Anatomical considerations and surgical technique of porcine cardiac xenotransplantation

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Central Message
A thorough understanding of the subtle differences from human heart is essential for donor pig-heart procurement. Size matching especially of the great vessels is crucial for xenotransplantation.

Central Picture Legend
Preparation of donor pig heart for xenotransplantation

Abstract
The availability of genetically modified pigs and a more thorough understanding of immunosuppression has reinvigorated interest in cardiac xenotransplantation. Despite the anatomical similarities between human and porcine hearts, certain subtle differences exist that deserve deliberation. Herein, we describe our experience with donor pig-heart procurement, as well as anatomical considerations and also detail the surgical technique for implantation in the human recipient.
Keywords: porcine heart, procurement, cardiac xenotransplantation, genetic modification, immunosuppression.

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Introduction

Due to a perennial shortage of donor hearts, recent research efforts into suitable alternatives has focused on pigs as an ideal species for unlimited supply of organs [1]. Concurrently, there has been major advancements in the ability to modify the pig genome, specifically the α1,3-galactosyltransferase gene knockout (GTKO), which has led to renewed interest in the potential clinical application of xenotransplantation [1,2]. As we approach the potential for a clinical trial in heart xenotransplantation, there are significant anatomical differences between porcine and human hearts which need thorough understanding by the transplant teams. Here, we describe our surgical technique of donor heart procurement in genetically modified pigs, as well as the alterations required to perform recipient orthotopic heart xenotransplant. As a research purpose, the, orthotopic heart xenotransplantation was performed in two brain-dead patients, who were observed for sixty-six hours postoperatively, utilizing standard medications and without additional mechanical circulatory support [3]. Written informed consent was obtained, and the decedent were transferred to the intensive care unit (ICU) at the New York University (NYU) Langone Hospital for initial assessment and stabilization. The NYU Research on Decedents Oversight Committee reviewed our xeno-heart transplant protocol and provided oversight [4].

Anatomical differences between human and porcine heart (Fig 1)

The porcine heart has a classic ‘Valentine heart’ shape in contrast to the human heart, which is trapezoidal in silhouette with a markedly eccentric apex due to the pig’s orthograde posture. The
superior and inferior caval veins open into the atrium at right angles to one another, whereas in human hearts the orifices are directly in line (Figure 1) [5]. Furthermore, the morphological right atrium of the pig has tubular shaped appendage, as seen in the left atrium in humans. In general, both the right and left atria are more prominent in the pig with thicker muscle and this makes enlarging the atria easier for appropriate size-matching to the recipient during biatrial anastomosis. A prominent left azygous vein enters on the left side of the pig heart and drains via the coronary sinus (Figure 2) [5].

The porcine left atrium receives only two pulmonary veins, whereas four orifices are generally observed in human hearts. With respect to the coronary circulation, the porcine heart is characterized by right-coronary dominance (origin of the posterior descending artery) (Figure 3.a, b, c) [6]. The anterior displacement of the porcine aortic trunk compared with that of humans is significant. Additionally, the pulmonary trunk is more at right angles with the inlet component of the right ventricle, reflecting the upright stance. In pigs, the orientation of pulmonary valve is more directly aligned with the orientation of the inlet component, an adaptation to the unguligrade stance of the pig. Consequently, the aorta and pulmonary artery are somewhat rotated in orientation and have to be taken care of during xenotransplant procedure.

**Difference between conduction system of human and porcine heart**

In the pig heart, the sinoatrial (SA) node lies at the right side of the terminal crest, at the junction of the cranial vena cava and the right atrial appendage; furthermore, the SA node is relatively lower on the septum than in humans. The SA node appears to be rectangular in its longitudinal direction but has a flattened appearance perpendicularly (Figure 4) [7]. The atroventricular node resides on the right side of the ventricular septum, an anatomic position similar to that of human hearts, but is more densely innervated (Figure 5) [8].
One of the most prominent features in the right ventricle of the porcine heart is the trabecula septomarginalis (TSM), formerly known as moderator band. This muscular strand connects the septal wall of the right ventricle to its free wall and carries Purkinje fibers from the right atrioventricular bundle across the right ventricle’s lumen. Compared with human hearts, the trabecula septomarginalis of swine is more prominent and situated more proximal relative to the base of the heart explaining the faster activation of right ventricle in pig heart. (Figure 6).

Consequently, there is a variation of the Purkinje fiber network in porcine hearts, which results in differences in ventricular conductivity and contractility [8]. The shorter PR interval and different activation sequence of its ventricles, make the swine heart easily excitable and markedly susceptible to ventricular fibrillation. This variability could account for frequent rhythm disturbances observed after xenotransplantation.

**Surgical technique of pig donor heart procurement**

Our technique of pig heart procurement resembles that of standard human donor heart procurement, with certain important modifications [9]. Essentially, in human heart procurement the SVC is ligated, the inter-atrial groove is vented and the anterior wall of the IVC is transected. Once the entire cardioplegia is delivered, the IVC posterior wall transection is completed. The left atrium is then excised ensuring adequate cuff for suturing the anastomosis. The Aorta is transected at the level of the innominate artery and the pulmonary artery at the level of bifurcation. Finally, the SVC is transected confirming its separation from the right pulmonary artery and the entire heart is removed. Pigs to be utilized for xenotransplant had 10 gene modifications and were placed in barrier containment in a sterile facility where the procurement was performed. The donor pig was brought to the operating suite and placed under anesthesia by trained veterinarians. Familiarity with anatomy of airway of pigs is of utmost importance; as
swine have thick tongues, long, narrow oropharyngeal spaces and elongated soft palate which can obscure or hide the epiglottis and thereby complicate endotracheal intubation [10, 11]. Pigs are also prone to laryngospasm, especially if lightly anesthetized. Prior to surgical incision, a femoral arterial line was placed for continuous hemodynamic monitoring. The manubrium in pigs is thicker than in humans. With the innominate vein in its close proximity posteriorly careful blunt dissection in the superior aspect of manubrium is required to avoid torrential venous bleeding which can obscure the surgical field. Median sternotomy was performed using a Lebsche knife. A pericardial well was created, and care taken not to ‘pull-up’ the pericardial sutures, given increased susceptibility of pig hearts to ventricular fibrillation and/or hypotension. Every effort should be made to minimize handling the donor heart. In pigs, the aorta and pulmonary artery are rotated as compared to their positions relative to the human heart. Additionally, the proximal aorta is short and narrow, thereby making the standard technique of aortic cross-clamp challenging (Figure 7, 8).

In our modified technique, the aorto-pulmonary window was carefully dissected, separating the two great vessels. Using a right-angle clamp, an umbilical tape was passed between the aorta and pulmonary artery. Next, the clamp was passed behind the innominate artery from the left side, and the right-end of the umbilical tape was pulled from behind the innominate artery thereby encircling the proximal aortic arch. The two ends are then passed through a tourniquet, allowing the aortic arch to be ‘clamped’ just beyond the innominate artery. The inferior vena cava (IVC) is relatively long in pigs and can be easily encircled above the diaphragm. The standard dose of IV heparin (30,000 units) was administered. In order to preserve adequate length of the ascending aorta, an 18-Fr cardioplegia catheter was inserted in the proximal innominate artery. Distal control of the innominate artery was obtained. The innominate artery was noted to be
small and fragile in the genetically modified pigs. When present, the left azygous vein is small and drains directly into the coronary sinus near the left atrial appendage. Care was taken to identify the opening of the left azygous vein close to the appendage and ligate it using fine silk suture. Once the surgical team was prepared to cross-clamp, the innominate artery was tied off distal to the cardioplegia catheter. The SVC was ligated close to the innominate vein and IVC was divided below the diaphragm, preserving as much length as possible. The left atrium was vented by incising the right pulmonary vein as it entered the heart. The aortic cross-clamp was applied by bringing the tourniquet down around the aortic arch and heart preserved by delivering two liters of cold preservation solution (UW® Solution; Organ Recovery Systems, Inc.; Itasca, IL), and the mediastinum packed with ice slush. The aortic root pressure and left ventricular distension was monitored manually. Donor cardiectomy was performed in sequential fashion, beginning with incising the IVC, left atrial cuff then the ascending aorta proximal to the clamp, the pulmonary artery at the level of bifurcation, and finally the SVC below the ligated part. In the first case, the heart-lung block was excised enbloc, and the heart separated from the lungs at the back-table. However, in the second case, isolated heart procurement was performed. The heart was transported to the recipient center in cold static preservation (CSP) in UW solution. Cold ischemia time (CIT) was 3 hours 11 minutes in the first case and 2 hours 40 minutes in the second case. With isolated heart procurement, CIT was considerably reduced in the second case.

**Technical considerations of pig-to-human heart implantation**

For both of our procedures, in the decedent the sternotomy, dissection and cardiectomy were performed per routine as is done in adult orthotopic heart transplantation retaining the right atrial cuff (right atrium is opened from lateral IVC towards the right atrial appendage) for biatrial anastomosis. Briefly, in biatrial technique the left atrial anastomosis is initiated at the base of the
left atrial appendage adjacent to the left superior pulmonary vein. The two ends of the suture are run inferiorly and superiorly and eventually joined in the middle of interatrial septum. A long 3-0 Prolene suture is then used and the suture ends are carried both inferiorly and superiorly to first complete the septal anastomosis, and then they are joined at the lateral wall of the septum. The PA and aortic anastomosis are then performed.

There are several important technical aspects to be considered for recipient pig-to-human heart implantation. First, there is inherent size mismatch between the human recipient in chronic heart failure and the standard donor pig heart. Cardiomegaly seen in advanced heart failure patients leads to asymmetric chest expansion and significant enlargement of all chambers and major vessels. With human donor hearts, the bicaval technique has shown to be superior to biatrial in both early and late outcomes [12]. However, at least in our first two procedures, the anatomic mismatch between the recipient and donor pig heart necessitated the classic biatrial anastomosis. Given the anatomical differences and the relative orientations of the vena cavae in the pig heart to that of humans, we thought it would be technically easier and less time consuming to perform a biatrial rather than a bicaval anastomosis. Additionally, the great vessels of the pig heart can have significant size mismatch relative to human aorta and pulmonary artery (PA). This can be addressed by simple patch augmentation so as to widen the anastomosis. In the first decedent xenoheart procedure, pericardial patch augmentation was necessary on the anterior wall of the pulmonary artery and the aorta. In the second decedent, it was only necessary for the aorta given the better size matching. It is our understanding that donor pig heart preoperative imaging using a 3D echocardiogram or CT/MR angiogram will be absolutely essential in planning the xenoheart procedure. As our ability to evaluate the size of the pig donor heart and great vessels improves and we have access to a larger inventory of genetically modified pigs,
some of the above alterations may not be necessary. Cardiac xenografts exhibit growth and
diastolic heart failure. Hence, xenografts with growth hormone receptor (GHR) knockout are
utilized which show reduced post-transplantation hypertrophy of the heart, thereby eliminating
the need for medications such as temsirolimus and after-load reducing agents in the recipient.
The long-term impact of this GHR knockout is not known yet [13]. Enhanced preoperative
imaging will remain the cornerstone to achieve better size matching of donor pigs to human
recipients.

Conclusion
Recent advances in genetic modifications of porcine hearts have ushered in a new era in cardiac
xenotransplantation. Detailed understanding of the anatomy and conduction pathway of porcine
hearts is essential to mastering donor procurement and implantation in humans. Better size
matching between the donor heart and recipient may negate some of the modifications that we
had to perform to augment the diameter of the great vessels, however, at present the data on the
relative size of the pig heart and great vessels is lacking. It is possible that in the future, with
larger availability of pigs, better anatomical characterization will be necessary prior to heart
xenotransplantation. One such approach may involve better characterization of size by 3D
echocardiography or CT/MR angiogram.

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Figure Legends

Figure 1: Dorsal view of the heart showing the relationship of the superior vena cava (SVC) and the inferior vena cava (forceps within its lumen) opening into the right atrium at right angles.

Figure 2: Dorsal view of the heart showing large azygous vein on the left side draining into the coronary sinus.

Figure 3: a) anterior aspect of pig heart with a “valentine” shape, blunt LV apex and large triangular left auricle. b) dorsal surface of the heart showing the LV, RV and the left auricle and c) pulmonary veins with the forceps passing through it, opening into the left atrium.

Figure 4: Position of the sinoatrial (SA) node at the junction of superior vena cava and right atrial appendage

Figure 5: The right atrial (RA) cavity opened and showing the coronary sinus orifice, the right atrial appendage (RAA) with thick pectinate muscle, septal leaflet of tricuspid valve (STL) and the location of the membranous septum (black arrow).

Figure 6: The RV (right ventricle) cavity opened up showing the moderator band (MB), originating higher on the septal wall and attached to the anterior papillary muscle (APM). The trabeculae prominent and coarse and towards the apex (black arrows).

Figure 7: Preparation for donor pig procurement showing short and narrow proximal aorta with cardioplegia needle in innominate artery, umbilical tape around proximal arch, and inferior vena cava (blue arrow) looped with umbilical tape.

Figure 8: Illustration showing the donor pig heart with the great vessels and cardioplegia catheter in distal ascending aorta.