Off-pump tricuspid valved stent implantation: the next step

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Abstract

OBJECTIVES: Off-pump transcatheter valved stent implantation could be a treatment option for patients suffering from symptomatic tricuspid regurgitation (TR) who are classified as inoperable. In this study, we present our recent short-term results of transventricular tricuspid-valved stent implantation and compare different stent types for atrial anchorage.

METHODS: Fifteen pigs received a self-expandable valved stent implantation off-pump via a transventricular access. Successfully implanted pigs were observed over a period of 6 h (n = 9), 48 h (n = 1) and 4 weeks (n = 1). Haemodynamic and full transoesophageal echocardiographic (TOE) evaluations were done before, 1 h, 3 h (n = 11; all successfully implanted pigs), and 6 h (n = 9; acute group) after implantation. Nine days postimplantation, one pig received additional angiography, computed tomography (CT) and transthoracic echocardiography (TTE). Post-mortem, gross examination was conducted to analyse the stent position and deformation. In two pigs (48 h and 4 weeks survival) histological staining and immunohistochemistry of surrounding myocardium was performed.

RESULTS: The heart rate significantly increased in all pigs postimplantation from 66.8 ± 13.6 to 101.8 ± 24.6 bpm, whereas cardiac output and pressure levels remained unchanged. Orthotopic positioning was reproducibly achieved. TOE showed an efficient reduction of para-valvular leakages from a mean grade of 1.4 ± 1 h postimplantation to a mean of 0.9 at 6 h postimplantation due to a special sealing pouch. The ratio early and late ventricular filling velocities remained constant and the valvular gradient across the valved stent stayed low during the observation period. Angiography, CT and TTE confirmed orthotopic positioning and mild grade of paraavalvular leakage after 9 days (n = 1). Only mild TR was observed here. The ventricular part of the stent was deformed to an oval shape in 7 of 14 animals as shown via post-mortem examination. The surrounding tissue after 1 month (n = 1) showed normal morphology, without inflammation or calcification.

CONCLUSION: This study shows the feasibility of catheter-based replacement of the tricuspid valve by a valved stent in an off-pump procedure. The successive enhancements in this tricuspidvalved stent design lead to a prototype being ready for mid- to long-term evaluations.

Keywords: Transventricular • Tricuspid valve • Transthoracic echocardiography • Off-pump • Valved stent

INTRODUCTION

Tricuspid regurgitation (TR) is a common finding upon echocardiographic examination [1–3]. In a population-based study, TR was found in 80–85% of the cohort with a prevalence of mild or higher grade TR of 14.8% in men and 18.4% in women [3]. Among tricuspid valve (TV) diseases, 94% involve regurgitations (TR), and only 2% stenosis [4]. Nevertheless, TV surgery is only considered to be an effective treatment for symptomatic TR [5]. Seventy-four percent of TV diseases in North America involve secondary to left heart pathology, such as mitral valve disease and left heart failure, right ventricular (RV) dysfunction or pulmonary hypertension [4].

Patients who previously underwent left-sided valve surgery and suffer from symptomatic TR have a worse perspective than patients initially treated at both mitral and TVs. Repeat surgery is associated with a high late-mortality rate, which is predicted by age and number of previous cardiac surgeries [6–8]. Minimally invasive replacement of the TV could be a treatment option for patients who are classified as inoperable.

Off-pump replacement of atrophicventricular (AV) valves has been the focus of recent investigations. However, invasive treatment of TV diseases has been rarely investigated and is limited to case reports which either require preliminary TV operations (replacement or annuloplasty) [9] or suggest heterotopic implantation within the vena cava [10]. In 2005, Boudjemline et al. [11] reported first progress on replacement of the TV using a transcatheter method under angiographic guidance noting difficulties of deploying and securing a stent in the tricuspid position. We have previously reported on the feasibility of replacing AV valves by transcatheter implanted valved stents under transoesophageal echocardiographic (TOE) guidance [12–14] and presented our first preliminary results of TV replacement via transventricular access in an acute study including seven animals [15].

In contrast to our first preliminary study, the aim of this study was to observe animals after transcatheter tricuspid valved stent implantation with different types of stents. Furthermore, special attention was paid to imaging procedures and the post-mortem evaluation in a mid-term survival setting.
MATERIALS AND METHODS

Tricuspid valved stent

A self-expandable nitinol stent consisting of a disc-like atrial cuff and a tubular ventricular body was developed (Fig. 1A–C). The stent was covered with a polytetrafluoroethylene (PTFE) membrane. Stent dimensions were varied and analysed. A glutaraldehyde-preserved native porcine (Medtronic Hancock II®) or pericardial bovine (Perimount Magna®) heart valve was mounted into the tubular ventricular body. Four tethers for stent fixation were attached to the ventricular column of the stent. The valved stent was stored in glutaraldehyde until implantation. A circular pouch made of a micro-perforated tissue was prepared and filled with 0.1 g super-absorbent polymer (SAP) [15]. Before implantation, the stent was thoroughly washed in saline solution and the SAP pouch was attached to the outside of the ventricular part by a running suture. The stent was crimped and placed into a custom-made delivery system with an outer diameter of 42-Fr (Fig. 1D).

Study design

The study cohort included 15 pigs of the German landrace and the German ‘Edelschwein’ race (66% female, mean weight 51.9 ± 1.9 kg). The study end points were separated into two groups: 6 h (h) survival (n = 13) and longer than 6 h survival (n = 2). The expected study end point in the second group was 4 weeks after implantation. Four different types of stents were used and evaluated in this study. Atrial and ventricular diameter, the connecting angle \( \alpha \) and the height of the stent were modified (Table 1) according to their previous outcomes. Table 1 also presents TOE measurements of end-diastolic annular diameters.

In vivo study preparation

All animals received humane care, according to the protocols published by the Center for Experimental Animal Research at the University of Kiel, Germany, in compliance with the Guide for the Care and Use of Laboratory Animal Resources, National Research Council, published by the National Academy Press, revised 1996 and 2011.

Anaesthesia was induced with ketamine 10% (12.6 ± 1.3 mg/kg body weight) and midazolam (1.4 ± 0.2 mg/kg body weight) and maintained with propofol 2% (7.1 ± 0.5 mg/kg body weight/h) and Fentanyl® 0.05 mg (0.015 ± 0.003 mg/kg body weight/h). Loading dose of potassium chloride (40 mmol) and magnesium sulphate (4 g 10%) in 500 ml Ringer’s solution and antiarrhythmic agent Amiodaron (150 mg Cordarex® in 100 ml 5% Glc-solution) and antibiotic therapy (Cefuroxim 1500 mg) were preventively administered. In two pigs surviving longer than 6 h, antibiotic treatment was continued with Enrofloxacin i.m. (Baytril® 10%, 5 mg/kg body weight) for 10 days postimplantation and analgesics were administered with NSAID Carprofen i.m. (Rimadyl®, 4 mg/kg/day) for 5 days postimplantation.

Measurements

Continuous electrocardiography and invasive blood pressures were monitored. Pressures from right atrium pressure (RAP), right ventricle pressure (RVP), pulmonary artery pressure (PAP) and pulmonary capillary wedge pressure (PCWP) as well as the cardiac output (CO) were measured by use of a Swan-Ganz catheter before and after the implantation process. The positioning of the stent was guided via TOE (Vivid i, GE Healthcare, Chalfont St Giles, England). TOE evaluation included
two-dimensional, pulsed-wave, continuous-wave and colour Doppler imaging. Paravalvular leakages (PVLs) were expressed as an overall grade. Hence, with colour jet extension, PVLs were classified into four grades: absent (0), trace or mild (1), mild to moderate (2), moderate to severe (3) and severe (4).

Surgical procedure and follow-up

The transventricular access was prepared as previously described [15]. The deployment system was inserted into the RV through an incision within the purse-string sutures at the RV wall close to the apex. The tip of the deployment system was pushed forward into the right atrium (RA) under TOE guidance. First, the atrial elements were released in the RA and the atrial cuff was positioned exactly above the native tricuspid annulus. In a second step, the delivery system was gently moved back in order to deploy the ventricular part of the stent into the tricuspid annulus and below. After full deployment, the delivery system was removed from the ventricle and the incision was closed by tightening the purse-string sutures. The tethers connected to the ventricular body of the stent were fixed outside the RV incision.

Haemodynamic and full TOE evaluation took place at defined evaluation points: before implantation (0 h), as well as 1 h and 3 h postimplantation. The acute study group (n = 13) was additionally evaluated at 6 h postimplantation and the animals were sacrificed afterwards in profound anaesthesia and the hearts were explanted for post-mortem investigation.

Additional treatment for the two pigs surviving longer than 6 h. After implantation, 7500 IU of protamine sulphate was administered, a surgical drain was placed and the chest was closed. Monitoring the pigs was continued for 3 h prior to returning them to the intensive care unit of the animal facility. The drain was removed 2 days postimplantation and general health was checked daily during the subsequent follow-up period of 10 days. Transthoracic echocardiography (TTE), right heart angiography and computed tomography (CT) with a multislice CT scanner (Siemens MDCT Somatom, 64 slice/min, Erlangen, Germany) were performed at 9 days postimplantation in one pig. Both hearts were explanted for post-mortem investigation.

Post-mortem evaluation

Gross examination was performed in all implanted animals. The position of the stent and the SAP pouch in relation to the native annulus and the right ventricular outflow tract (RVOT), as well as the expansion of the SAP were evaluated. Furthermore, mammography (Hologic Selenia, Medicor, Inc., Kerpen, Germany) and microscopic analysis of surrounding tissue were performed for the pigs surviving longer than 6 h post implantation. Tissue samples for histological examination were fixated in 4% formalin and embedded in paraffin, and microscopic slides (5 µm) were prepared. Slides were deparaffinized and rehydrated and stained with haematoxylin and eosin (H&E) stain for general morphology and other stainings as follows. Calcification was estimated by von Kossa staining (precipitation reaction with silver ions and counterstaining with nuclear fast red) and immunohistochemistry with anti-CD3 (DCS GmbH & Co. KG, Hamburg, Germany), anti-CD20 (BioLogo, Dr H. Schultheiß e.K., Kronshagen, Germany), anti-CD45 (AbD Serotec MorphoSys UK Ltd, Oxford, UK) and anti-CD68 (BioLogo, Dr H. Schultheiß e.K., Kronshagen, Germany) was conducted to detect inflammatory signs: T-lymphocytes, B-lymphocytes, leucocytes and macrophages, respectively. Sections were analysed and documented using brightfield light microscopy.

Statistical analysis

Statistical analysis included haemodynamic evaluation of the general monitoring parameters heart rate (HR), mean arterial pressure (MAP) and mean central venous pressure (CVP), as well as pulmonary artery catheter measurements: mean RAP, diastolic right ventricular pressure (diaRVP), systolic pulmonary artery pressure (sysPAP) and CO. Central tendency is expressed by mean value, and dispersion by standard deviation (±). Statistically significant changes in individual haemodynamic values (HR, MAP, CVP, RAP, diaRVP, sysPAP and CO) over time were assessed using the Friedman χ² test and post hoc multiple Conover comparison with Bonferroni-Holm correction. A P-value of <0.05 was considered to indicate statistical significance.

RESULTS

Within the group of 6 h, implantation was successfully accomplished at first attempt in 8 of 13 pigs and 2 attempts were needed in 1 pig. In this case, no SAP pouch was used due to expansion of the pouch during the first attempt. Implantation failed in four animals due to fatal arrhythmia, which occurred before implantation (n = 1) due to introduction of the deployment system; and while implantation due to reposition of the stent being necessary after stent migration during apical fixation (n = 2); and stent migration into the ventricle during implantation (n = 1).

Table 1: Overview of the different types of stents in chronological order of implantation

<table>
<thead>
<tr>
<th>Stent type</th>
<th>Implanted in case</th>
<th>Atrial Ø (mm)</th>
<th>Ventricular Ø (mm)</th>
<th>Ventricular height (mm)</th>
<th>Atrioventricular angle α (°)</th>
<th>Mean end-diastolic annular Ø (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>36</td>
<td>27</td>
<td>19</td>
<td>90</td>
<td>31.0</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>53</td>
<td>25</td>
<td>18</td>
<td>110</td>
<td>29.0</td>
</tr>
<tr>
<td>III</td>
<td>3&amp;4</td>
<td>36</td>
<td>27</td>
<td>15</td>
<td>90</td>
<td>30.5</td>
</tr>
<tr>
<td>IV</td>
<td>5–15</td>
<td>48</td>
<td>30</td>
<td>13</td>
<td>110</td>
<td>31.4</td>
</tr>
</tbody>
</table>

Ø: diameter.
Two additional pigs were operated on for a 4-week follow-up. One of them had to be sacrificed 48 h postimplantation because of worsened general health and a second animal reached the expected mid-term study end point of 4 weeks. In total, implantation was successful in 11 pigs. Due to ventricular arrhythmia during apex incision, two pigs had to be defibrilated. However, these pigs remained stable throughout the surgical procedure. Unsustained atrial and ventricular ectopic beats occurred during Swan-Ganz catheter application and device deployment in all pigs, but neither haemodynamically relevant arrhythmias nor AV blocks were present after implantation in all successfully implanted animals.

The mean overall application time was 249 ± 169 s and the mean releasing time of the stent was 99 ± 32 s. Electrolytes and pH value remained within physiological ranges of tolerance during surgery. Blood loss ranged from 50 to 1400 cc during operation. The high amount of blood loss was the result of stent explantation and a successful second attempt in one animal; without this value, the mean blood loss was at 117 ± 122 cc.

Haemodynamics

Table 2 shows a comparison of haemodynamic parameters preimplantation, 1, 3 and 6 h, as well as 9 days postimplantation. The HR increased significantly during the observational period from 66.8 bpm before implantation to 103.9 ± 25.6* bpm at 1 h postimplantation, respectively (Fig. 2). All other parameters remained stable and did not show any significant alterations. Examination 9 days postimplantation in one pig revealed the RAP, the diaRVP and the HR to be slightly increased from 5 mmHg, 2.9 ± 2.2 mmHg and 4.4 ± 3.3 bpm before implantation to 22.2 ± 1.9 mmHg, 21.1 ± 4.6 mmHg and 43 ± 0.7 bpm at 6 h postimplantation, respectively. The HR increased from 87 bpm at 1 h postimplantation to 98 bpm at 3 h postimplantation. Systolic PAP remained stable at all points of evaluation. The pig surviving 4 weeks showed a heart and breathing rate of 120 bpm and 26/min, respectively, before euthanasia.

Transoesophageal echocardiography

TOE provided adequate imaging for successful guidance and orientation during the deployment procedure, which was visualized in a four-chamber view. Particular attention was paid to the PVL.

Immediately after implantation, PVL were observed in all cases (range from Grade 1–4, mean 1.4), with reductions at 6 h postimplantation (range from Grade 0–3, mean 0.9). The ratio early and late ventricular filling velocities remained constant with 1.36, 1.42 and 1.48 at 0, 1 and 6 h, respectively. The transvalvular gradient across the valved stent remained low throughout the entire observation time.

The TOE evaluation before implantation showed a mean end-diastolic annular diameter of 31.1 mm (Table 1). An oversizing of the atrial stent diameter with respect to the annulus (20.1% by stent type I, 82.8% by stent type II, 18.3% by stent type III and 53.2% by stent type IV) was used for diastolic anchorage of the stent.

Further cardiac imaging procedures

Nine days postimplantation, TTE, angiography and CT were performed in one pig surviving 4 weeks. In echocardiographic imaging, the RVOT was free of obstruction and only mild TR and PVL were recorded. The stent was orthotopic and no signs of embolism or tricuspid stenosis were noticed. During angiographic visualization (Fig. 3A), the pulmonary arteries contrasted immediately

Table 2: The table shows stable haemodynamic conditions. At least n = 9 animals were analysed for each of the parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before implantation</th>
<th>1 h postimplantation</th>
<th>3 h postimplantation</th>
<th>6 h postimplantation</th>
<th>9 days postimplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>66.8 ± 13.6</td>
<td>103.9 ± 25.6*</td>
<td>102.5 ± 23.1*</td>
<td>101.8 ± 24.6*</td>
<td>72</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>67.8 ± 13.0</td>
<td>61.8 ± 13.9</td>
<td>71 ± 2.3</td>
<td>78.2 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>meanCVP (mmHg)</td>
<td>6.6 ± 2.8</td>
<td>7.8 ± 3.2</td>
<td>7.0 ± 2.1</td>
<td>7.8 ± 2.7</td>
<td>11</td>
</tr>
<tr>
<td>meanRAP (mmHg)</td>
<td>8.1 ± 2.8</td>
<td>7.3 ± 1.9</td>
<td>2.4 ± 2.3</td>
<td>2.7 ± 2.7</td>
<td>4</td>
</tr>
<tr>
<td>diaRVP (mmHg)</td>
<td>4.4 ± 3.3</td>
<td>2.9 ± 2.2</td>
<td>21.1 ± 4.6</td>
<td>19.3 ± 5.0</td>
<td>22</td>
</tr>
<tr>
<td>sysPAP (mmHg)</td>
<td>22.0 ± 2.8</td>
<td>22.2 ± 1.9</td>
<td>3.7 ± 0.9</td>
<td>3.9 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>CO [l]</td>
<td>3.9 ± 0.8</td>
<td>4.3 ± 0.7</td>
<td>4.3 ± 0.7</td>
<td>4.3 ± 0.7</td>
<td></td>
</tr>
</tbody>
</table>

* Significant change P ≤ 0.05.

HR: heart rate; MAP: mean arterial pressure; CVP: central vein pressure; RAP: right atrial pressure; diaRVP: diastolic right ventricular pressure; sysPAP: systolic pulmonary artery pressure; CO: cardiac output.
and properly. A TR, Grade 1–2, induced by the catheter, was observed.

The CT with contrast medium 9 days postimplantation (Fig. 3B–D) showed a significant dilatation of the RA. Good stent positioning within the native tricuspid annulus was shown. The atrial crown had a good circular anatomical fit within adjacent structures. The ventricular tube-shaped stent body was deformed to an oval shape measuring 19 × 33 mm, long axis in septal to lateral direction. CT-investigation did not reveal any stent fractures. The SAP pouch expanded uniformly underneath the tricuspid annulus. The RVOT was not obstructed by the ventricular body of the stent. The RV myocardium was free of hypertrophy or dilatation.

Post-mortem investigation

Post-mortem investigations of the animals were performed in 14 animals (all but one pig, which died before implantation) (Fig. 4). Gross examination confirmed desired positioning of the stent in all successfully implanted cases (n = 11). In detail, the atrial crown was positioned above the native annulus and the ventricular part pushed the native valve to the side in these cases. Stent deformation was assessed and showed an oval shaping in 50% of all cases (7 of 14). In the group of pigs surviving >6 h, 64% (7 of 11) of the stents showed an oval deformation. Stent fracture (one strut) was found in one case, not associated with deformation of the stent or fatal outcome after implantation (successful 6 h case, no oval deformation of stent observed). An obstruction of the RVOT was not found in any of the successfully implanted animals (n = 11). The examination of the three deceased pigs showed anterior (n = 1) and septal (n = 2) migration of the stent into the ventricle. The atrioventricular angle α = 110° was found to be superior for anchorage to the angle of 90°. Therefore, the sizes and angles were adapted (Table 1). The expansion and location of the SAP pouch was assessed in only 13 cases due to one implantation without a pouch. Complete expansion was achieved in 8 of 13 animals. In five cases, the pouch was partly located above the annulus.

Post-mortem investigation of the pigs surviving >6 h confirmed the desired position and no fracture of the device. After 4 weeks (Fig. 4 C and D), an oval deformation was observed. Furthermore, the SAP pouch was located at the expected position below the annulus and was completely ingrown. Obstruction of the RVOT or myocardial induration was not found. However, only one of three leaflets showed adequate mobility, and the two other leaflets were indurated and immobile. A large thrombus was detected in the RA, obstructing the valve. The thrombus had an expansion of 2 × 3 cm, and the RA showed a mild dilatation. In the mammography (Fig. 5), calcification could not be identified around the stent at the ventricular part due to shadowing from the SAP pouch and only a slightly calcified base of the bioprosthetic valve was seen.

Histological analysis

Tissue around the native TV annulus was excised for histological analysis in two pigs surviving longer than 6 h. H&E stain (Fig. 6A and B) showed regular cell configuration of myocytes with normal nuclear proportions and morphology in both cases. Immunohistochemistry with anti-CD3, anti-CD20, anti-CD45 and anti-CD68 (Fig. 6C–F, respectively) revealed no inflammatory signs of T-lymphocytes, B-lymphocytes, leucocytes and macrophages in varying regions of the myocardium, respectively. von Kossa staining exposed calcification of the granulation tissue on
the atrial crown of the stent, but no calcification of the tissue around the in-grown stent was detected (Fig. 5C).

**DISCUSSION**

The feasibility of implanting a self-expandable nitinol stent under TOE guidance into the mitral position has been reported previously by our group with the omission of X-ray exposure and rapid ventricular pacing [12, 16]. Although Boujoumline et al. [11] and Iino et al. [15] reported on experimental studies of orthotopic TV replacement, progress in humans is so far limited to selected cases: Lauten et al. [17] reported on the possibility of heterotopic implantation of custom-made valve stents into the superior vena cava and the inferior vena cava (IVC) in an animal study and the first implantations in human IVC [10]. However, this procedure only reduces severe venous backflow in the IVC and is limited to patients with preserved RV function. Due to the induced ventricularization of the RA, the preload increases and may support RV failure as well as noisome effects on cardiac rhythm. Roberts et al. [9] described a series of percutaneous TV implantations in 15 patients with prior TV replacement, and hence with sufficient structures for anchorage of the implanted Melody percutaneous pulmonary valve (Medtronic, Inc., Minneapolis, MN, USA). The unique anatomy of the native TV annulus complicates the anchorage of a valved stent [18]. In contrast to our previous study [15], we examined different stent types and presented mid-term results including histological analysis, which is rare after valved stent implantation so far.

This study shows the results of 15 animals with implanted tricuspid valved stents. The transventricular approach grants a safe access to the TV and enables a secure implantation and a proper

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Figure 4: Macroscopic evaluation. (A) RV laterally opened demonstrates free RVOT, (B) opened RA shows oval deformation of the valved stent. (C and D) After 4 weeks: (C) opened right ventricle, RVOT on top, totally ingrown and oval formed stent (D) atrial view after resection of the atrium, tissue ingrowth demonstrated by the forceps.

Figure 5: Evaluation of calcification after 4 weeks. Mammography of the stent showed calcification around the SAP (A) and a mildly calcified base of the leaflets (B). Tissue stained with von Kossa and counterstaining with nuclear fast red (C) revealed calcification of the tissue on the atrial crown.
fixation of the stent. Starting the application process in the RA minimizes the risk of getting the stent entangled in the chordae tendineae [11]. The application of tethers reduces the irritation of the trabeculae carneae that may occur using a ventricular disc for fixation [11]. In 10 successfully implanted pigs, the SAP pouch reduced PVL regardless of the pouch’s position above or below the native annulus.

Fatal ventricular fibrillation occurred in three pigs during implantation. They died while repositioning the stents after migration during fixation of the ventricular tethers (n = 2) and removing the cone of the delivery system (n = 1). The migration of the stent into the ventricle was caused by too small a diameter of the atrial cuff (n = 2, diameter of 36 mm) and an oversized ventricular height (n = 1, ventricular height of 18 mm). In the latter case, the anterior side of the atrial cuff was pushed further back into the RA resulting in a bulging of the opposite side into the RV. Thus, less irritating fixation systems, such as a clip for the tethers outside the myocardium, need to be evaluated in further studies. In one case, implantation could be repeated after ventricular migration of the stent while positioning, resulting in perfect position of this stent at the second attempt, confirmed upon gross examination. Explantation of tricuspid valved stents is not practicable due to impaired lesions and involved high blood loss.

Insufficient adjacent structures at the native annulus [18] and movement of the annulus [19–21] complicate stent anchorage. Therefore, large diameters of the atrial element were used in order to minimize risk of stent migration into the ventricle. The atrial oversizing of 53.2% by stent type IV and its atrioventricular angle of α = 110° demonstrated satisfactory fixation of the stent without myocardial constriction and enabled physiological pressure values. The coronary sinus flow was not restricted in all animals. An oval deformation of the stent was observed in 50% of all cases, which is likely linked to these large diameters. However, in patients with TR the annulus was found to be rather circular and more flattened and might work well with our proposed stent device [21, 22].

In all successfully implanted pigs, hemodynamic evaluations showed significantly increased HR, but within ranges of tolerance. The increase might be induced by atrial irritation of the conduction system, since it has not been seen in off-pump transapical mitral implantation [16]. Besides, the pig surviving 4 weeks showed normal levels of the HR at follow-up. Pressure levels and CO showed normal values and showed no significant changes in all animals.

The pig surviving 4 weeks showed an increased mean RAP after 9 days due to atrial thrombus formation and malfunction of two leaflets. Hence, an increase in the diaRVP was seen. Nevertheless, sysPAP remained constant during the observational period and demonstrated a normal systolic ventricular function. The pig had not been under anticoagulative therapy after implantation, which might have contributed to thrombus formation as well as low pressure gradients and high turbulences in the RA. Most likely, the thrombus contributed to an increased RAP and dilatation of the RA, confirming the CT results, although a thrombus was not observable during the visualization. Adequate positioning of the atrial crown above the native valve area was achieved at the first attempt in this pig. The RVOT was not obstructed and the native chordae tendineae remained intact at implantation. Conclusively, a proper myocardial movement was observed in the TOE and TTE at all points of evaluation. The RVOT remained unimpaired 9 days after implantation as seen in angiography and in CT. A central regurgitation was found in the angiography, which was likely aggravated by the catheter. The SAP and the PTFE-membrane ensured proper sealing of the stent without obstructing the RVOT.

The normal anatomy as presented in the H&E staining and the immunohistochemistry findings showed no inflammatory in- duction neither after 48 h nor after 4 weeks and suggests that the oversizing of the stent does not irritate the myocardium and furthermore, SAP is not harmful to the surrounding tissue. Results of microscopic examinations of the cardiac structures around nitinol stents are rare. We can confirm the results from another study of our group, which demonstrated the absence of inflammatory

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**Figure 6:** H&E stain and immunohistochemistry. (A): H&E after 48 h, (B): H&E after 4 weeks. (C–F) immunohistochemistry after 4 weeks with anti-CD3 (C), anti-CD20 (D), anti-CD45 (E) and anti-CD68 (F).
processes at the native myocardial tissue around the percutaneously replaced pulmonary valve in six sheep after 3 months of survival [23]. In addition to the anti-CD3 which was used in their paper, we used anti-CD20, anti-CD45 and anti-CD68 in order to verify our results for innate and adaptive immune system. von Kossa staining and mammography showed a calcification process of the tissue on the atrial crown and at the SAP pouch, whereas von Kossa staining was negative at the tissue around the SAP, although it seemed contrasted in the mammography. This suggests that the SAP pouch itself appears contrasted and that mammography is less sensitive compared with von Kossa staining.

Study limitations

In this study, only healthy animals were included and the pathological anatomy of diseased human TVs were not considered. Since only two animals were followed up for >6 h, further investigations will include a larger group of animals in order to investigate the reproducibility of the long-term outcomes of the valved stent and to analyse whether the HR increase is transient.

CONCLUSION

These results demonstrate the feasibility of transfemoral-based replacement of the TV under TOE guidance in an off-pump setting. Stent failure was caused by small atrial diameters and a large ventricular height. The presented fixation system was able to achieve a stable position in the orthotopic site, although fail-safe transventricular fixation needs to be established in order to minimize the risk of migration. Moreover, the sealing-pouch reduced PVL shortly after implantation by expansion due to its hygroscopic quality. The mid-term results show the potential of good valvular function of this novel device, but this has to be confirmed by further mid-term studies.

Conflict of interest: none declared.

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