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Two- and three-dimensional transoesophageal echocardiography in large swine used as model for transcatheter heart valve therapies: standard planes and values

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Abstract

OBJECTIVES: Swine models are widely used to develop new techniques and materials for the treatment of heart valve disease like aortic valve and mitral valve transcatheter interventions and to train physicians in these techniques. Transoesophageal echocardiography (TOE) is crucial in these models. We defined standard planes of 2D and 3D TOE in healthy pigs undergoing transcatheter heart valve interventions.

METHODS: Twenty healthy pigs (weight 56–106 kg) underwent different mitral and aortic valve interventions (transcatheter aortic valve implantations, implantations of a mitral band, bicuspidization of the aortic valve, trans-septal punctures). For image guidance of the procedures, an adult TOE probe was introduced under direct vision in the oesophagus. Before the procedure itself was performed, a standardized protocol was used to determine normal values for anatomical and functional echocardiographic parameters.

RESULTS: Positioning of the probe was possible in all animals and ideal when achieving a distance from the front teeth (incisors) of 40–60 cm. Anteflexion and lateroflexion of the probe was necessary to achieve optimal imaging quality. 2D visualization of all relevant cardiac structures was possible. The aortic annulus diameter was 24.1 ± 2.5 mm, the sinus of valsalva diameter was 30.6 ± 4 mm and the sinotubular junction diameter was 25.2 ± 4 mm. The ascending aorta had a diameter of 24 ± 4 mm and the descending aorta a diameter of 16 ± 5 mm. The mitral valve anterior–posterior diameter was 31.8 ± 4 mm and the commissure to commissure diameter was 40.5 ± 5 mm resulting in a mitral valve area of 10.7 ± 1.5 cm². 3D visualization was possible for the aortic and the mitral valve. None of the animals showed any pathology except one that had a dilated left ventricle and moderate mitral valve insufficiency. Left and right ventricular dimensions and the anatomy of the aortic-, mitral-, tricuspid and pulmonary valve as well as of the aorta were comparable with those of the human anatomy.

CONCLUSIONS: 2D and 3D TOE can be routinely applied as image guidance in pigs used as a model for the development and training of new techniques to treat heart valve disease.

Keywords: Animal model • Transoesophageal echocardiography • Minimally invasive surgery • Transcatheter valve therapy

INTRODUCTION

Domestic pigs (*Sus Scrofa*) are used as an animal model for preclinical evaluation and investigation of new heart valve prostheses and therapies due to their similarities to the human physiology and anatomy of the heart and vessels, and their good availability [1–3]. Furthermore, good correlation between weight and sizes of the valvular annuli, short but well-defined ascending aorta and aortic arch curvature stronger than the ones found in cloven-footed animals have been described in pigs [4, 5]. These models are also widely used to train physicians in these new techniques. Important imaging modality for aortic valve and mitral valve interventions is transoesophageal echocardiography (TOE). The use of TOE in transcatheter aortic valve implantation (TAVI) procedures and transcatheter

mitral valve interventions (i.e. the MitraClip procedure) is extensively described in the literature [6–9]. For the use of TOE in large swine models, the literature is very limited [10]. For the development and the training of new transcatheter techniques in large swine models, TOE for image guidance of the procedure is crucial as well.

The aim of this study was to show the feasibility of TOE in swine to develop a TOE approach and establish standard values for a large swine animal model that can be used during the development and training of transcatheter heart valve therapies.

METHODS

The animal housing and experimental protocols were approved by the Cantonal Veterinary Department, Zurich, Switzerland, under

licence no. ZH 152/2013, and were in accordance with Swiss Animal Protection Law. Housing and experimental procedures were also conforming to the European Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes and to the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, National Academy of Sciences, 2011).

The animals were included in different projects within the licence covering transcatheter aortic valve replacement with standard transcatheter heart valve prosthesis (i.e. Symetis ACURATE TA Aortic prosthesis) and transcatheter mitral valve repair techniques (Valtech Cardioband [11], Cardiosolutions Mitra-Spacer [12]).

TOE was part of the study protocols. The examinations were performed in the operating room before the interventions were started; no additional animals were needed for this study.

Twenty domestic pigs with a median weight of 92.5 kg (56–106 kg) were included in the study. All animals received premedication with ketamine (20 mg/kg), azaperone (1.5 mg/kg) and atropine (0.75 mg) intramuscularly. After the loss of postural reflexes, the animals were transported to the surgical preparation room. The anaesthesia was deepened by a bolus injection of propofol (1–2 mg/kg)

and the trachea of the animal was intubated. Anaesthesia was then maintained with 2–3% of isoflurane in 1 : 1 oxygen/air mixture (4–5 l/min). Amiodarone (2–3 mg/kg bolus i.v.) was administered to stabilize the heart rhythm. Animals were placed on the operating table in dorsal recumbence.

After the induction of anaesthesia, real-time 3D TOE Philips iE33 (Philips Medical Systems, Andover, MA, USA) was performed using a standard adult matrix array TOE probe X7-2t (Philips Medical Systems). The probe was covered with a latex coat that was floated with ultrasonic gel (Skintact, Leonhard Lang GmbH, Innsbruck, Austria) and was introduced under laryngoscopic sight, lifting the tongue and the lower jaw with the spatula. The TOE probe was introduced from the right side of the pig's pharynx to find the oesophagus below the trachea. For introduction, the probe was kept unblocked in neutral position.

Two-dimensional transoesophageal echocardiography

The examination was started at around 55 cm from the incisors. The exact depth of the probe, the flexion of the tip of the probe and the rotation of the transducer were adapted for optimal imaging. An issue of TOE in pigs is the anatomy of the bronchial system. The main bronchus is located between oesophagus and the heart (Fig. 1). Therefore, strong anteflexion and lateroflexion with the tip of the probe is needed to avoid the interferences with the bronchus. After anteflexion and lateroflexion of the tip of the probe and forward rotation of the transducer between 0 and 20° or 160 and 180° (Fig. 2), a modified four-chamber view was generated. Although the left cavities were well visualized in this view, the right heart structures, particularly the tricuspid valve and the right ventricle could be partially hidden behind the main bronchus. Systolic and diastolic wall thickness of the free walls of the left and the right ventricle as well as of the septal wall was measured. Further anteflexion and lateroflexion of the tip of the probe at this level allowed the visualization of a short-axis view of the aortic valve and an inflow-outflow view of the right ventricle (Fig. 3). To assess mitral valve function and anatomy in a two-chamber view, a rotation of the transducer angle to 40–60° was used and transmittal Doppler obtained (Fig. 4). The probe was left at the same level in the oesophagus, and the transducer was rotated between 50 and 70° to achieve a mid-oesophageal long-axis view (Fig. 5). The diameters of the aortic annulus, sinus of Valsalva, sinotubular junction and ascending aorta were measured

Figure 1: Computed tomography of a pig. MV: mitral valve; LV: left ventricle; Oe: oesophagus; Br: bronchus.

Figure 2: Four-chamber view. Left side shows a schematic. Right side shows an exemplary image. MV: mitral valve; TV: tricuspid valve; IVS: inter-ventricular septum; LV: left ventricle; RV: right ventricle; LA: left atrium; RA: right atrium; PA: pulmonary artery.

Figure 3: Aortic valve in SAX and right ventricular inflow-outflow view. AV: aortic valve; TV: tricuspid valve; PV: pulmonary valve; SAX: short-axis.

Figure 4: Mitral valve in a two-chamber view. MV: mitral valve; LV: left ventricle; LA: left atrium.

Figure 5: Mid-oesophageal LAX view. Left side shows a schematic. Right side shows an exemplary image. LAX: long-axis; IAS: interatrial septum; MV: mitral valve; AV: aortic valve; LV: left ventricle; RV: right ventricle.

in this view. The left ventricular mid-papillary end-systolic and end-diastolic diameters (LVESD and LVEDD) and the left ventricular end-systolic and end-diastolic volumes (LVESV and LVEDV) using Simpson's rule were assessed and the left ventricular ejection fraction (LVEF) was calculated. Cardiac output was obtained from the multiplication of the ejection volume and the heart rate. Using the 3D software X-Plane on the aortic valve in long-axis, a short-axis of the aortic valve was obtained, with the inflow-outflow view of the right ventricle. From this position, the probe was then pulled back of 10 cm and the transducer angle rotated between 0 and 60°. The aortic and the pulmonary valve could then be seen in long-axis view, parallel to each other and parallel to the probe. Small rotations of the probe were necessary to focus on each valve separately. In this position, Doppler echocardiography was performed to measure the peak and the mean gradient,

the peak and the mean jet velocity and the velocity time integral through the aortic and the pulmonary valve (Fig. 6).

Three-dimensional transoesophageal echocardiography

3D imaging of aortic, pulmonary and mitral valves was always possible; 3D imaging of the tricuspid valve was generally more difficult to obtain because of its anterior position and the extremely fine quality of its leaflets. Main focus was laid on the aortic and the mitral valve. Examples are shown in Videos 1 and 2. 3D views of the mitral and the aortic valve were obtained from the long-axis bidimensional imaging (rotation of the probe between 50 and 70° as shown in Fig. 5). For 3D measurement of the mitral valve

Figure 6: Aorta and pulmonary valve shown in parallel. Left image shows a schematic. Right image shows an exemplary TOE image and the Doppler measurement in the pulmonary valve. AA: ascending aorta; PV: pulmonary valve; TOE: transoesophageal echocardiography.

Video 1: 3D Transoesophageal echocardiography of the aortic valve.

opening area, we used the 3D quantification software (3DQ-QLab) by Philips Medical Systems. 3DQ is widely used for quantification in 3D. The mitral valve was displayed in three orthogonally orientated planes and the plane in which the measurement was performed was orientated perpendicularly to the valve leaflets. An example of these measurements is shown in Fig. 7.

Statistics

All variables were tested for normal distribution with the Kolmogorow-Smirnow and the Shapiro-Wilk test. If normal distribution was present, the mean with standard deviation was calculated. In all other cases, the median with the range was calculated. To test the correlation of weight and cardiac dimensions, Pearson's

Video 2: 3D Transoesophageal echocardiography of the mitral valve.

R was calculated. A *P*-value of <0.05 was defined as high enough to decline the null hypothesis.

RESULTS

Insertion of the TOE probe was possible in all animals. The depth of insertion from the front teeth (incisors) row varied from 40 to 60 cm. The mean depth for visualization of the aortic, the mitral and the tricuspid valves was 53 ± 5 cm. To visualize the pulmonary valve, the probe had to be pulled back ~ 10 cm to a mean depth of 45 ± 4 cm (range from 35 to 55 cm). Mean heart rate during the examinations was 67 ± 8 beats per minute. Except in one animal, which had a dilated left ventricle with consecutive mitral valve insufficiency, no pathologies were found. Values for left ventricular dimensions and

Figure 7: Measurement of the mitral valve dimensions with Philips' QLab. D1: commissure-to-commissure diameter; D2: antero-posterior diameter; A1: mitral valve area.

function, aortic root and aortic dimensions as well as functional parameters for the aortic and the mitral valve are presented in Table 1. With more experience, the pulmonary valve was assessed as well. Results are available for 13 animals.

There was a significant correlation of body weight with the diameters of the aortic annulus ($R = 0.6$; $P < 0.01$), left ventricular outflow tract ($R = 0.7$; $P < 0.001$), sinus of Valsalva ($R = 0.6$; $P < 0.01$) and sinotubular junction ($R = 0.7$; $P < 0.001$). Animals with a body weight of 56–65 kg had annulus diameters of 20–24 mm. All animals with a body weight of >100 kg had annulus diameters of at least 25 mm (up to 30 mm) except one animal with an annulus diameter of 24.4 mm. There was also a correlation of the body weight with the mitral valve annulus area ($R = 0.6$; $P < 0.01$), the antero-posterior ($R = 0.5$; $P < 0.05$) and inter-commissural diameters ($R = 0.7$; $P < 0.001$) of the mitral valve. Animals with a body weight of >95 kg had a mitral valve annulus area of >10.5 cm².

DISCUSSION

In this study, we demonstrate feasibility, show landmark data and suggest a method, closely resembling actual TOE standard in humans that can be used as the imaging modality in large porcine model for preclinical testing and training of transcatheter heart valve therapies.

2D and 3D TOE in experimental, closed chest porcine models is feasible. To the best of our knowledge, there is only one study available that systematically describes transoesophageal echocardiographic findings in pigs [11]. At this time, 3D echocardiography was not yet available. In that publication, TOE and intracardiac ultrasound were used to describe cardiac dimensions. Left ventricular diameters and

wall thickness were comparable with those of our findings. In contrast, end-systolic and end-diastolic volumes were found to be lower in our study. Consequently, we also found a lower stroke volume but the same LVEF. We used Simpson's rules to measure the ventricular volumes whereas the biplane area-length formula was used previously by Ren *et al.* The use of the modified Simpson's rules to assess left ventricle volume has been shown to be reliable in humans [15]. The ventricular volume might be underestimated because of the foreshortening of the apex in TOE.

Ascending and descending aortic dimensions were similar in both studies. Aortic root dimensions as well as mitral valve dimensions were not published previously in [11]. However, these parameters are important nowadays for the planning of animal experiments. Porcine models play an important role in the development of transcatheter heart valve therapies. The first implantation of a valve in a stent for closed chest aortic valve implantation in a pig model was described in 1992 [16]. In 2002, the first human case of a balloon expandable stent-valve implantation was described [17]. The first human implantation of a CoreValve prosthesis was performed in 2004 [18] after extensive testing in a porcine model [19]. Since then, TAVI grew rapidly and numerous new prostheses appeared on the market. In this setting, acute porcine experiments played and still play an important role. One major topic is the development of new prosthesis. Another main issue is the need for training of the physicians performing the procedures. Many companies request an animal training before the first human implantations for new implanters.

After the rise of TAVI, the mitral valve is the next target for transcatheter therapies. Different devices are under preclinical and clinical testing. Among others, a transcatheter mitral annuloplasty band has been developed and tested heavily in a porcine model [11].

Table 1: Echocardiographic findings in pigs

	Study values pig (n = 20)	Normal values human
Heart rate (bpm)	67 ± 8 [55–81]	
Left ventricular dimensions and function		1.
LVESD (mm)	34 ± 4 [27–41]	22–35 ^a
LVEDD (mm)	49.6 ± 4.7 [39–59]	38–52 ^a
LVESV (ml)	37.1 ± 10 [22–62]	14–42 ^a
LVEDV (ml)	88.4 ± 21.5 [61–147]	46–106 ^a
LVEF (%)	58.1 ± 7.4 [47–71]	≥55 ^a
BV (ml)	51.9 ± 15 [30–85]	
CO (ml/min)	3421 ± 815.7 [2040–5346]	
CI (ml/min/kg)	43.4 ± 11.5 [19–65]	
IVS wall thickness syst. (mm)	15.5 ± 0.7 [10–21]	6–9 ^a
IVS wall thickness diast. (mm)	10.9 [7–13]	6–9 ^a
Aortic root and aorta		
Aortic annulus diameter (mm)	24.1 ± 2.5 [20–30]	20–31 ^b
Sinus of Valsalva diameter (mm)	30.6 ± 4 [24–39]	29–45 ^b
Sinotubular junction diameter (mm)	25.2 ± 4 [20–33]	22–36 ^b
Ascending aorta diameter (mm)	24 ± 4 [19–33]	22–36 ^b
Descending aorta diameter (mm)	16 ± 5	20–30 ^b
Max. jet velocity aortic valve (m/s)	0.75 ± 0.2 [44–128]	
Mean jet velocity aortic valve (m/s)	0.5 ± 0.1 [19–87]	
Max. gradient aortic valve (mmHg)	2 [1–6]	
Mean gradient aortic valve (mmHg)	1 [0–4]	
Mitral valve		
Mitral valve diameter ap (mm)	31.8 ± 4 [25–38]	
Mitral valve diameter cc (mm)	40.5 ± 5 [32–51]	
Mitral valve area (cm ²)	10.7 ± 1.5 [8–13]	
Max. jet velocity mitral valve (cm/s)	57.6 ± 12 [39–83]	
Mean jet velocity mitral valve (cm/s)	29.4 ± 5.5 [20–41]	
Max. gradient mitral valve (mmHg)	1 [1–3]	
Mean gradient mitral valve (mmHg)	0 [0–1]	
Pulmonary valve		
Max. jet velocity pulmonary valve (cm/s)	45 ± 8	
Mean jet velocity pulmonary valve (cm/s)	30 ± 5	
Max. gradient pulmonary valve (mmHg)	0 [0–1]	
Mean gradient pulmonary valve (mmHg)	0 [0–1]	
RV wall thickness systole	8.5 ± 2.1	
RV wall thickness diastole	4.6 ± 1.7	

LVESD: left ventricular end-systolic diameter; LVEDD: left ventricular end-diastolic diameter; LVESV: left ventricular end-systolic volume; LVEDV: left ventricular end-diastolic volume; LVEF: left ventricular ejection fraction; BV: beating volume; CO: cardiac output; CI: cardiac index; IVS: inter-ventricular septum; syst.: in systole; diast.: in diastole; Max.: maximum; ap: anterior-posterior; cc: commissure-to-commissure. RV: right ventricular. All animals and the animal with the pathologic mitral valve are included in Table 1.

^aLang *et al.* [13].

^bEvangelista *et al.* [14].

Transcatheter mitral valve replacement is on the rise. For these therapies, preclinical testing [20, 21] and training are crucial as well. Especially for these new mitral valve therapies, echocardiography is indispensable and has to be started in the preclinical phase. The advantage of TOE in pig models is that, compared with human anatomy, several similarities can be found: the aortic valve is tricuspid and the aortic root dimensions are comparable with the human anatomy as well as cardiac chamber dimensions and the mitral valve anatomy. The mitral valve has an anterior and a posterior leaflet and the annulus has a similar shape. These findings underline that 2D and 3D TOE in animal experiments with pigs represents a useful tool for preclinical testing of transcatheter valve therapies under 'human-like' conditions. Beating heart mitral valve repair procedures, TAVI, as well as atrial and ventricular septal defect device closure all rely on limited intraprocedural visualization that could be enhanced with this guide.

In the present study, we demonstrate feasibility, show landmark data and suggest a method, closely resembling actual TOE

standard in humans that can be used in large porcine model for preclinical testing of devices and training of clinicians.

Conflict of interest: none declared.

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