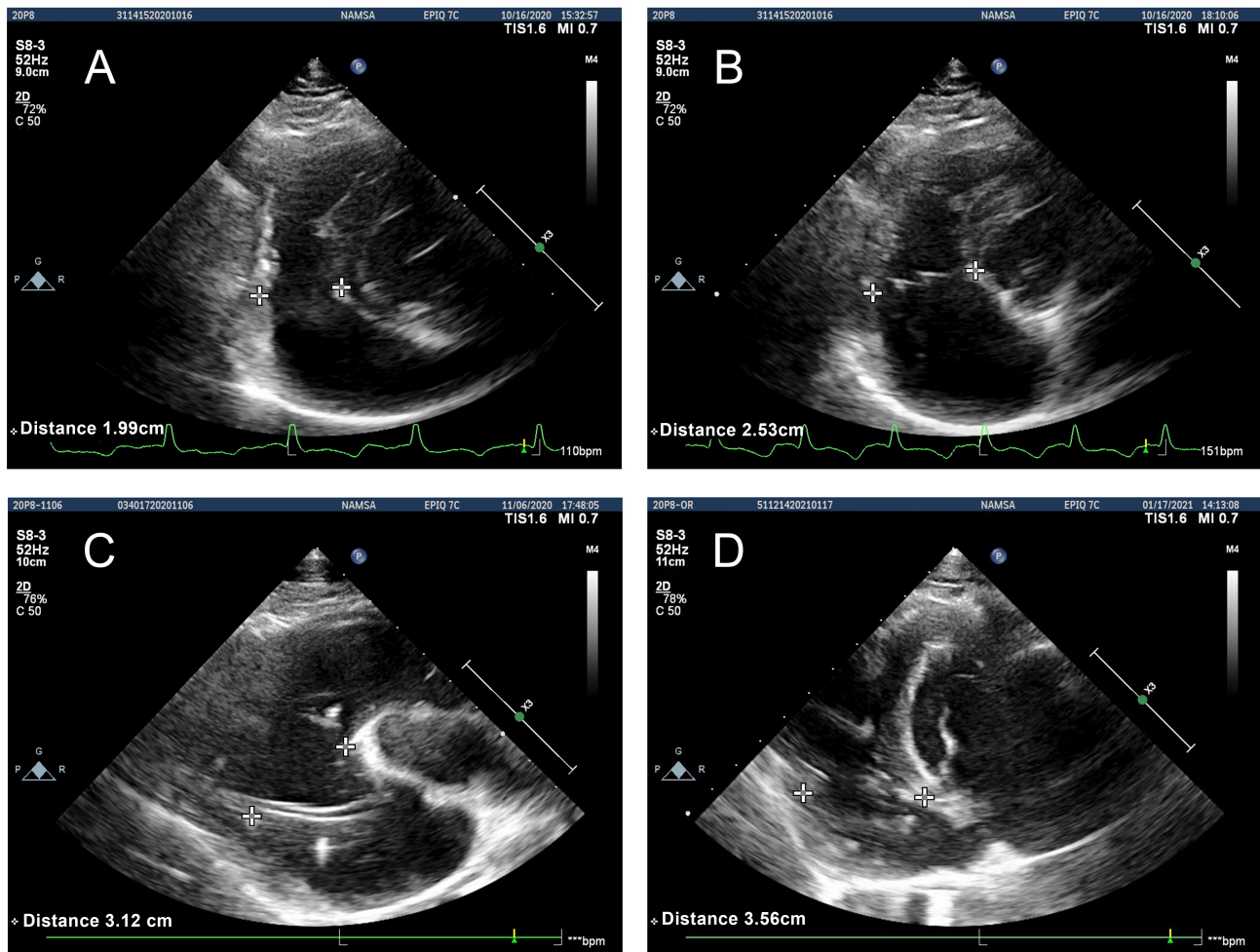


Fig. 4. The diameter of the tricuspid valvular ring: (A) preoperative; (B) within 1 hour postoperatively; (C) 1 month postoperatively; and (D) 3 months postoperatively.

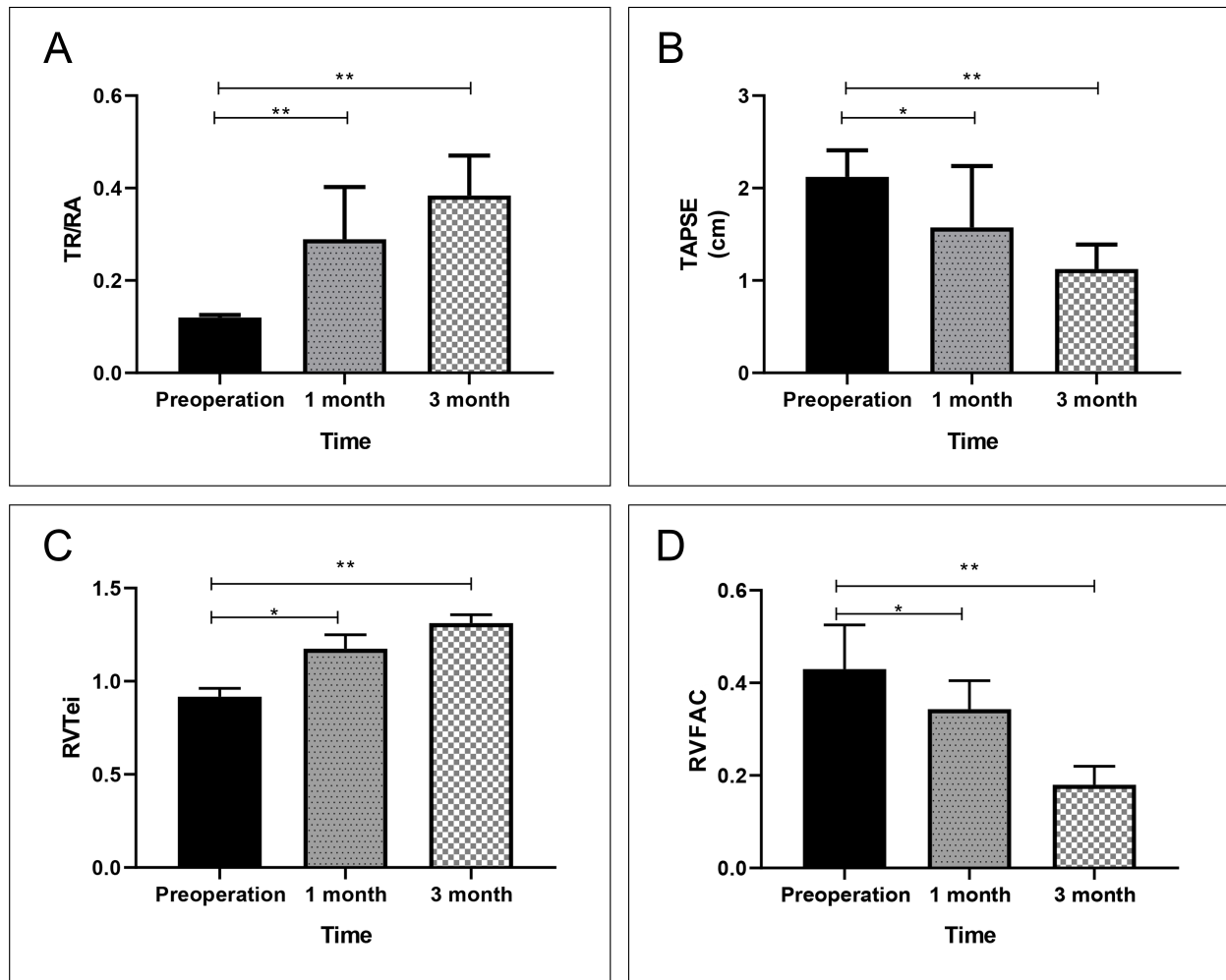


Fig. 5. Comparison of cardiac ultrasound results in the experimental group before surgery and 1 month postoperation and 3 months postoperation. (A) TR/RA; (B) TAPSE; (C) RVTei; (D) RVFAC. * $p < 0.05$, ** $p < 0.01$.

Discussion

Although TR is a prevalent cardiac issue, the pathophysiological mechanism underlying this condition remains underexplored. At present, the development of animal models for tricuspid valve insufficiency is crucial for advancing research in tricuspid valve diseases. Of note, numerous animal models of tricuspid valve insufficiencies have been reported. Hoppe *et al.* [21] induced TR in sheep using a percutaneous catheter by utilizing the guide wire to tear the tendinous cords and papillary muscle tissue of the tricuspid valve. However, this technique has a poor modeling consistency and is associated with a high risk of ventricular rupture. Bai *et al.* [13] employed a specially designed grasping forceps to grasp chordae tendineae or the tricuspid valve leaflets through a catheter to damage the tricuspid valve in the process of establishing a regurgitation model. Nevertheless, it is challenging to estimate the extent of regurgitation and attain surgical consistency with this method. Similarly, this method is also facing high risk of ventricular rup-

ture and massive hemorrhage. Xie *et al.* [14] used surgical methods to directly block the superior and inferior vena cava in dogs, followed by the rapid excision of the anterior and posterior lobes of the tricuspid valve, septal lobes, papillary muscles and chordae tendineae, with subsequent suturing of the heart wound. Nonetheless, while the degree of TR achieved by this method was optimal and consistent, the procedure in itself was complex, risky, and difficult to standardize.

Herein, a TR model with high stability and high return flow was established by inserting self-made, self-expanding stents into the tricuspid valvular ring via the IJV. Three months after the stent was implanted, dissection of the experimental animals revealed that the self-expanding stent was closely attached to the tricuspid ring, with a portion of the stent embedded into the ventricular muscle.

It is worthwhile acknowledging that although self-expanding stent had marginally displaced and transversed in one animal, the TR condition remained significant.

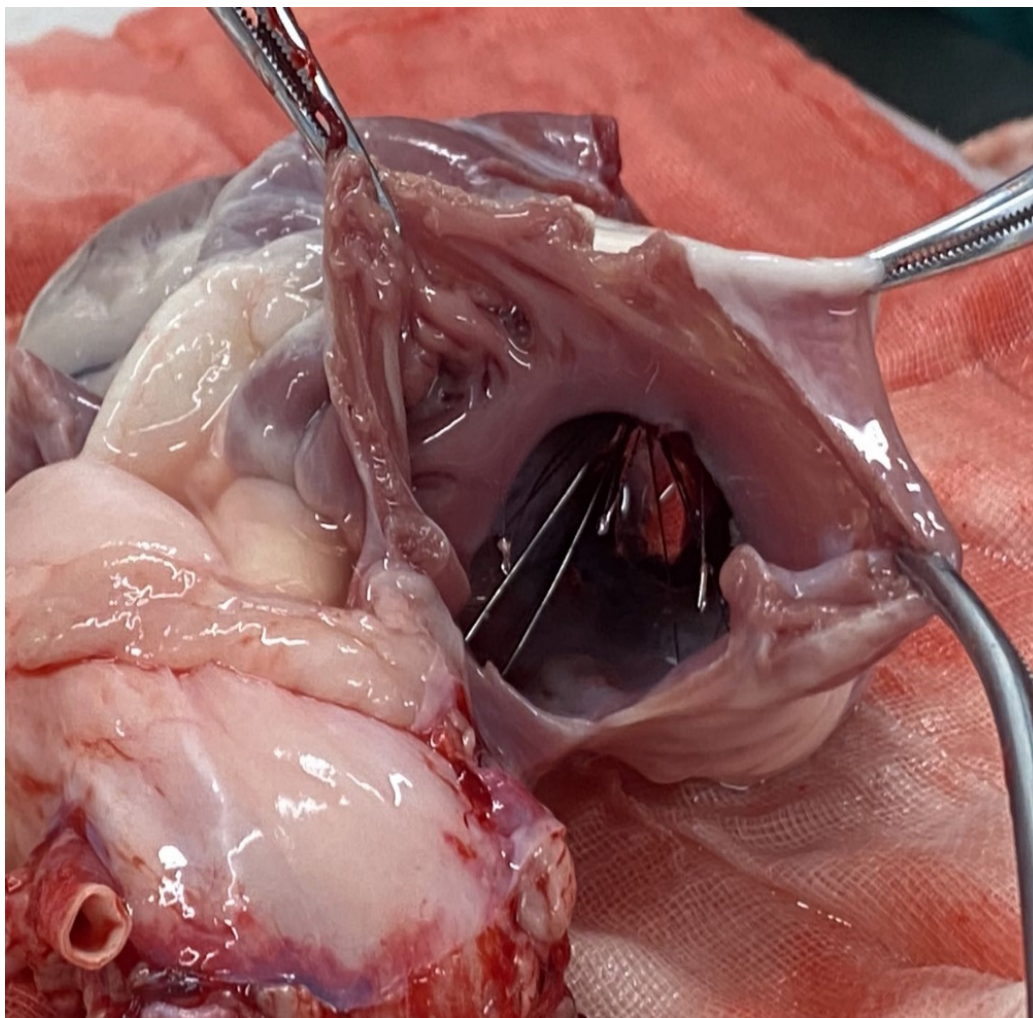


Fig. 6. Incision of the right atrium depicting the stent supporting the tricuspid valve ring and partially wrapping around the tricuspid tendinous cords.

In addition, for animals in the experimental group, no significant changes in body weight and no edema in the abdomen and limbs were observed, probably due to the prolonged duration of right heart failure induced by TR [22]. There was no significant change in body weight in the control group.

However, echocardiography illustrated changes in the structure and function of the right heart in the experimental group before surgery, as well as at 1 month and 3 months postoperatively. It is well known that the structure of the right heart is more complex than that of the left heart, and its function index cannot solely rely on the ejection fraction of the right heart [23,24]. Herein, tricuspid valve insufficiency (TR/RA), RVFAC, TAPSE, RVTei, and tricuspid annular diameter were used to quantify the functional and structural changes of the right heart. Our results unveiled significant increases in TR and the TR/RA ratio in the experimental group after surgery, reflecting tricuspid regurgitation. In addition, RVFAC and TAPSE decreased, while the tricuspid annular diameter and RVTei increased, indi-

cating dilation of the tricuspid annulus, structural changes in the right ventricle, and decreased right heart function. In contrast, there were no significant differences in these indexes before and after the operation in the control group.

Conclusions

The IJV self-expanding stent made of nickel-titanium shape memory metal alloy can be used to establish a simple, safe, and reproducible animal model of TR, providing a novel option for modeling TR in animals.

Availability of Data and Materials

The data supporting the findings of this study are included in the article.

Author Contributions

PX and JQZ designed the research study; PX, WZF, HXX, and YHJ performed the experiments; YHJ and HXX performed data collection and analysis. PX and JQZ were involved in drafting the manuscript. All authors have been

involved in revising it critically for important intellectual content. All authors have approved the final version of the manuscript for publication. All authors have participated sufficiently in the work to assume public responsibility for appropriate sections of the content and agree to be accountable for all aspects of the work, including addressing questions regarding its accuracy or integrity.

Ethics Approval and Consent to Participate

This study protocols were approved by the Academic Committee of Shaoxing People's Hospital. The care and use of animals in this study were reviewed and approved by the Animal Ethics Committee and Animal Management Committee of Zhejiang University (No. IACUC-2020-030).

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Conflict of Interest

The authors declare no conflict of interest.

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