Aus der
Herzchirurgischen Klinik und Poliklinik
der Ludwig-Maximilians-Universität München
Direktor: Prof. Dr. med. Christian Hagl

Application of Telemetry System in Xenogeneic Cardiac Transplantation Using a Heterotopic Thoracic Model

Dissertation
zum Erwerb des Doktorgrades der Medizin
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität München

vorgelegt von
Jianping Fan
aus Huanggang, V.R. China
2013

Mit Genehmigung der Medizinischen Fakultät

der Universität München

Berichterstatter: Prof. Dr. med. Paolo Brenner

Mitberichterstatter: Prof. Dr. med. Christian Kupatt

Priv. Doz. Dr. med. Sieglinde Kofler

Mitbetreuung durch den
promovierten Mitarbeiter: Dr. med. Jan-Michael Abicht

Dekan: Prof. Dr. med. Dr. h. c. M. Reiser, FACR, FRCR

Tag der mündlichen Prüfung: 18. 07. 2013

CONTENTS

Abbreviations

ACR..... Acute cellular rejection
AHXR ..... Acute humoral xenograft rejection
alloTx..... allotransplantation
APA ..... Anti-pig antibody
ATG..... Antithymocyte globulin
aPTT ..... activated partial thromboplastin time
AVR..... Acute vascular rejection
AUC..... Area Under Curve
α-Gal Galactose-α-1, 3-galactose
α-Gal KO ..... Galactose-α-1, 3-galactose knockout
α1, 3GalT-KO ..... α1, 3-galactosyl transferase-knockout or
CFD..... Complement fixation test diluent
cTnI ..... Cardiac troponin I
CXTx ..... Cardiac xenotransplantation
C1..... Complement 1
DXR..... Delayed xenograft rejection
EA ..... Elevated Anti-Pig antibody serum level
ECG ..... Electrocardiography
EDTA..... Ethylenediaminetetraacetic acid
e.g. ..... For example
EMB ..... Endomyocardial biopsy
ET..... Elevated troponin serum level
Fc ..... Fragment, crystallisable
FDA..... Food and Drug Administration
..... of the United States
FXa..... Factor Xa
GM-CSF ..... Granulocyte-macrophage colony
..... stimulating factor
GPIb..... Platelet glycoprotein Ib
HAR..... Hyperacute rejection
hCD46 ..... Human CD46
hCRP..... Human complement-regulatory proteins

HR ..... Heart rate
HTCXTx ..... Heterotopic thoracic cardiac
..... xenogeneic transplantation
hTM ..... Human Thrombomodulin
HTx ..... Heart transplantation
IA ..... Immunoadsorption
IgA ..... Immunoglobulin A
IgG ..... Immunoglobulin G
IgM ..... Immunoglobulin M
IL ..... Interleukin
ISHLT ..... The International Society for Heart and
..... Lung Transplantation
LFA-1 ..... Lymphocyte function-associated antigen-1
LV ..... Left ventricle
LVP ..... Left ventricular pressure
LVPSP ..... Left ventricular peak systolic pressure
LVEDP ..... Left ventricular end diastolic pressure
Max ..... Maximum
Min ..... Minimum
MMF ..... Mycophenolate mofetil
NF ..... Nuclear factor
NK cell ..... Natural killer cell
POD ..... Postoperative day
PRBC ..... Purified red blood cell
QRSA ..... Amplitude of QRS complex
QTc ..... Corrected QT Interval
TBM ..... Thrombomodulin
TNF ..... Tumor necrosis factor
UT ..... Unelevated troponin serum level
UA ..... Unelevated Anti-Pig antibody serum level
vWF ..... Von Willebrand factor
XNA ..... Xenogeneic natural antibody

IU ..... International unit
µl ..... microliter
ml ..... milliliter
ng ..... nanogram
µg ..... microgram
mg ..... milligram
kg ..... kilogram
mV ..... millivoltage
ms ..... millisecond
s ..... second
min ..... minute
hr ..... hour
d ..... day
Hz ..... Hertz

Ca^{2+} ..... calcium ion

Mg^{2+} ..... magnesium ion

K^{+} ..... potassium ion

1 Introduction

Though the increasing use of ventricular assist devices has contributed to a declining mortality of patients with end-stage heart failure in recent years, the concomitant life-threatening complications such as thromboembolism and infection limit the widespread clinical application of this category of mechanical devices in sustaining lives of patients with terminal heart failure. So far, heart allotransplantation (alloTx) has remained the definitive therapy for end-stage heart failure despite its limitations for the patient, such as a long waiting time and long period of hospitalization before a suitable human donor is available. However, despite concerted, persistent societal efforts to increase the availability of organs from cadaveric donors, we are still faced with increasing discrepancy between the availability of donated human organs and the demand for transplantation. Therefore, the need for a new source of organs including hearts for clinical transplantation has been increasing for decades and xenotransplantation is widely considered to open a new pathway to resolve this issue.

1.1 History of xenotransplantation

Xenotransplantation refers to any procedure that involves the transplantation, implantation, or infusion into a human recipient of either living cells, tissues, or organs from a non-human animal source or human body fluids, cells, tissues, or organs that have had ex vivo contact with living non-human animal cells, tissues or organs.

The history of xenotransplantation dates back to 1682 when a Russian physician reportedly repaired the skull of a wounded nobleman using a piece of bone from a dog [1]. Blood transfusions from various animals to humans were attempted as early as the seventeenth century despite being associated with major complications and even death [2]. It was not until early in the twentieth century that scientists began to attempt to transplant tissues across the species barrier. In 1905, a French surgeon transplanted slices of rabbit kidney into a child with renal failure [1] followed by other doctors who attempted transplantation of organs from the pig, goat, lamb and non-human primate into patients. It is no surprise that all of the attempts

failed due to a lack of the fundamental knowledge of the immunological mechanism of organ rejection. Consequently, scientific interest in the transplantation of tissues or organs from animal to human waned.

In the 1960s a revival of xenotransplantation was witnessed in the world, pioneered by Keith Reemtsma who transplanted chimpanzee or baboon kidneys into thirteen patients with terminal renal failure when human organs were not available. One recipient survived for nine months but the others died within days. In 1964, James Hardy attempted the transplantation of a chimpanzee heart and other cardiac attempts of such kind followed [3]. All of these attempts were ultimately unsuccessful with unacceptable rates of organ failure and early mortality. Thereafter, this revival eventually died as well.

Recent advances in the understanding of organ rejection and development of new immunosuppressive drugs again rekindled interest in xenotransplantation. The best known clinical cardiac xenotransplantation was that performed by Leonard Bailey, who transplanted a baboon heart into an infant girl, known as Baby Fae, in 1983. The surgical procedure in Baby Fae was technically successful, but the graft underwent acute rejection and the patient died 20 days later [3].

1.2 Immunological barriers to xenotransplantation

1.2.1 Hyperacute rejection

Pigs are thought to be the best source animal species for clinical xenotransplantation. The selection of pigs as the preferable donor species is based on its ready availability, the physiologic and organ size compatibility, and the fact that use of non-human primate species would pose unexpected ethical, infectious, and logistical issues [4-6]. Moreover, in 1999, there was a moratorium on the use of non-human primates as organ donors imposed by FDA (Food and Drug Administration of United States) citing the risk of cross-species infection.

Transplantation of an unmodified pig heart into a non-immunosuppressed human or non-human primate results inevitably in destruction of the graft within minutes or hours, which is known as a process termed hyperacute rejection (HAR). In the event of HAR, the binding of pig antigens to human or primate natural

(preformed) antibodies initiates activation of the complement and coagulation systems, leading to a constellation of events characterized by intravascular thrombosis, endothelial injury, interstitial hemorrhage and edema, and cellular infiltration in the tissue [7–9].

The main target for human preformed antibodies is galactose-α-1,3-galactose (α-Gal) [10,11], an oligosaccharide that is not present in human beings, apes, and Old World monkeys [12]. This carbohydrate epitope is synthesized by the enzyme, α-1,3-galactosltransferase, which glycosylates N-acetyllactosamine of various glycoproteins on the surface of many porcine cells, among which are vascular endothelial cells [13–15].

HAR is primarily mediated by preformed xenogeneic natural antibody (XNA) against the α-Gal epitope, most of which is immunoglobulin M (IgM). It is reported that more than 80% of the total amount of XNAs in human serum is directed against the α-Gal epitope and around 1% of peripheral B cells synthesize anti- α-Gal antibodies [16–18]. Among XNAs against α-Gal epitope IgM is thought to initiate HAR other than other immunoglobulin isotypes such as immunoglobulin G (IgG) and immunoglobulin A (IgA) [19–20]. Hyperacute rejection may not occur in xenotransplantation between closely-related species, a so called concordant transplantation (e.g. transplantation from non-human primate to human). As opposed to concordant transplantation, discordant transplantation represents another category of xenotransplantation accomplished between two distantly-related species (e.g. transplantation from pig to human, transplantation from pig to rat). Concordant species usually have a closer phylogenic relationship than discordant animals. In discordant TX, the recipient normally harbours preformed antibodies against surface antigens on the cells of the donor organ, which initiate vigorous HAR shortly after xenotransplantation. Nonetheless, some therapeutic strategies prove to be effective in preventing HAR such as removing natural antibody or neutralizing complement.

1.2.2 Acute humoral xenograft rejection

A delayed antibody-mediated rejection after xenotransplantation is called “acute humoral xenograft rejection” (AHXR , also termed delayed xenograft rejection (DXR) or acute vascular rejection (AVR)) [21]. In this case, elicited (IgM and IgG) antibodies recognize and bind to Gal and other non-Gal antigens on the vascular endothelium.

This leads to complement and subsequent vascular endothelial cell activation, followed by injuries caused by the complement and cellular components of the innate immune system.

Natural killer (NK) cells play a role in AHXR [22–24] as well as macrophages [25], but their exact implication remains unclear. AHXR may occur despite the administration of conventional immunosuppressive agents, and is particularly observed following a T-cell-dependent elicited antibody response.

1.2.3 Acute cellular rejection

Cellular immunity plays a significant role in the development of xenograft failure [26–30]. However, few studies of acute cellular rejection (ACR) in discordant xenotransplantation have been reported, possibly because T-cell activation normally results in a rapid elicited antibody response that leads to AHXR before significant T-cell infiltration develops in the xenograft. T-cell response contributes to the development of both ACR and AHXR through both direct and indirect pathways, but elicited antibody response often masks T-cell response and obscures the pathological findings of ACR in xenotransplantation. As a consequence, the role of T-cell immunity in xenotransplantation is often underestimated.

Even though the T-cell response is thought to be more vigorous in the xenograft than in the allograft [31–35], potent immunosuppressants can largely suppress ACR as well as T-cell-dependent AHXR. Therefore, acute cellular rejection is not typically seen under intense immunosuppressive protocol either [36–41].

1.2.4 Chronic rejection

Chronic rejection is a major cause of graft loss in allotransplantation and is also considered to occur in xenotransplantation setting. However, very little information is available in the context of pig-to-primate xenotransplant, possibly due to the fact that long-term survival of xenografts is not routinely achieved owing to the preceding occurrence of AHXR. The development of chronic vasculopathy, which is considered to be a characteristic of chronic cardiac xenograft rejection, is associated with both humoral and cellular rejection. It is characterized by intimal thickening, fibrin exudation, complement and immunoglobulin deposition and cellular infiltration, similar to those seen in long-surviving allografts [42].

The causative factors of chronic rejection remain poorly understood. It is assumed that both chronic immune and non-immune elements are involved in this process and that the former is associated with both cellular and humoral immunity.

1.2.5 Coagulation dysregulation

It is known that there are definite molecular incompatibilities between the coagulation systems of pig and human, which may contribute to coagulation dysregulation, leading to thrombotic microangiopathy and eventual failure of pig xenograft.

In a molecular analysis it is shown that pig thrombomodulin (TBM) is a poor cofactor for activation of human protein C in spite of its ability to bind human thrombin [31]. Pig TBM is estimated to possess only 1–10% of the capacity of human TBM in terms of generating activated protein C (APC) in the context of xenotransplantation [43–46].

The second pig–human molecular incompatibility has also been found to lie between von Willebrand factor (vWF) and platelet glycoprotein Ib (GPIb). Human platelets aggregate upon contact with pig vWF due to an aberrant interaction between the O-glycosylated A1 domain and human platelet GPIb, even in the absence of shear stress [47].

The third one is associated with tissue factor pathway inhibitor expressed by porcine endothelial cells, which does not effectively neutralize human factor Xa (FXa) [48, 49].

1.3 Strategies to overcome rejection in xenotransplantation

Xenograft rejection is mediated by mechanisms that differ from those involved in alloreactivity and which are inadequately controlled solely by conventional immunosuppressive agents. Therefore, in addition to the use of immunosuppressive pharmaceutical therapy, the development of specific strategies is also required to overcome rejection episodes in the setting of xenotransplantation. This includes modification of the host immunity or production of genetically engineered organs, such as utilization of complement inhibitors, depletion of natural antibodies, and deletion of specific xenoantigens or introduction of protective transgenes by genetic modification of donor animals.

1.3.1 Immunosuppressive pharmaceutical therapy

Immunosuppression can be achieved by depleting lymphocytes, diverting lymphocyte traffic, or blocking lymphocyte response pathways by a variety of immunosuppressive drugs, which include small-molecule drugs, depleting and non-depleting protein drugs, intravenous immunoglobulin and glucocorticoids. Small-molecule immunosuppressive agents include calcineurin-inhibitors (e.g. tacrolimus), target-of-rapamycin Inhibitors (e.g. sirolimus), inhibitors of nucleotide synthesis (e.g. mycophenolate mofetil), alkylating agent (e.g. cyclophosphamide) and azathioprine. Protein immunosuppressive drugs consist of polyclonal and monoclonal antibodies represented by polyclonal antithymocyte globulin, anti-CD3 monoclonal antibodies, anti-CD20 antibodies,anti–interleukin (IL)-2 receptor (anti-CD25) antibodies, anti-CD52 antibodies, anti–tumour necrosis factor (TNF) reagents and LFA-1 (lymphocyte function-associated antigen-1) inhibitors. The antibodies deplete circulating lymphocytes by direct cytotoxicity (both complement and cell-mediated) and block lymphocyte function by binding to cell surface molecules involved in the regulation of cell function.

1.3.2 Complement inhibitors

One effective approach to prevent hyperacute rejection is to administer complement inhibitory molecules. For instance, soluble complement receptor 1 [50] or cobra venom factor [50, 51] both inhibiting complement activity, have been used to obtain prolonged survival of the xenografts in xenogeneic transplantation models. Because of effective blockade of activation pathways, these factors are more efficient than complement 1 (C1) inhibitor [51]. As expected, their efficacies are inferior to those obtained in allotransplant.

1.3.3 Depletion of natural antibodies

Plasmapheresis represents one of the most common approaches to deplete XNAs in the periphery of the recipient. Plasmapheresis involves the replacement of recipient plasma by substitutes such as albumin. Thus, in addition to XNA, non-immunoglobulin plasma proteins such as complement and coagulation factors are also depleted by this approach. To avoid these drawbacks, an antibody-based immunoadsorption (immunoapheresis) has been proposed, which allows depletion of total plasma IgG, IgA and IgM through immunoaffinity columns [52].

Immunoadsorption offered some advantages in pig-to-baboon combination by lowering the anti-pig IgG, IgA and IgM antibody titters dramatically [53]. However, both plasmapheresis and immunopheresis methods are hampered by the removal of immunoglobulin non-specific for xenoantigens that leads to an increase in recipient vulnerability to opportunistic infections in the post-transplant period.

1.3.4 Genetically engineered pigs

As mentioned above, α-Gal epitope is the major target for human and non-human primate anti-pig antibodies and its binding to preformed XNAs mediates the hyperacute rejection. To disable the expression of α-Gal epitope on the surface of pig cells, deletion of α1,3-galactosyltransferase using a genetic engineering method was proposed. With the introduction of techniques of nuclear transfer and embryo transfer [54,55], knock-out of the gene for α1,3-galactosyltransferase in pigs was achieved, which proved to be one of the most significant advances that have been made in this field. Genetically modified αGalT-knockout (GTKO) pigs were first available for experimental studies in 2003 [56]. The first transplantation of organs from GTKO pigs into non-human primates was reported in 2005 [57–59], in which the incidence of hyperacute rejection of pig grafts was greatly reduced.

Although the availability of GTKO pigs has been a major step forward, there are well documented natural antibodies to non-Gal antigens in humans and non-human primates [60–62], which are associated with hyperacute rejection or AHXR [40, 63], but the nature of which remains uncertain. Recent study suggested the elicited non-Gal antibody response is directed to a limited number of non-Gal antigens, which comprise members of the heat shock and annexin protein families, porcine complement, and thromboregulatory proteins [64]. It is believed that substitution of the genes for these non-Gal targets could abrogate the elicited antibody response and maintain endothelial cell function so as to prolong graft survival.

The presence of a complement-regulatory protein on the surface of vascular endothelial cells in the pig largely protects the graft from hyperacute rejection. Expression of a human complement-regulatory protein (hCRP) by genetically modified pigs might be effective in protecting the pig cells from lysis by activated complement system. Actually, incidence of hyperacute rejection was brought down when organs from pigs expressing one or more human complement-regulatory

proteins, such as CD46, CD55, or CD59 were transplanted into non-human primates [65–69]. It was further reduced in transplanted organs from GTKO pigs that also express hCRPs (GTKO/hCRP pigs) [70].

1.4 Orthotopic and heterotopic cardiac xenotransplantation

The abdominal heterotopic cardiac xenotransplantation (CXTx) is an established model in the study of pig-to-non-human primate cardiac xenotransplantation, in which xenograft is perfused and beating but does not contribute to circulation. Specifically, the left ventricular cavity is not or only partially perfused via an atrial septum defect while coronary sinus blood is drained into the right ventricle. Thus, the heterotopic abdominal cardiac xenotransplantation is a non-working model especially with regard to the left ventricle. So far a median heterotopic cardiac xenograft survival of 3 months has been achieved [71], with individual survival in excess of 8 months [72]. If these results can be replicated with life-supporting orthotopic transplantation the clinical application might be justified in the future. However, due to inherent problems of this orthotopic model predominantly in the early peri- and postoperative period a survival of more than 3 months has not been achieved (the survival ranges from 1 to 57 days [64]). This is one requirement of the ISHLT (The International Society for Heart and Lung Transplantation) Xenotransplantation Advisory Committee guidelines for initiation of a clinical trial [73].

In our pig-to-primate cardiac xenotransplantation project we demonstrated the feasibility of a heterotopic thoracic cardiac xenotransplantation technique (HTCXTx), which represents a life supporting model in which both the donor and recipient heart contribute to circulation. Good porcine cardiac graft function was maintained for up to 50 days using clinically applicable immunosuppression [74]. Thereby, HTCXTx is a possible alternative to the orthotopic and abdominal heterotopic xenotransplantation models, which combines the advantages of both models.

1.5 Application of telemetry system in cardiac xenotransplantation

Evaluation of cardiac xenograft function in a pig-to-baboon xenotransplant model is difficult and time consuming, which has been traditionally accomplished by means of palpation, ultrasound and biopsy. However, those methods require frequent

application of anesthesia to the baboon, increasing the risk of complications such as bleeding, infection, and graft injury, leading to increased morbidity and possibly mortality rate and compromising the experiment sometimes.

In recent years, telemetry has been widely applied in the life science research involving a wide variety of animal models, optimizing experiment conditions and facilitating data collection. The telemetry system enables the monitoring of animals while they move freely within their cages. Technically, a miniature transmitter implanted in each animal is capable of measuring one or more parameters (e.g. blood pressure, temperature, heart rate, ECG) with the data transmitted to a nearby receiver via radio frequency signals. The data may be collected and processed readily using the corresponding data acquisition system.

The monitoring of heterotopic transplantation by telemetry has been described [75–78]. This system provides reliable measurement of graft function and advanced clues in case of infection, rejection, or loss of graft function. Importantly, this system also obviates repeated anesthesia of recipient baboons to evaluate the heart function required by traditional means, rendering measurements free from the effects of anesthesia. The ability to evaluate several parameters retrospectively is an added advantage of this system.

Furthermore, this implantable system prevented manipulation by the primate recipients, which usually do not tolerate external leads and monitoring devices well unless the animals are heavily sedated or restrained. The technique provides more humane treatment of the recipients compared with traditional means [75].

In this study, we sought to assess the use of continuous telemetry as a non-invasive method to monitor cardiac xenograft function in pig-to-non-human primate heterotopic thoracic cardiac xenotransplantation. By comparing the changes of telemetric signals with biochemical, immunological evidence obtained during the posttransplant period, we aimed to validate the role of this technique in detecting the onset of myocardial damage or acute immune response.

2 Materials and Methods

2.1 Animals

Care of animals was taken in accordance with the Guide for the Care and Use of Laboratory Animals based on the German Law for the Care of Experimental Animals (German Legislation for the Welfare of Laboratory Animals, article 5, §7 - §9a, Project -70-11).

2.1.1 Donor animals – Pigs

In this study, one double- (α-Gal KO + hCD46) and six triple-transgenic (α-Gal KO + hCD46 + hTM) pigs (fig.2.1) were used as organ donors. The Institute of Molecular Animal Breeding and Biotechnology, Gene Centre, Ludwig Maximilian University, Munich, Germany and Revivicor Inc., Blacksburg, VA, USA were jointly responsible for breeding them.

Figure 2.1: Transgenic pigs before xenotransplantation.

After birth, the animals were examined by auscultation and echocardiography to ensure they are healthy as donors. The transgenesis was validated by ear biopsy and immunohistochemistry. At an age of 6-8 weeks, the animals, approximately weighing10-15 kg, were used for the experiment. With this age and body weight, the heart size of the donor pig matches the recipient baboon best.

2.1.2 Recipient animals – Baboons

The baboons (fig.2.2) were provided by the German Primate Center in Göttingen. Prior to delivery of the baboons, a broad variety of microbiological screening tests (e.g. Simian immunodeficiency virus, Simian T-Cell leukemia virus, Herpes papionis virus-2, Tuberculosis, Rabies) were performed.

Figure 2.2: Baboon housed in a cage preoperatively.

About six weeks before the transplant, the animals were taken to the Walter-Brendel-Centre, which was used as a host laboratory for future reference. Postoperatively, each of them was kept in a large mobile cage specially designed to facilitate therapeutic treatment during the postoperative period. Once their physical

condition was stable, they were brought back into the normal cages. The general information of the recipients and donors is present in table 2.1.

	Baboon Recipient					Pig Donors	
No.	Sex	Type	BG	Age	Weight	Weight	Transgenes
1	Male	P.anubis	AB	5 years	18kg	14kg	GalT-KO/hCD46
2	Male	P.anubis	B	6 years	24kg	16 kg	GalT-KO/hCD46/hTM
3	Male	P.anubis	AB	6 years	27kg	13 kg	GalT-KO/hCD46/hTM
4	Male	P.anubis	B	10 years	28kg	16 kg	GalT-KO/hCD46/hTM
5	Male	P.anubis	B	10 years	27kg	17 kg	GalT-KO/hCD46/hTM
6	Male	P.anubis	B	7 years	23kg	22 kg	GalT-KO/hCD46/hTM
7	Male	P.anubis	B	8 years	34kg	17 kg	GalT-KO/hCD46/hTM

Table 2.1: General information of recipient baboons and donor pigs.

2.2 Preoperative treatment

Central venous catheters were placed two days prior to transplantation (Arrow®, 14 G, Arrow International Inc, USA). Jackets and tethering systems (Lomir™, Lomir Biomedical Inc, Canada) were used to protect the infusion system from damage by the baboons.

Then, immunoadsorption was performed through the newly implanted catheters and repeated on the day of operation.

2.3 Technique of heterotopic thoracic xenogeneic heart transplantation

2.3.1 Explantation of donor heart

Anesthesia was initiated by intramuscular injection of 10-20 mg / kg ketamine hydrochloride (Ketavet®, Pharmacia GmbH, Berlin, Germany), 10 mg / kg azaperone

(Stresnil®, Janssen Animal Health, Neuss, Germany) and 0.025 mg / kg atropine sulphate (Atropine sulfate Braun®, B. Braun Melsungen AG, Melsungen, Germany). After endotracheal intubation, the animals were mechanically ventilated with oxygen-air mixture of 100% (Siemens Servo Ventilator 900C, Siemens-Elema system, AB, Solna, Sweden). Anesthesia was maintained using 7 mg / kg / h propofol (propofol 2%, Fresenius Kabi GmbH, Bad Homburg, Germany) and 0.05 mg / kg / h fentanyl dihydrogencitrate (Fentanyl-Janssen®, Janssen Pharmaceuticals Inc, USA).

After median sternotomy and systemic heparinization (500 IU / kg heparin (Heparin-Natrium-25000-ratiopharm®, ratiopharm GmbH, Ulm, Germany)), cardioplegic solution (50 ml / kg HTK Bretschneider solution (Custodiol®, Dr. Franz Köhler Chemie GmbH, Alsbach-Hähnlein, Germany)) was used to induce cardiac arrest. Before explantation of the heart, both venae cavae were ligated and then the ascending aorta and pulmonary trunk were separated as well as the pulmonary veins. Afterwards, the heart was harvested and stored in a sterile bag soaked in ice water before being weighed.

2.3.2 Heterotopic thoracic cardiac transplantation

2.3.2.1 Anesthesia

Anesthesia was initiated by an intravenous dose of 3 mg / kg ketamine hydrochloride and 0.15 mg / kg midazolam (Midazolam-ratiopharm®, Ratiopharm GmbH, Ulm, Germany) and maintained by intravenous administration of 0.05 mg / kg / h fentanyl dihydrogencitrate and 5 to 10 mg / kg / h propofol. Thereafter, the baboon was endotracheally intubated (5.0 to 6.5 Hi-Lo Lanz™, Mallinckrodt, Athlone, Ireland) and mechanically ventilated with oxygen-air mixture of 40%.

2.3.2.2 Surgical Technique

After median sternotomy, the heart was exposed and then the aorta, vena cava superior and inferior were canullated. Subsequently, cardiopulmonary bypass (Hilite 2800, Medos, Stolberg, Germany) was established following administration of 500 IU / kg heparin.

Anastomosis was performed between the left atria of both hearts and then the right atria. By means of an end-to-side anastomosis, the ascending aorta of the donor's heart was linked to the recipient's aorta. An end-to-side and side-to-side

anastomosis was also established to connect the two pulmonary trunks with a vascular prosthesis (Terumo Vascutek Gelweave, Ø 10 mm, Hamburg, Germany) (Fig. 2.3a, 2.3b, 2.3c) interposed.

During reperfusion of the hearts, the telemetry monitoring system (DSI Monitoring, Data Science International Inc, St. Paul, MN, USA) was also implanted (please refer to 2.7 Telemetry system for details of the surgical technique).

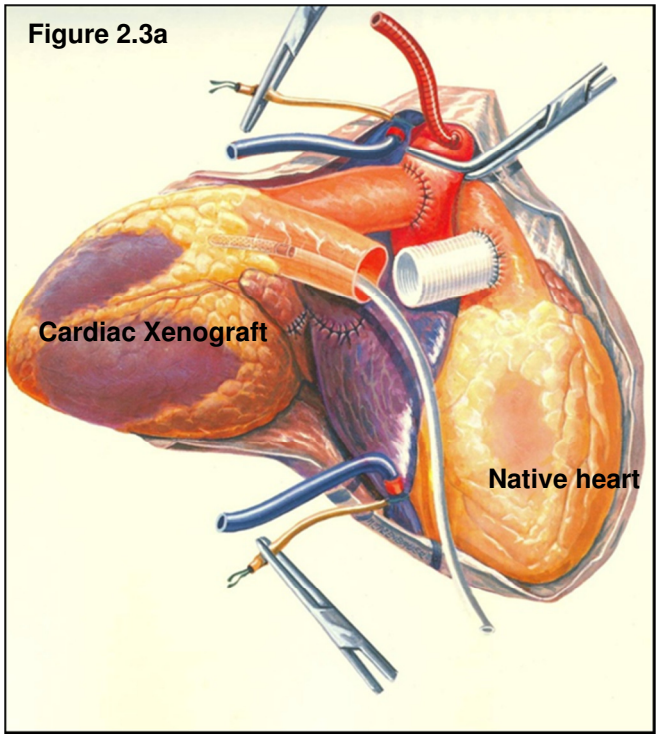
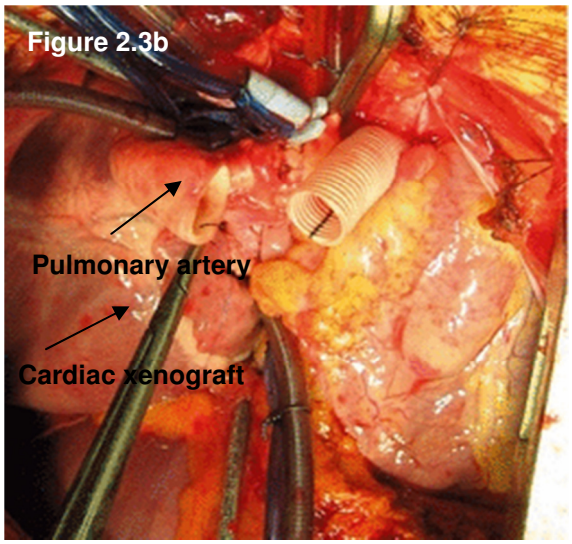


Figure 2.3a: Thoracic heterotopic cardiac transplantation (On the right is the native heart and the cardiac xenograft is on the left).



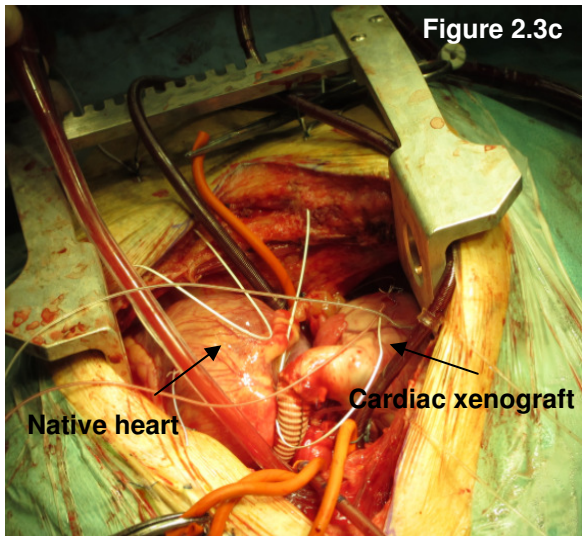


Figure 2.3b: Thoracic heterotopic cardiac transplantation (Operative findings during anastomosis of the pulmonary arteries). Figure 2.3c: Thoracic heterotopic heart transplantation (Illustration of the position of the xenograft in the thorax of the recipient).

2.4 Immunosuppressive therapy

2.4.1 Immunosuppressant application

To achieve a reduction of B-cells, T-cells and plasma cells and prevent secondary antibody production, we used a quadruple combination of rituximab, bortezomib, dexamethasone and cyclophosphamide, which had been effective in the treatment of multiple myeloma and was already used in clinic. The treatment was initiated about four weeks before operation.

The immunosuppressive regimen is outlined in table 2.2.

Drugs	**Dose**	**Timing**
Anti CD20	19 mg/kg	Preoperative days -28, -21, -14, -7, 0, +7 and +14
Bortezomib	1,3 mg/m^2	Preoperative days -28, -25,-21 and -18
Dexamethasone	2 mg/kg	3 cycles of 4 days following preoperative days -28, -20 and -13
Antithymocyte globulin (ATG)	1.5 mg/kg/d iv infusion	Preoperative days -2, -1 ,0 and postoperative days +1, +2
Tacrolimus	0.01 mg/kg/d iv infusion	Postoperative days
MMF	20 mg/kg/ hr iv infusion	Twice per day postoperatively
Methylprednisolone	10 mg/kg iv injection	Twice per day postoperatively, tapered off in 7 weeks

Table 2.2: Pharmaceutical regimen applied perioperatively.

2.4.2 Immunoadsorption

This treatment was performed on the preoperative second day as well as the day of operation.

Immunoadsorption (TheraSorb Life18, MACS, Bergisch Gladbach) was utilized to reduce antibodies and immune complexes in the blood of the baboon recipients to around 20%.

2.5 Non-immunosuppressive medication

2.5.1 Acetylsalicylic acid

To improve the coagulation system of recipient animals and thus to reduce the risk of pathological events such as embolism and infarct, baboons were given 50 - 75 mg acetylsalicylic acid intravenously a day from the 4th or 5th postoperative day onward (Aspirin®, Bayer Schering Pharma, Germany).

2.5.2 Heparin

Heparin was intravenously given to animals continuously by infusion pump to reduce the risk of thrombotic events post cardiac transplantation. The dose of heparin was adjusted to achieve aPTT (activated partial thromboplastin time) level of 35 to 45s.

2.5.3 Gastrointestinal symptomatic medications

To prevent gastrointestinal side effects caused by medication, the animals were given proton pump inhibitors (e.g. pantoprazol), H_2 blockers (e.g. ranitidine) or 5 -HT_3 blockers (e.g. ondansetron).

2.5.4 Antibiotics

A variety of broad-spectrum antibiotics (e.g. Tazobac, Cefuroxim, Meropenem, Ciprofloxacin) were applied to prevent or treat the postoperative infection. The selection and dosage of antibiotics were based on body weight and antibiotic sensitivity test.

2.5.5 Electrolyte supplementation

According to the results of the regular assay of serum electrolyte post operation, electrolytes such as Ca^{2+}, K^{+}, Mg^{2+} were selectively given to animals by syringe pump continuously.

2.5.6 Analgesia

Ketamine hydrochloride and metamizole sodium (Novaminsulfon-ratiopharm®, ratiopharm GmbH, Ulm, Germany) were administered for up to 10 days postoperatively. Dosages were adjusted to the condition of the animal.

2.6 Laboratory tests

Among a variety of laboratory tests which were not all involved in this study, troponin and anti-pig antibodies (APA) serum levels were determined on a daily basis. Troponin is analysed by the Institute of clinical chemistry of the University Hospital Grosshadern, University of Munich.

Anti-pig hemolytic assay was performed according to an established protocol detailed in the appendix.

2.7 Telemetry system

2.7.1 Setup

The telemetry equipment from Data Sciences International (DSI Inc, St. Paul, MN, USA), includes an implantable transmitter (Model TL11M3-D70-PCTP) (fig.2.4), a receiver (Model RMC1) (fig.2.5), a data processing device (Data Exchange Matrix) and an ambient pressure reference monitor (APR-1). The transmitter has two pressure catheters and two biopotential leads. The pressure catheter is a 14-cm fluid filled catheter with a terminal sensing region containing non-compressible fluid and a plug of biocompatible gel. This sensing region relays pressure waves to the DSI transmitter, which transmits both the pressure and biopotential signals to the receiver via radio frequency. The data processing system, which has a sampling frequency of 500 Hz, is programmed to acquire and process these signals into data that is subsequently relayed to the system computer in a real time manner. As a result, real time pressure waves are displayed on the computer monitor as well as ECG obtained in a similar way. Ambient pressure reference monitor ensures accurate pressure measurements in animals when using DSI transmitters, by providing dynamic corrections to the digital signals acquired by the system.

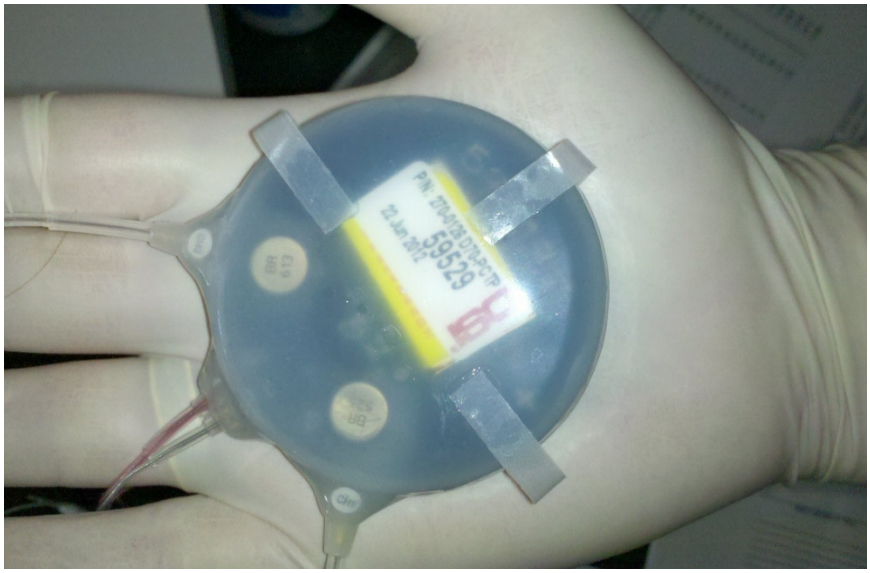


Figure 2.4: The transmitter of a telemetry system.

Figure 2.5: Receiver of the telemetry system in use.

2.7.2 Surgical implantation of telemetry system

One pressure catheter was inserted into the xenograft's left ventricle (fig.2.6) through the apex and secured using a 6-0 purse-string suture with pledgets. The other pressure catheter was implanted into the aorta. The two biopotential leads were sutured and fixed onto the ventricular epicardium for ECG monitoring.

The telemetry transmitter was placed in a subcutaneous pocket in the chest wall before the thoracic cavity was closed. Finally, the transmitter was externally activated by a magnet.

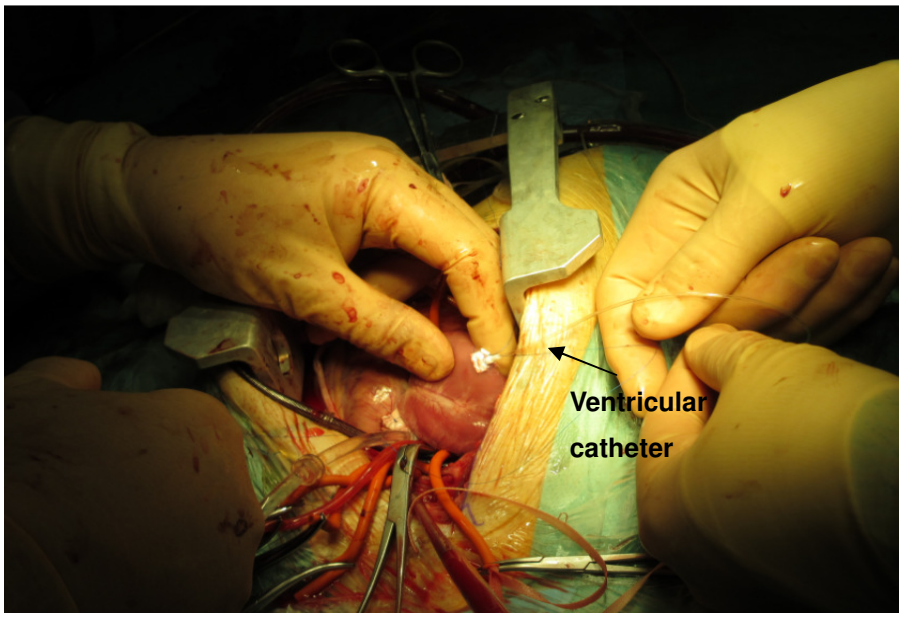


Figure 2.6: Implantation of the ventricular pressure catheter into cardiac graft.

2.7.3 Continuous telemetric monitoring

The telemetric data were acquired, processed and recorded continuously (fig.2.7). The following parameters were displayed online:

- Blood pressure waves of left ventricle of the graft
- Blood pressure waves of the aorta
- Epicardial ECG of the graft
- Systolic, diastolic and mean pressure of left ventricle of the graft
- Systolic and diastolic pressure of the aorta
- HR derived from pressure waves of left ventricle of the graft
- HR derived from ECG of the graft
- Temperature measured at the transmitter housing.

Two baboons could be monitored simultaneously if necessary.

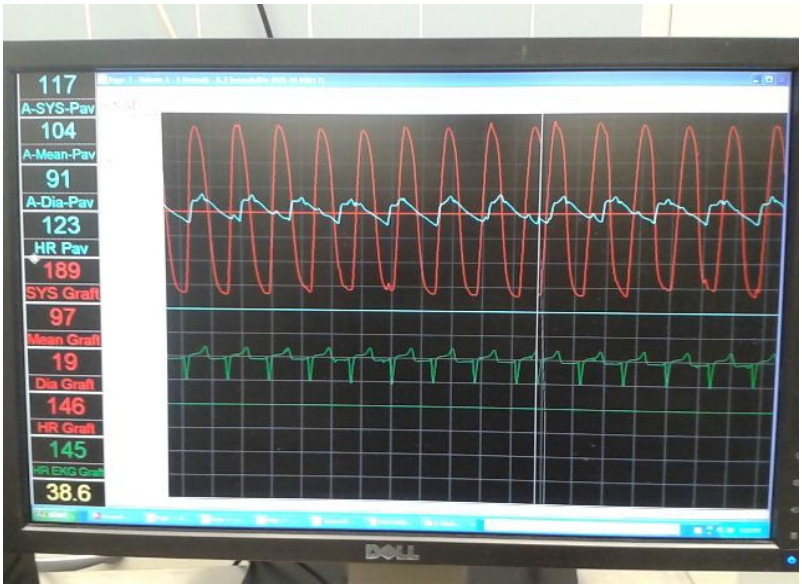


Figure 2.7: Monitor of the telemetry system displaying multiple telemetric parameters of a recipient baboon simultaneously.

Generally, this system was used to monitor cardiac hemodynamics and detect cardiac events, allowing timely initiation of therapy. Due to wireless signal transmission, no impairment was inflicted on the animal.

2.7.4 Derived parameters

Ponemah (DSI Ponemah Physiology Platform, Data Sciences International Inc, MN, USA) was used for data acquisition and analysis. These following parameters were derived from recorded data:

LVPSP: denotes the peak values of left ventricular pressure (figure 2.8).

LVEDP: represents the pressure in the left ventricles at the end of diastole. End of diastole was derived from the ventricular pressure waveform (figure 2.8).

HR: based on the R-R intervals of the ECG.

+dP/dtmax: defined as the maximal rate of rise of left ventricular pressure within one pulse (figure 2.8).

-dP/dtmax: defined as the maximal rate of decline of left ventricular pressure within one pulse (figure 2.8).

QRSA: defined as the amplitude between the lowest trough and highest peak of a QRS complex in ECG (figure 2.9).

Absolute Values of Deviation of S-T segment: deviation of S-T segment from baseline was measured 80ms after the endpoint of a QRS complex (figure 2.9).

Duration of QRS complex: defined as the time span between the start and end of a QRS complex (figure 2.9).

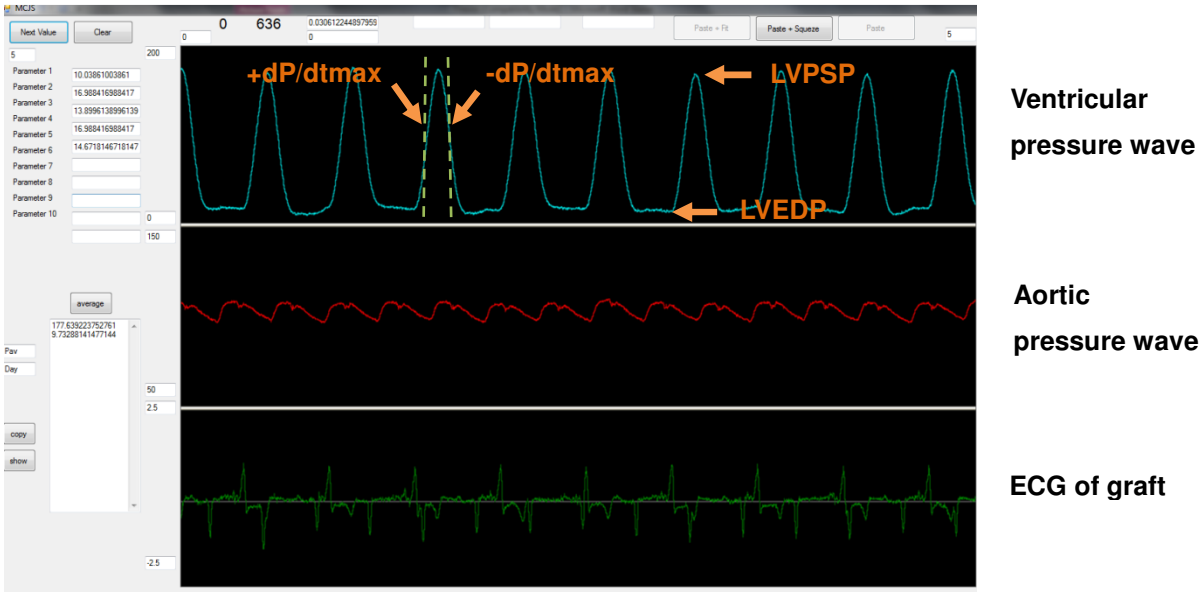


Figure 2.8: Measurement of hemodynamic and ECG variables.

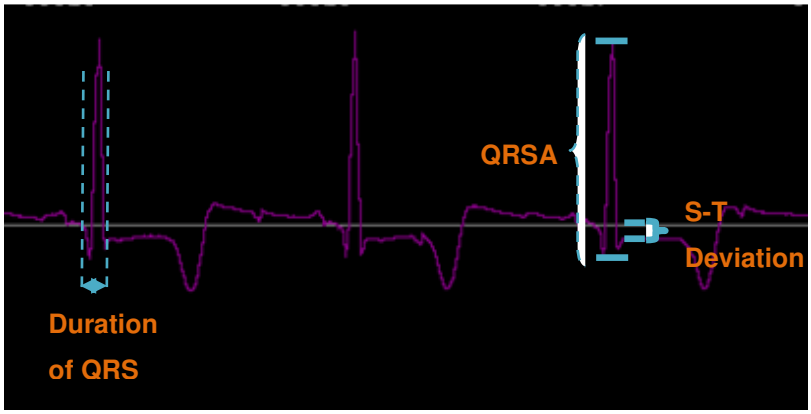


Figure 2.9: ECG of the graft.

2.7.5 Timepoints

Timepoints for data analysis were set daily at 6:00 in the morning, when these animals were not subject to manipulation or medication and artefacts caused by these possible factors were ruled out.

2.8 Group categorization

According to the postoperative troponin and APA serum level changes, the seven experiments were categorized as elevated troponin (ET) or unelevated troponin (UT) and as elevated (EA) or unelevated APA level (UA).

Elevation of troponin level was defined empirically as a rise in troponin serum concentration of more than 3ng/ml/24h. For each experiment the time point of elevation was defined by the highest increase between all observed days.

Elevation of APA was defined as an increase to over 1000 AUC. The time point of elevation was defined as the day on which the level of APA reached 1000 AUC. For technical reasons of the performed ELISA, the threshold of APA level was reduced to 100 AUC in baboon 3.

2.9 Endpoints of the study

Endpoints of the study are defined as follows:

A. Graft failure: Indicated by no bioelectrical signals in the ECG and no mobility of myocardium in TTE

B. Intolerable pain or stress of the animal: (evaluated daily, score sheet shown in Appendix B)

C. Death of the baboon

In case of condition A or B, the animal was euthanized. For euthanasia, the baboon was anesthetized by using 10 mg / kg ketamine hydrochloride and 0.5 mg / kg midazolam and an overdose of pentobarbital (80-160mg / kg Narcoren®, Merial, Halbergmoos, Germany) was given afterwards.

2.10 Statistical methods

The data were presented as Mean ± SD (standard deviation). The t-test and one-way analysis of variance (ANOVA) were applied for statistical analysis with $p<0.05$ considered significant. The statistical analysis and other representations were conducted by the software Sigmaplot 11.0 (SigmaPlot™, Systat Software Inc, USA).

3 Results

3.1 Basal analysis

In this study, the longest survival achieved among them amounted to 37 days with a median survival of 17 days. The endpoint events in this study consist of three endpoint A, three endpoint B and one endpoint C.

Using the telemetric system, we were able to acquire, process and record the experimental data continuously during the course of the study. However, due to technical failures, transmission of radio frequency signals between the transmitter and receiver were disrupted occasionally, resulting in loss of telemetric data for three timepoints in one baboon. Additionally, determination of APA and troponin are unavailable at some timepoints in four baboons because of failure to obtain serum samples from them. Based on the measurements of the investigated telemetric parameters available for individual baboon recipients during the post-transplant period, an overall descriptive analysis of all parameters was performed and summarized in table 3.1. Similarly, descriptive analysis for individual baboons was conducted and summarized separately in table 3.2 – 3.8.

Parameter	Max	Min	Median	Mean	Unit
LVPSP	304	12	167	164	mmHg
LVEDP	111	2	15	18	mmHg
QRSA	10.28	0.20	2.25	2.73	mV
Deviation of S-T Segment	0.92	0	0.17	0.21	mV
Duration of QRS	151	58	78	85	ms
Heart Rate	213	45	128	134	/min
Troponin	384.0	0.3	4.1	22.8	ng/mL
APA	13176	4	360	827	AUC
+dP/dtmax	4574	456	2612	2614	mmHg/s
-dP/dtmax	4729	244	2036	2124	mmHg/s

Table 3.1: An overall descriptive analysis of individual parameters.

These parameters were plotted against postoperative days and presented in figure 3.1 – 3.8. Generally, the visual observation of the changes of the investigated parameters revealed a high heterogeneity among the recipients and there seems to be no uniform pattern that visually fits their changes over time. The results for these recipients are briefly described individually as follows.

In baboon 1, elevation of both troponin and APA levels was recorded in the last postoperative week. As seen from figure. 3.1e, the levels of both troponin and APA stayed at a high level on POD 1 and declined sharply until POD 6 and POD 7 respectively. Afterwards, both of their levels maintained flat until they began to rise on POD 17 and POD 16. In contrast, LVPSP, +dP/dtmax, -dP/dtmax, HR and QRSA were shown to have been declining continuously from then on, until the end. As for LVEDP, duration of QRS complex and deviation of S-T segment their levels appeared to fluctuate modestly from the day of operation onward (due to technical failures, telemetric data of POD 9-11 were unable to be acquired) (figure 3.1a-d).

As for baboon 2, the level of APA began to rise on POD 11, while the level of troponin returned to a low level on POD 6 and stayed constant (figure 3.2e). From POD 14 onwards, LVPSP and +dP/dtmax increased, while QRSA appeared to decrease until the end. Concerning the changes of the other parameters, no clear trend lines were shown during the postoperative period (figure 3.2a-d).

Elevation of both APA and troponin was recorded postoperatively in baboon 3. Specifically, APA level began to begin to rise on POD 7 until POD 13 (The measurements of APA on POD 14-17 were unavailable), while troponin level started to increase on POD 16 (figure 3.3e). Coincidentally, downward trend lines were documented for the parameters LVPSP, +dP/dtmax, -dP/dtmax, HR and QRSA from POD 15 onwards. LVEDP, duration of QRS complex and deviation of S-T segment appeared to fluctuate during the entire postoperative period (figure 3.3a-d).

APA level of baboon 4 was observed to markedly rise from POD 9 onwards, while troponin level appeared unchanged during the same period (figure 3.4e). Apparently, LVEDP and ECG parameters maintained constant throughout the postoperative period (figure 3.4a, figure 3.4c, and figure 3.4d). Despite wide fluctuations in LVPSP, +dP/dtmax, -dP/dtmax and HR, there were no clear downward or upward trend lines demonstrated in the corresponding graphs (figure 3.4a-c).

In baboon 5, an upsurge of both APA and troponin levels was documented for the last few postoperative days, which started on POD 11 and POD 17 (figure 3.5e). LVPSP and HR appeared to decline continuously from POD15 onwards (figure 3.5a, figure 3.5c), while +dP/dtmax, -dP/dtmax and QRSA started to drop on POD 18. Regarding other parameters, no clear trends were detectable in their curve patterns except extremely higher LVEDP of the cardiac xenograft on POD 7, POD 8 and POD 9 (figure 3.5a-d).

Baboon 6 survived the longest in this group. Its APA level was suppressed at a considerably low level for the entire postoperative period (figure 3.6e). After the first week post operation, troponin level maintained low until a rise was recorded on the last two days. Simultaneously, there were decreases of LVPSP, +dP/dtmax, -dP/dtmax, QRSA and HR (figure 3.6a-c).

As for baboon 7, the measurements of APA and troponin were unavailable on POD 14 and POD 11 respectively. Levels of both APA and troponin maintained low for the last week of the postoperative period (figure 3.7e). HR, +dP/dtmax and -dP/dtmax dropped over the last two days, while LVPSP stayed at a high level. During the entire postoperative period, the other parameters appeared relatively unchanged (figure 3.7a-d).

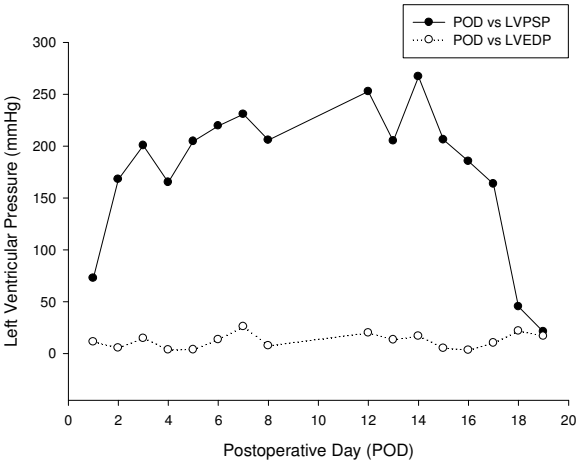


Figure 3.1a

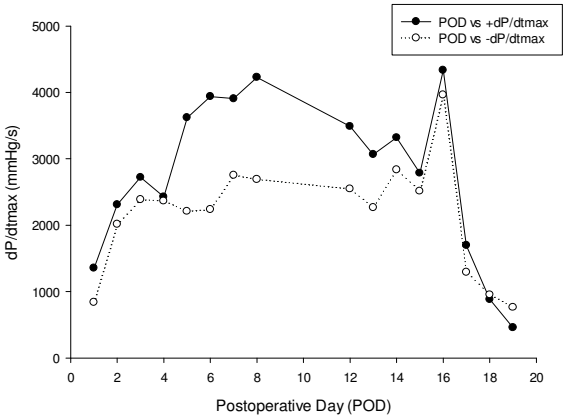


Figure 3.1b

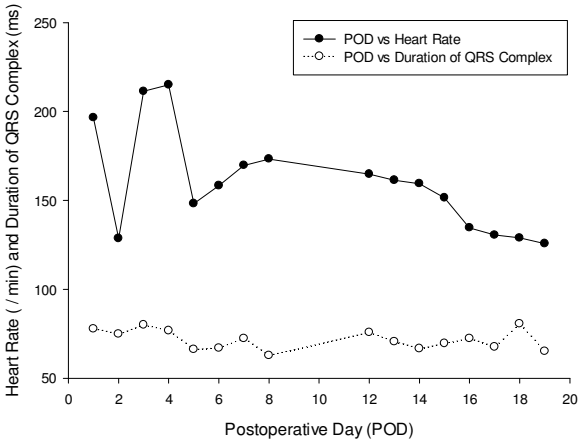


Figure 3.1c

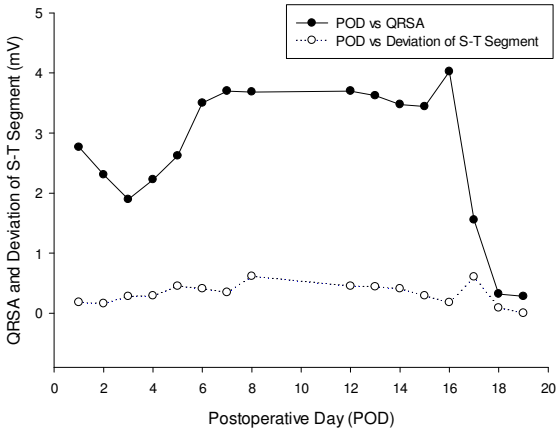


Figure 3.1d

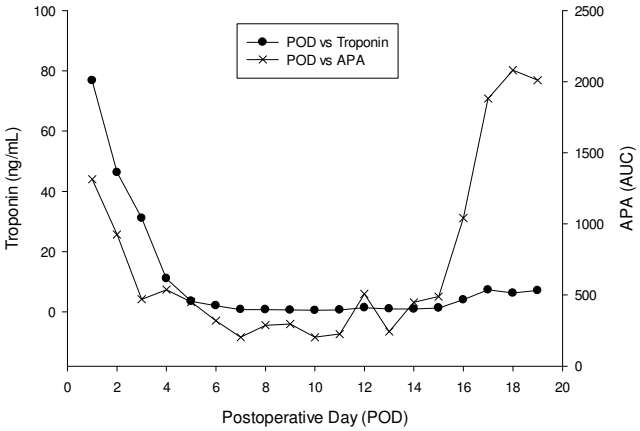


Figure 3.1e

Parameter	Max	Min	Median	Mean
LVPSP (mmHg)	267	21	203	176
LVEDP (mmHg)	26	3	12	12
+dP/dtmax (mmHg	4334	458	2925	2783
-dP/dtmax /s)	3962	763	2313	2163
Heart Rate (/min)	215	126	159	160
QRSA (mV)	4.03	0.28	3.10	2.70
Deviation of S -T Segment (mV)	0.62	0	0.32	0.33
Duration of QRS (ms)	81	63	72	72
Troponin (ng/mL)	76.8	0.5	1.8	10.3
APA (AUC)	2081	203	461	662

Table 3.2

Figure 3.1(a-e): Measurement of hemodynamic and ECG parameters and serum level of troponin and APA of baboon 1 during the postoperative period. Table 3.2: Descriptive analysis of individual parameters for baboon 1.

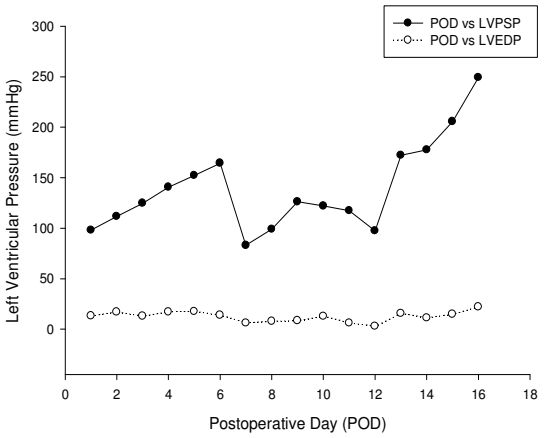


Figure 3.2a

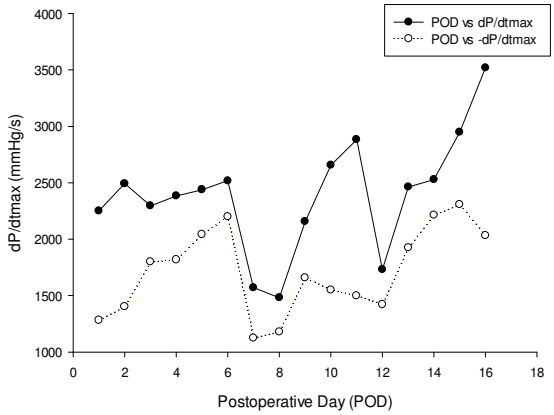


Figure 3.2b

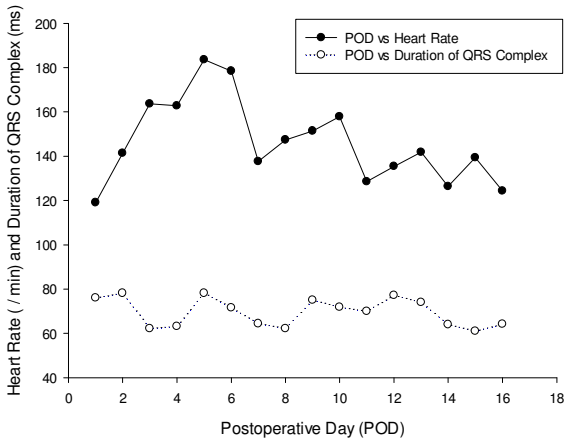


Figure 3.2c

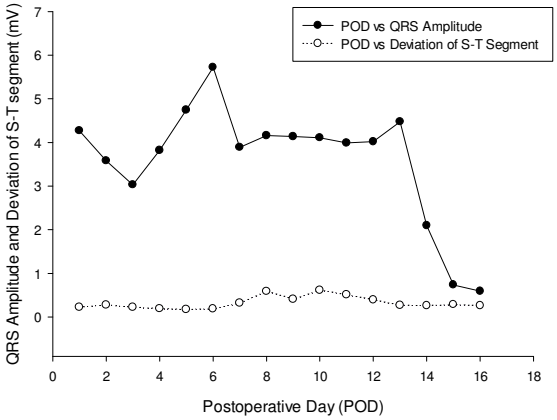


Figure 3.2d

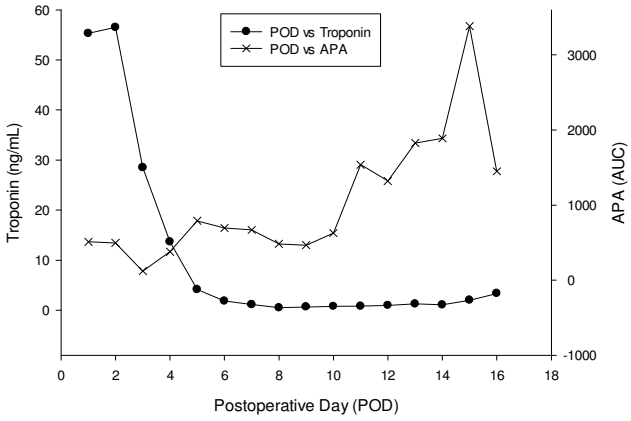


Figure 3.2e

Parameter	Max	Min	Median	Mean
LVPSP (mmHg)	249	83	126	140
LVEDP (mmHg)	22	3	13	13
+dP/dtmax (mmHg	3518	1482	2450	2395
-dP/dtmax /s)	2307	1126	1729	1716
Heart Rate (/min)	184	119	142	146
QRSA (mV)	5.72	0.59	4.00	3.59
Deviation of S -T Segment (mV)	0.62	0.18	0.28	0.33
Duration of QRS (ms)	78	61	71	70
Troponin (ng/mL)	56.5	0.5	1.6	10.8
APA (AUC)	3381	121	683	1040

Table 3.3

Figure 3.2(a-e): Measurement of hemodynamic and ECG parameters and serum level of troponin and APA of baboon 2 during the postoperative period. Table 3.3: Descriptive analysis of individual parameters for baboon 2.

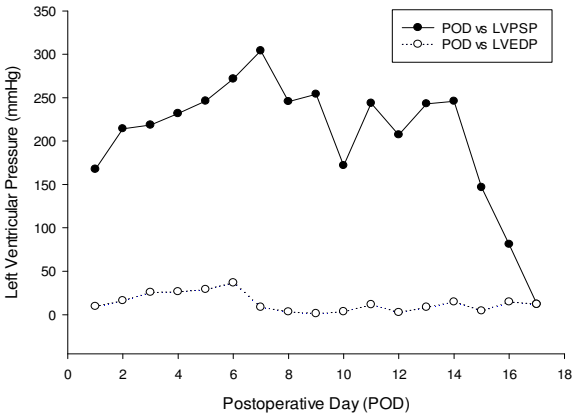


Figure 3.3a

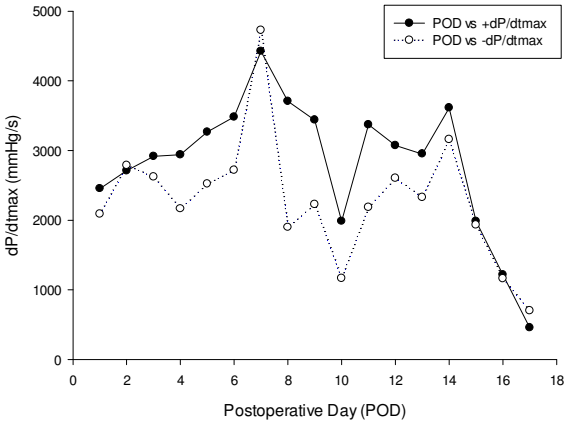


Figure 3.3b

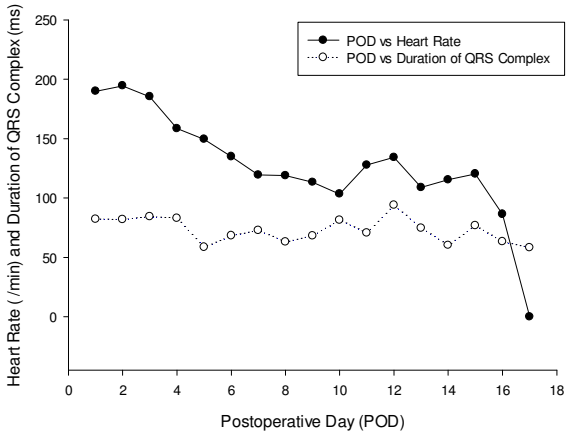


Figure 3.3c

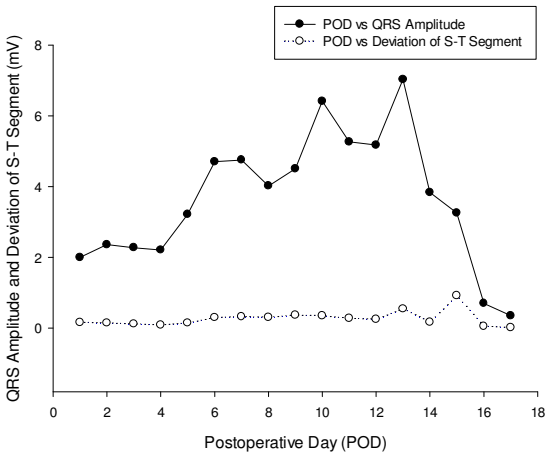


Figure 3.3d

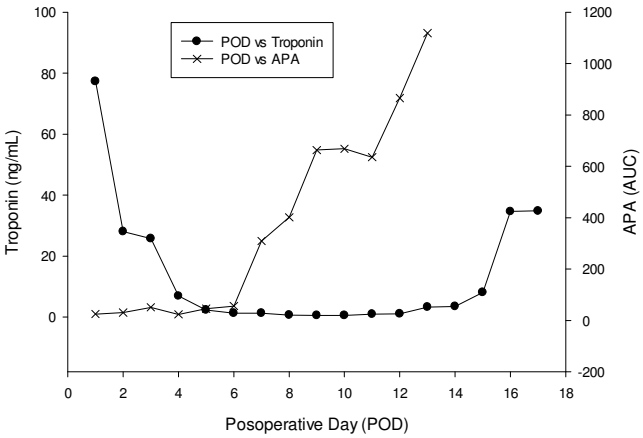


Figure 3.3e

Parameter	Max	Min	Median	Mean
LVPSP (mmHg)	304	12	225	195
LVEDP (mmHg)	39	5	12	13
+dP/dtmax (mmHg	4427	456	2952	2824
-dP/dtmax /s)	4729	700	2228	2294
Heart Rate (/min)	195	64	120	128
QRSA (mV)	7.03	0.50	3.55	3.45
Deviation of S -T Segment (mV)	0.92	0.02	0.25	0.27
Duration of QRS (ms)	94	42	72	69
Troponin (ng/mL)	77.3	0.5	3.5	13.7
APA (AUC)	1118	29	309	376

Table 3.4

Figure 3.3(a-e): Measurement of hemodynamic and ECG parameters and serum level of troponin and APA of baboon 3 during the postoperative period. Table 3.4: Descriptive analysis of individual parameters for baboon 3.

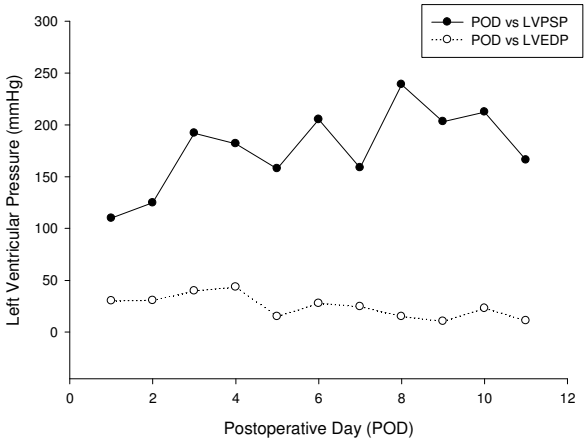


Figure 3.4a

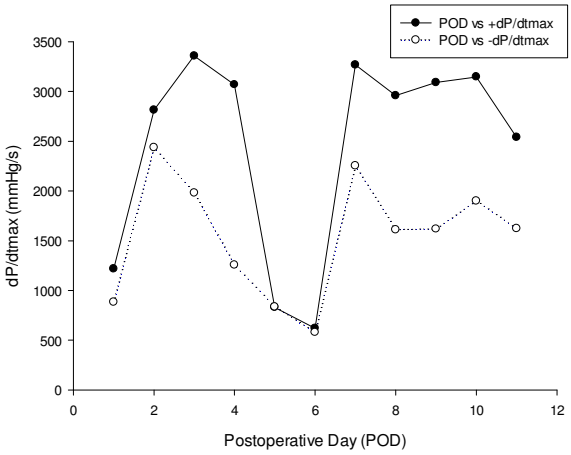


Figure 3.4b

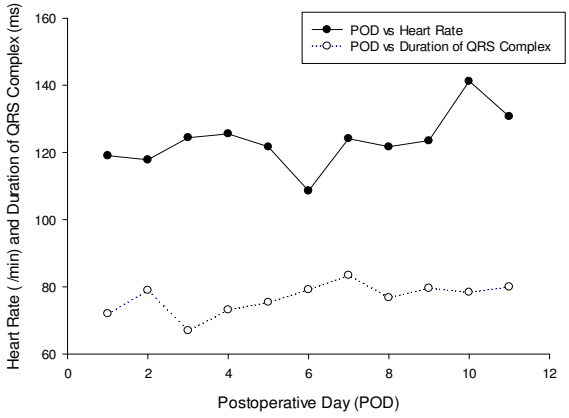


Figure 3.4c

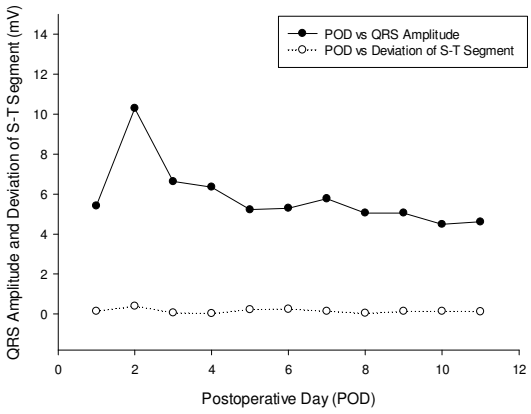


Figure 3.4d

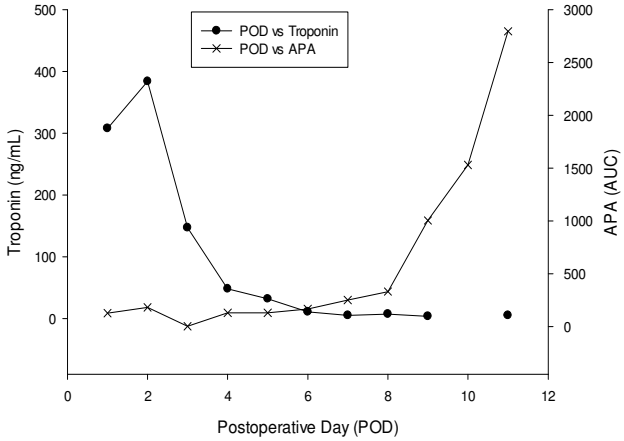


Figure 3.4e

Parameter	Max	Min	Median	Mean
LVPSP (mmHg)	239	110	182	177
LVEDP (mmHg)	44	11	25	25
+dP/dtmax (mmHg	3358	621	2959	2447
-dP/dtmax /s)	2438	579	1619	1544
Heart Rate (/min)	141	109	124	124
QRSA (mV)	10.28	4.48	5.30	5.83
Deviation of S -T Segment (mV)	0.40	0.03	0.12	0.15
Duration of QRS (ms)	83	67	78	77
Troponin (ng/mL)	384.0	3.8	21.5	95.2
APA (AUC)	2796	4	169	514

Table 3.5

Figure 3.4(a-e): Measurement of hemodynamic and ECG parameters and serum level of troponin and APA of baboon 4 during the postoperative period. Table 3.5: Descriptive analysis of individual parameters for baboon 4.

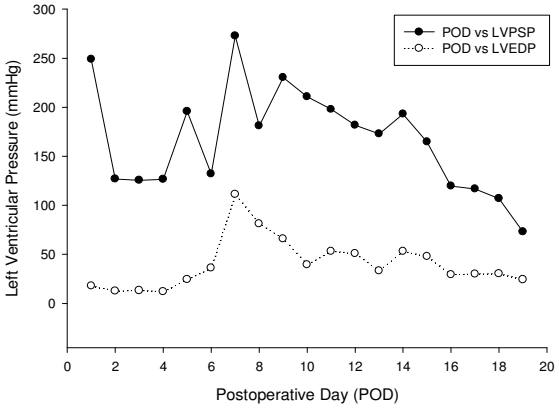


Figure 3.5a

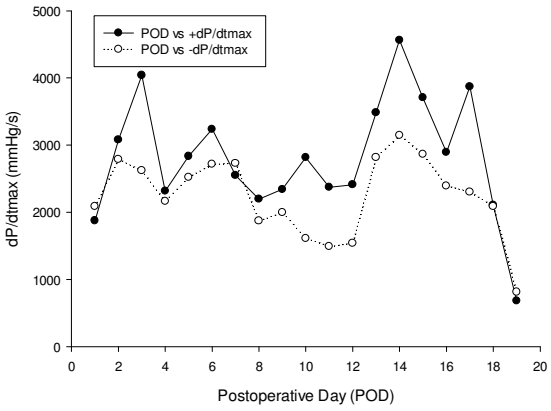


Figure 3.5b

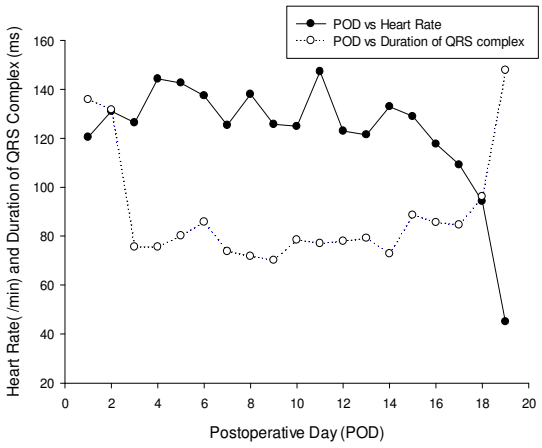


Figure 3.5c

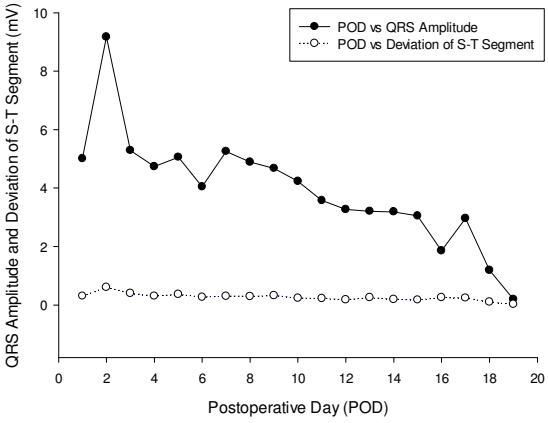


Figure 3.5d

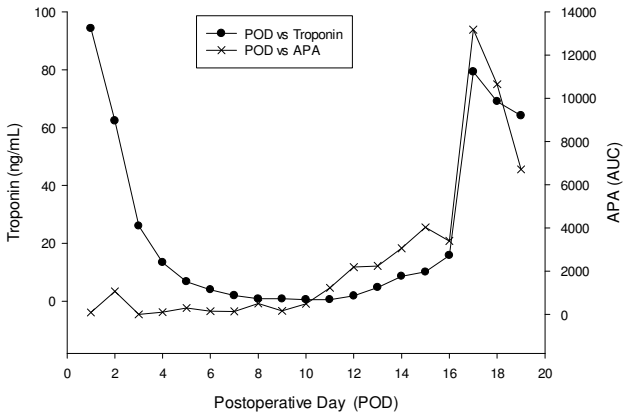


Figure 3.5e

Parameter	Max	Min	Median	Mean
LVPSP (mmHg)	349	73	173	172
LVEDP (mmHg)	111	12	33	40
+dP/dtmax (mmHg	4563	684	2814	2757
-dP/dtmax /s)	4146	813	2394	2867
Heart Rate (/min)	147	45	126	123
QRSA (mV)	9.17	0.20	4.04	4.00
Deviation of S -T Segment (mV)	0.60	0.02	0.25	0.27
Duration of QRS (ms)	147	70	80	91
Troponin (ng/mL)	94.3	0.6	8.6	24.5
APA (AUC)	13176	86	1561	2868

Table 3.6

Figure 3.5(a-e): Measurement of hemodynamic and ECG parameters and serum level of troponin and APA of baboon 5 during the postoperative period. Table 3.6: Descriptive analysis of individual parameters for baboon 5.

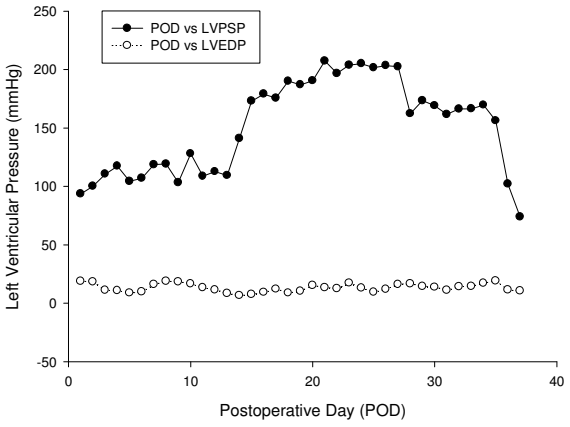


Figure 3.6a

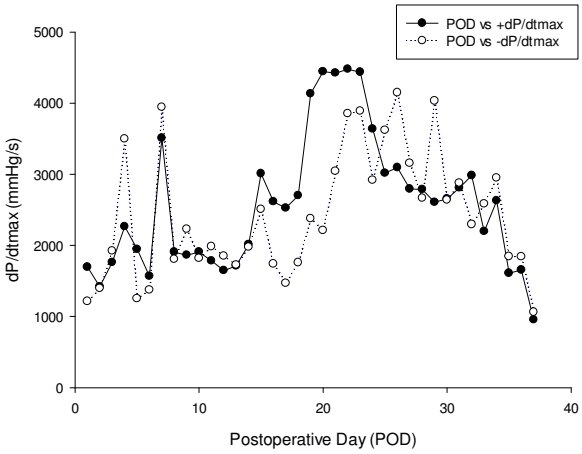


Figure 3.6b

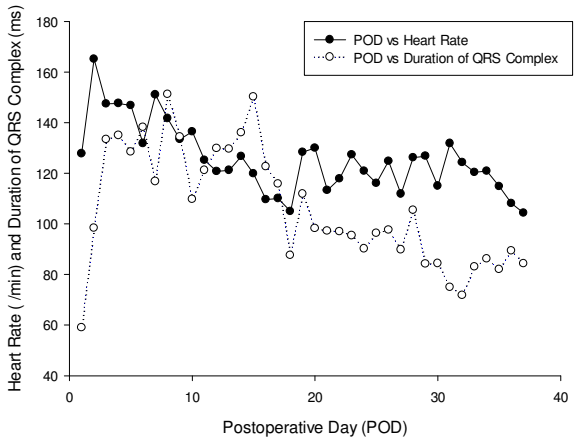


Figure 3.6c

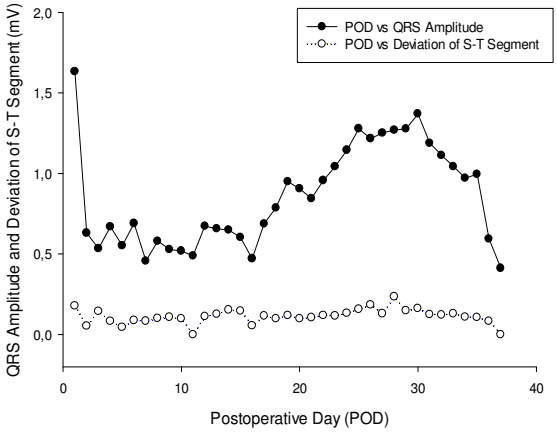


Figure 3.6d

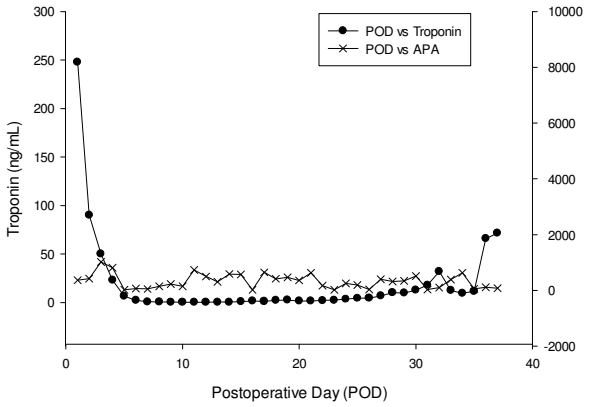


Figure 3.6e

Parameter	Max	Min	Median	Mean
LVPSP (mmHg)	207	74	162	151
LVEDP (mmHg)	19	7	13	13
+dP/dtmax (mmHg	4479	955	2609	2573
-dP/dtmax /s)	4153	1060	2509	2596
Heart Rate (/min)	165	104	125	126
QRSA (mV)	1.63	0.41	0.79	0.86
Deviation of S -T Segment (mV)	0.24	0	0.12	0.11
Duration of QRS (ms)	151	59	98	106
Troponin (ng/mL)	248.0	0.3	3.5	19.3
APA (AUC)	1017	15	313	325

Table 3.7

Figure 3.6(a-e): Measurement of hemodynamic and ECG parameters and serum level of troponin and APA of baboon 6 during the postoperative period. Table 3.7: Descriptive analysis of individual parameters for baboon 6.

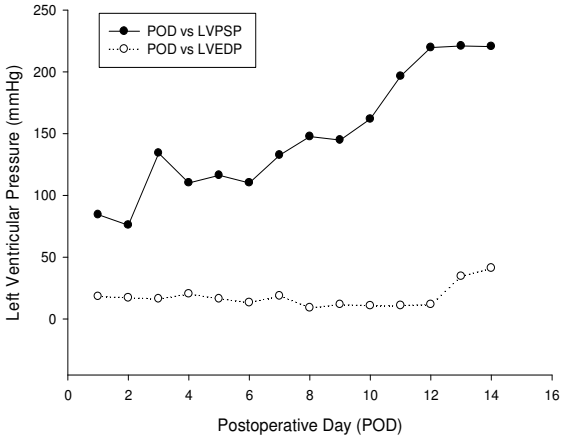


Figure 3.7a

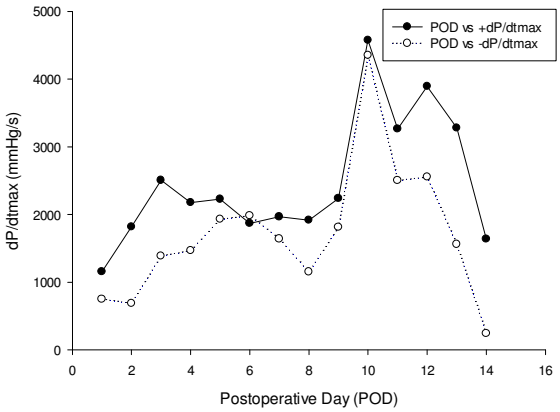


Figure 3.7b

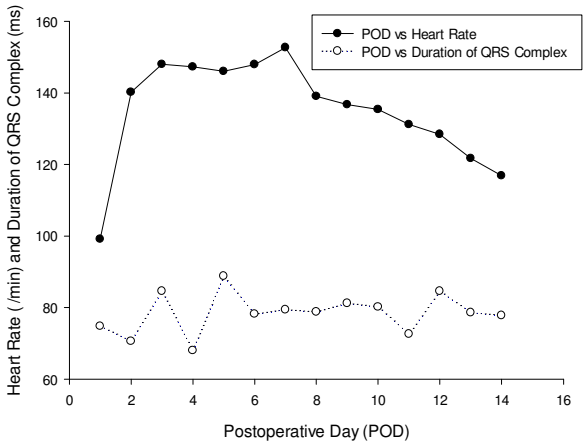


Figure 3.7c

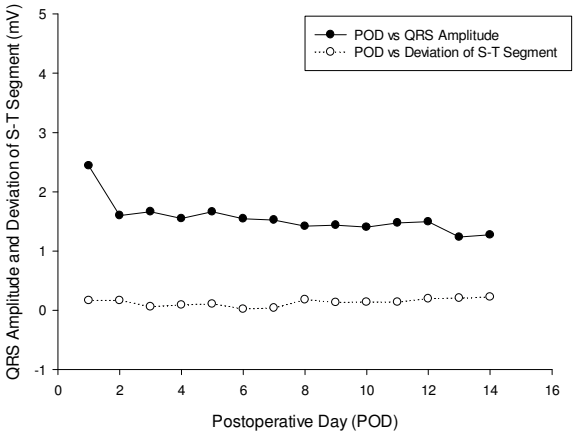


Figure 3.7d

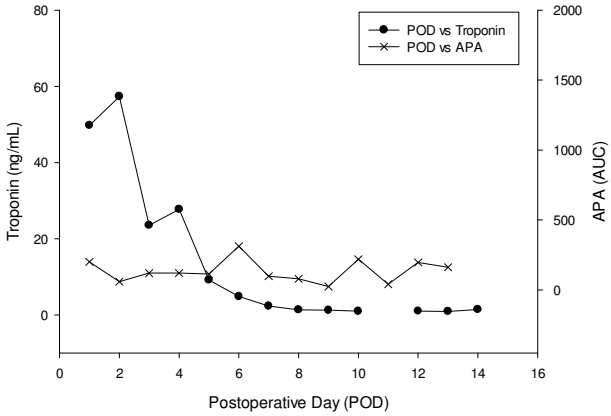


Figure 3.7e

Parameter	Max	Min	Median	Mean
LVPSP (mmHg)	221	76	140	148
LVEDP (mmHg)	41	9	16	18
+dP/dtmax (mmHg	4574	1155	2203	2465
-dP/dtmax /s)	4352	244	1601	1717
Heart Rate (/min)	153	99	138	135
QRSA (mv)	2.44	1.23	1.51	1.55
Deviation of S -T Segment (mV)	0.22	0.02	0.14	0.13
Duration of QRS (ms)	89	68	79	78
Troponin (ng/mL)	57.3	0.9	2.3	14.0
APA (AUC)	312	24.0	120	134

Table 3.8

Figure 3.7(a-e): Measurement of hemodynamic, ECG parameters and serum level of troponin and APA of baboon 7 during the postoperative period. Table 3.8: Descriptive analysis of individual parameters for baboon 7.

3.2 Group categorization based on profile of APA and troponin level elevation

Based on the criterion of group categorization described in the section 2.8, four recipients were classified into group ET and three into group UT. Additionally, five were classified as EA and two as UA.

For technical reasons of the performed ELISA, the antibody measurements of baboon 3 were one order of magnitude lower compared to the other recipients. Accordingly, the threshold was lowered to 100 AUC.

Recipient (Group)	Elevation of Troponin	Starting Day	Group	Elevation of APA	Starting Day	Group
Baboon 1	Yes	POD 17	ET	Yes	POD 16	EA
Baboon 2	No		UT	Yes	POD 11	EA
Baboon 3	Yes	POD 16	ET	Yes	POD 7	EA
Baboon 4	No		UT	Yes	POD 9	EA
Baboon 5	Yes	POD 17	ET	Yes	POD 11	EA
Baboon 6	Yes	POD 36	ET	No		UA
Baboon 7	No		UT	No		UA

ET= Group with elevated troponin serum level, UT= Group with unelevated troponin serum level.

EA= Group with elevated APA serum level, UA= Group with unelevated APA serum level.

Table 3.9: Profile of postoperative elevation of APA and troponin levels

3.3 Comparative analysis of hemodynamics and ECG during the first week and last week post operation

All hemodynamic and ECG parameters were compared between baboons of group ET (n=4) and those in group UT (n=3) for each day of both the first and last postoperative weeks. There was no significant difference between group ET and group UT during both the first and last postoperative weeks in terms of LVEDP and duration of QRS complex ($p>0.05$) (figure 3.8a, figure 3.8b, figure 3.8m, figure 3.8n). HR and QRSA were significantly lower in group ET compared to group UT on day 6 (104 ± 9 vs 134 ± 11beats/min, 0.70 ± 0.30 vs 2.15 ± 1.03 mV) ($p<0.05$) and 7 (82 ± 11 vs 124 ± 7 beats/min, 0.31 ± 0.09 vs 2.16 ± 1.15 mV) ($p<0.05$) of the last postoperative week (figure 3.8i, figure 3.8j, figure 3.8k and figure 3.8l). LVPSP of group ET didn't differ from that of group UT during the first and last postoperative

week with the exception of day 5 (145 ± 21 vs 200 ± 21 mmHg) ($p<0.05$), 6 (84 ± 28 vs 213 ± 8 mmHg) ($p<0.01$) and 7 (45 ± 33 vs 212 ± 42 mmHg) ($p<0.01$) of the last postoperative week. On these days LVPSP of group ET was significantly lower compared to group UT (figure 3.8c and figure 3.8d).

Accordingly, for +dP/dtmax, no significant difference was observed between the two groups during the first postoperative week, but it was significantly lower in group ET on day 6 (1466 ± 531 vs 3125 ± 166 mmHg/s) ($p<0.01$) and 7 (638 ± 237 vs 2565 ± 940 mmHg/s) ($p<0.05$) and higher on day 1 (3183 ± 306 vs 1801 ± 918 mmHg/s) ($p<0.05$) of the last postoperative week compared to group UT (figure 3.8e and figure 3.8f). In contrast, there was a significant difference in -dP/dtmax between group ET and group UT on day 4 (2548 ± 539 vs 1515 ± 283 mmHg/s) ($p<0.05$) of the first postoperative week and day 6 (1011 ± 540 vs 2023 ± 373 mmHg/s) ($p<0.05$) and 7 (834 ± 158 vs 1600 ± 437 mmHg/s) ($p<0.05$) of the last postoperative week (figure 3.8g and figure 3.8h). No significant difference was observed in deviation of S-T segment between group ET and group UT during both the first and last postoperative weeks ($p>0.05$) (figure 3.8o and figure 3.8p).

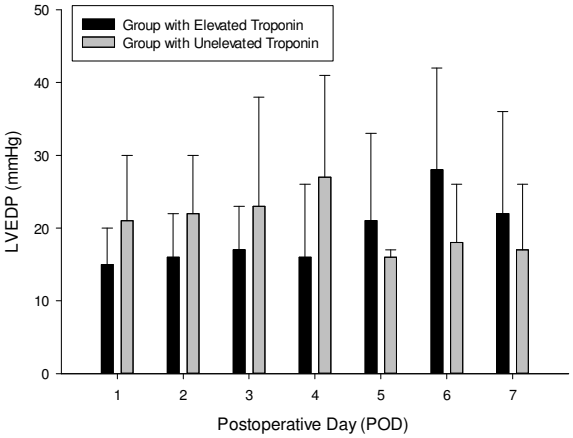


Figure 3.8a

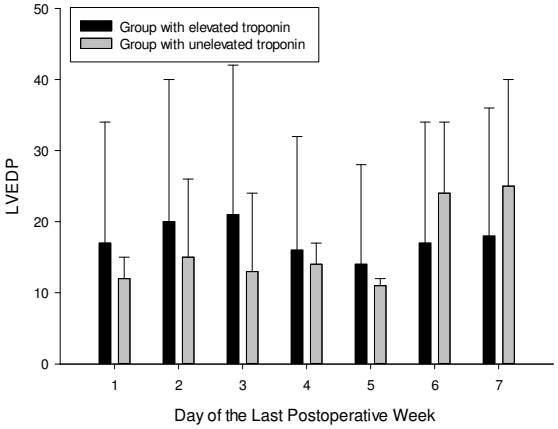


Figure 3.8b

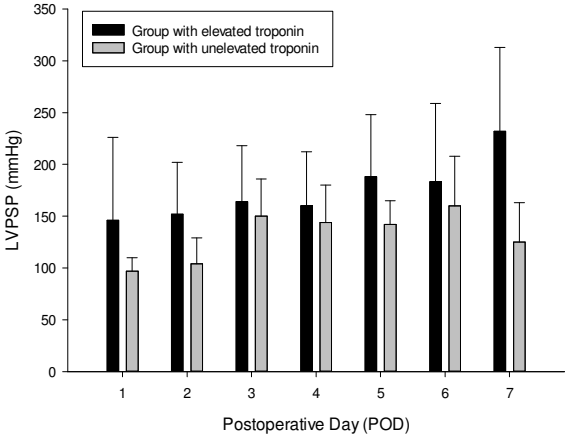


Figure 3.8c

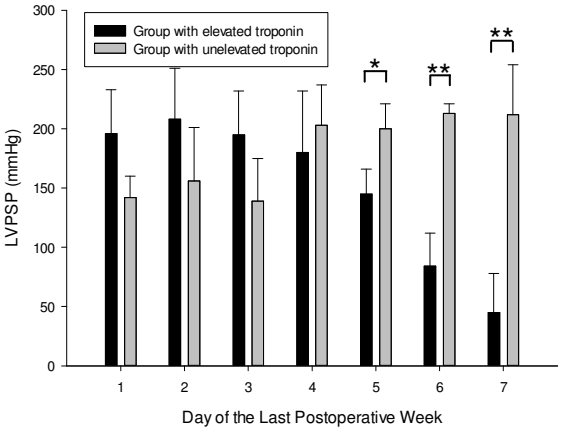


Figure 3.8d

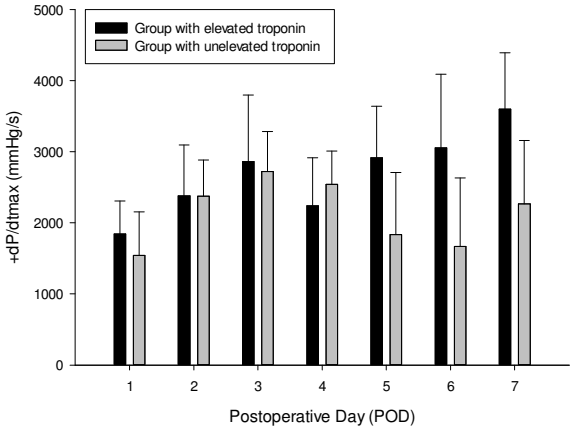


Figure 3.8e

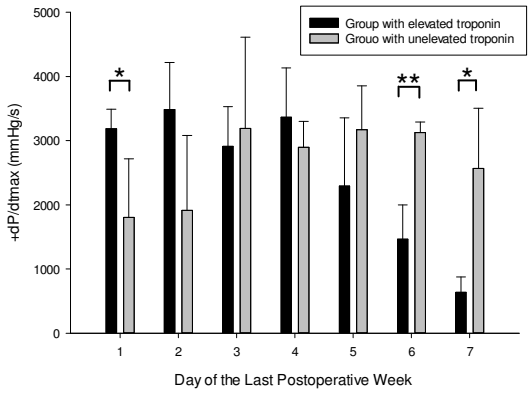


Figure 3.8f

Figure 3.8(a-p): Comparison of hemodynamic and ECG parameters between groups with elevated troponin (n=4) and unelevated troponin level (n=3) during the first week and last week post operation (* $p<0.05$ and ** $p<0.01$ are considered statistically significant).

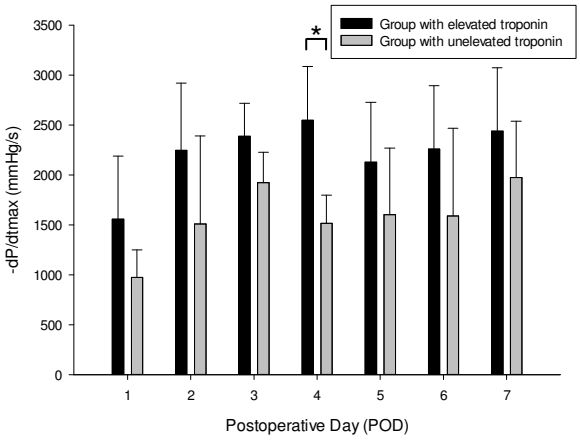


Figure 3.8g

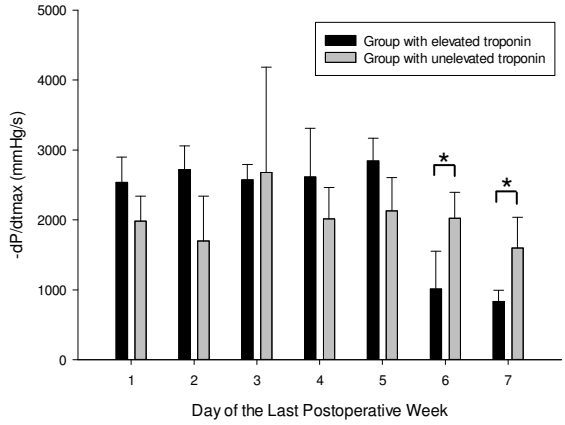


Figure 3.8h

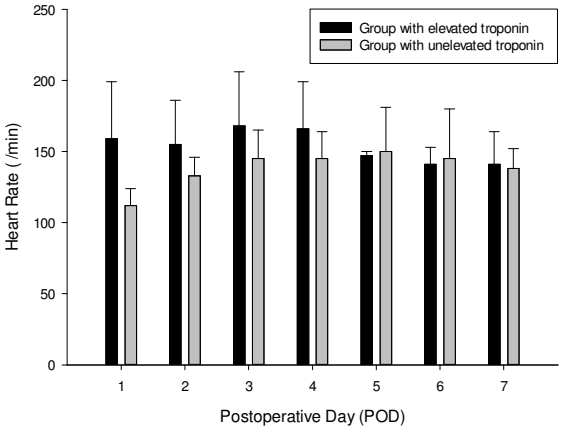


Figure 3.8i

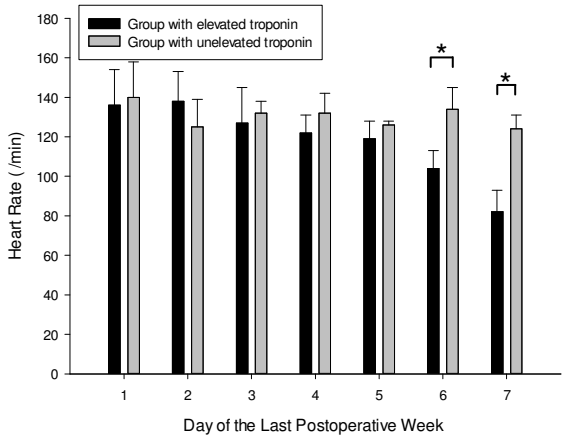


Figure 3.8j

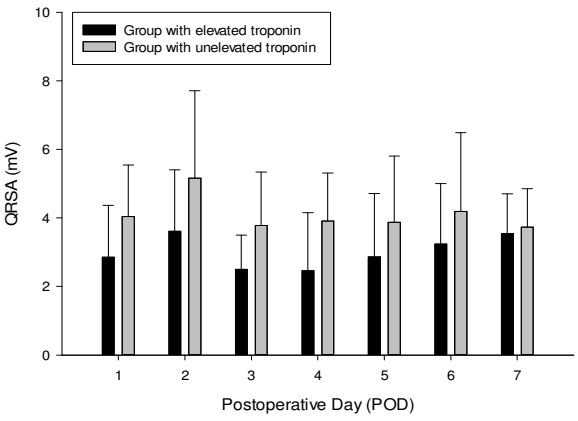


Figure 3.8k

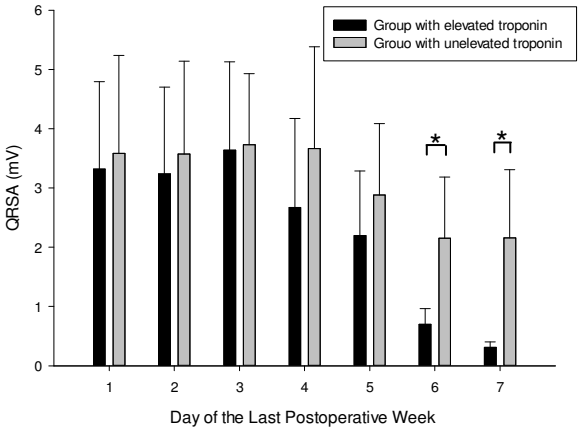


Figure 3.8l

Figure 3.8(a-p): Comparison of hemodynamic and ECG parameters between groups with elevated troponin (n=4) and unelevated troponin level (n=3) during the first week and last week post operation (* $p<0.05$ and ** $p<0.01$ are considered statistically significant).

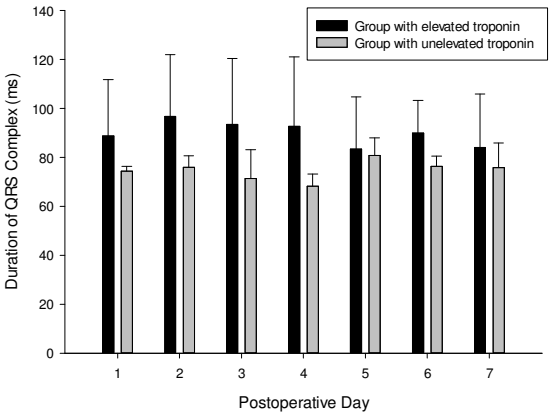


Figure 3.8m

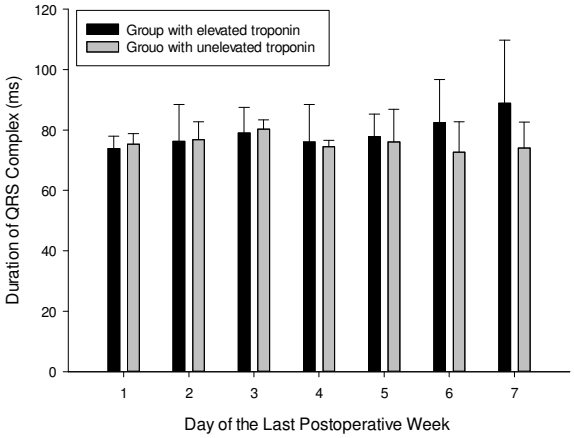


Figure 3.8n

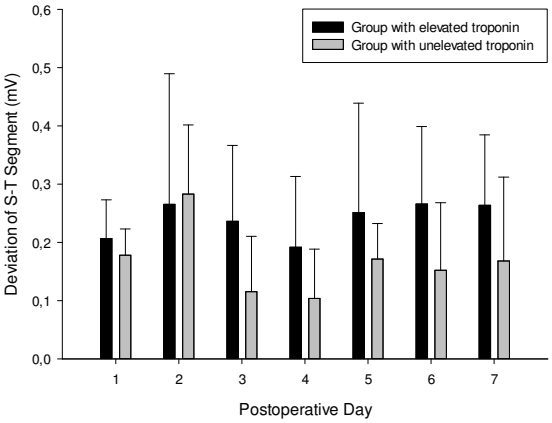


Figure 3.8o

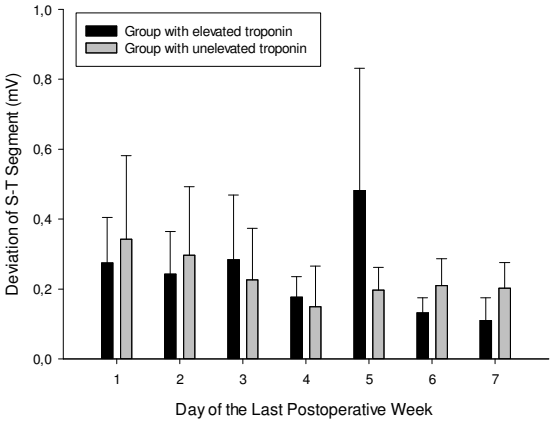


Figure 3.8p

Figure 3.8(a-p): Comparison of hemodynamic and ECG parameters between groups with elevated troponin (n=4) and unelevated troponin level (n=3) during the first week and last week post operation (* $p<0.05$ and ** $p<0.01$ are considered statistically significant).

3.4 Temporal changes of hemodynamic and ECG parameters in relation to elevation of serum level of troponin

Baboons of group ET were analysed based on the starting time points of elevation of troponin serum level presented (table 3.9). Data of hemodynamic and ECG parameters were compared between one day before and two days after elevation of troponin serum concentration (figure 3.9a-d).

LVEDP remained unchanged during the observed period. In contrast, the level of LVPSP was significantly lower on the 1st (84 ± 28 vs 146 ± 21 mmHg) ($p<0.05$) and 2nd (45 ± 33 vs 146 ± 21 mmHg) ($p<0.01$) day after elevation of troponin compared to the day before that (figure 3.9a).

Similarly, +dP/dtmax was significantly lower on the 2nd day (668 ± 329 vs 2337 ± 858 mmHg/s) ($p<0.05$) after elevation of troponin compared to the day before that. –dP/dtmax was significantly lower on the 1st (1511 ± 540 vs 2843 ± 418 mmHg/s) ($p<0.05$) and 2nd (834 ± 158 vs 2843 ± 418 mmHg/s) ($p<0.05$) day after elevation of troponin than on the day before (figure 3.9b).

As for QRSA, a downward trend was observed during these days. Significantly lower readings were obtained on the 1st (0.70 ± 0.30 vs 2.19 ± 1.09 mV) ($p<0.05$) and 2nd (0.31 ± 0.09 vs 2.19 ± 1.09 mV) ($P<0.01$) day after elevation of troponin compared to the day before (figure 3.9d). Absolute values of deviation of S-T segment were also compared, but no significant difference was observed between those days ($p>0.05$).

A gradual decline of heart rates of xenografts was recorded after elevation of troponin level and the difference was significant between its values obtained on the day before and the 1st (104 ± 7 vs 119 ± 9 beats/min) ($p<0.05$) and 2nd (82 ± 9 vs 119 ± 9 beats/min) ($p<0.01$) day after elevation of troponin, whereas changes of duration of QRS complex were not significant during the same period (figure 3.9c).

Analysis of APA serum level showed no significant difference in APA serum level between the day before and the two days after elevation of troponin in the 3 baboons from which serum samples were available ($p>0.05$).

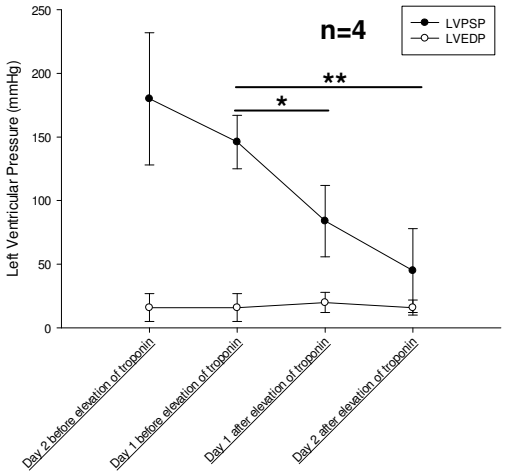


Figure 3.9a

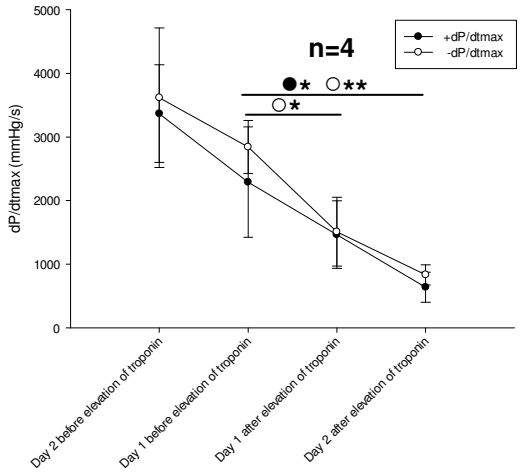


Figure 3.9b

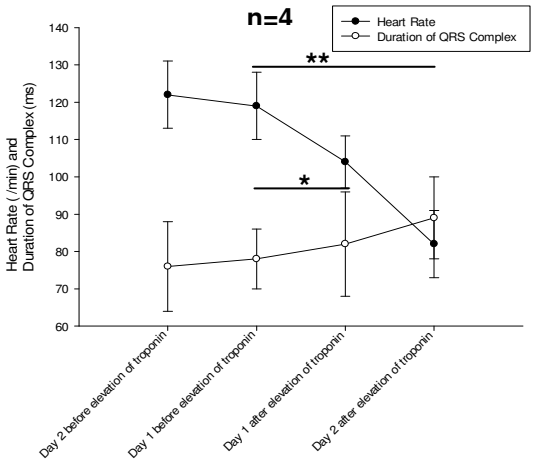


Figure 3.9c

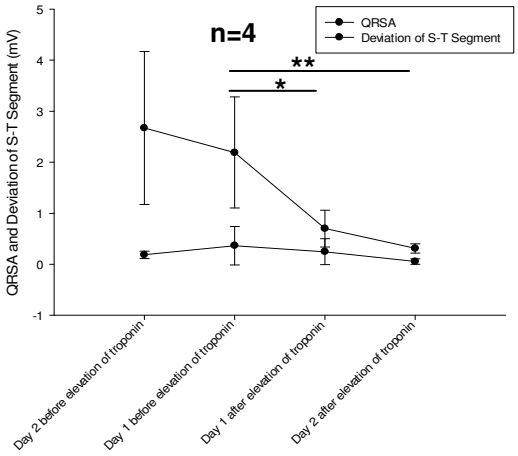


Figure 3.9d

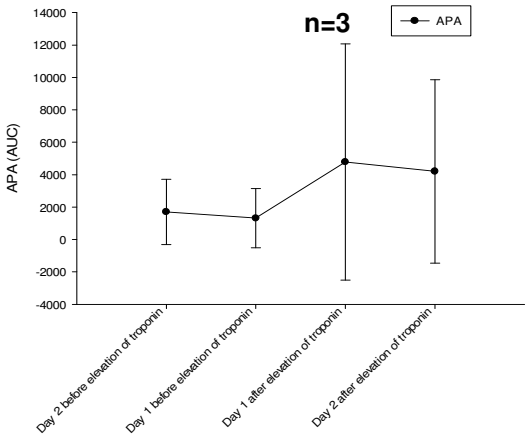


Figure 3.9e

Figure 3.9(a-e): Comparison of hemodynamic and ECG parameters before and after elevation of the serum level of troponin (*p<0.05, **p<0.01 mean significant).

3.5 Temporal changes of hemodynamic and ECG parameters in relation to elevation of serum level of anti-pig antibody (APA)

Marked increases of serum level of anti-pig antibody were observed in baboon 1, baboon 2, baboon 3, baboon 4 and baboon 5 during the postoperative period (table 3.9). Changes of hemodynamic and ECG variables after elevation of APA were analysed in this group.

Before and after elevation of APA, LVEDP and LVPSP of cardiac xenografts varied slightly and no significant difference was observed in these hemodynamic parameters ($p>0.05$) (figure 3.10a). Though decreased values of ±dP/dtmax were recorded for two consecutive days after APA started to rise, they were not significantly lower compared to the day before the rise of APA level ($p>0.05$) (figure 3.10b). Similarly, heart rates and duration of QRS complex appeared to fluctuate modestly throughout the period without any significant changes ($p>0.05$) (figure 3.10c).

Notably, during this period, troponin level rose moderately over time and a gradual drop of QRSA was observed. However, no significant changes were detected in both of them ($p>0.05$) (figure 3.10d and figure 3.10e).

Additionally, absolute values of deviation of S-T segment was compared between the day before and two days after elevation of APA level, but no significant difference was observed ($p>0.05$) (figure 3.10d).

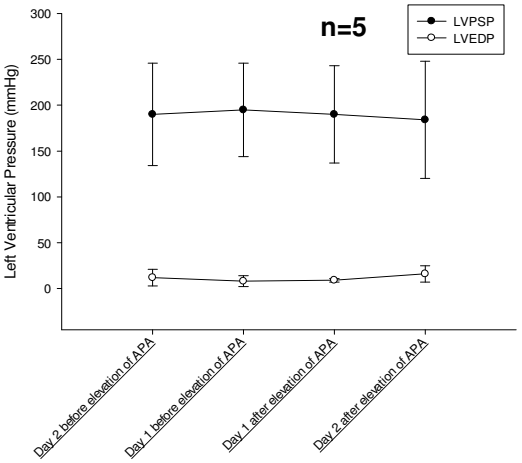


Figure 3.10a

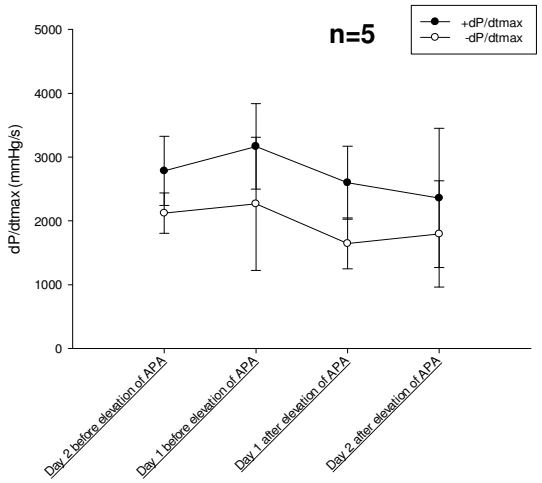


Figure 3.10b

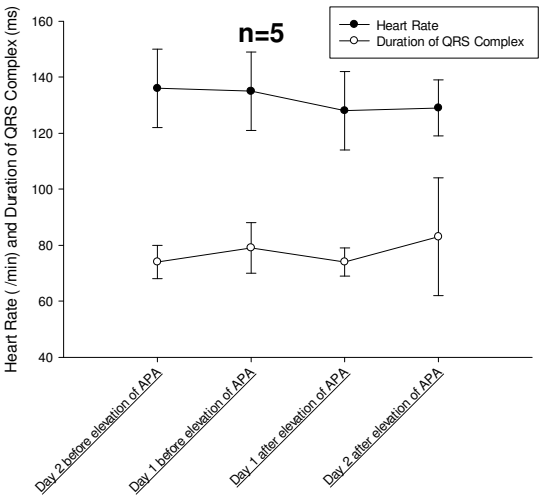


Figure 3.10c

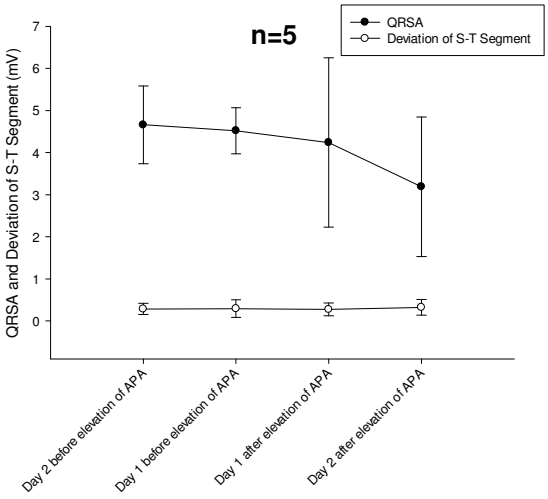


Figure 3.10d

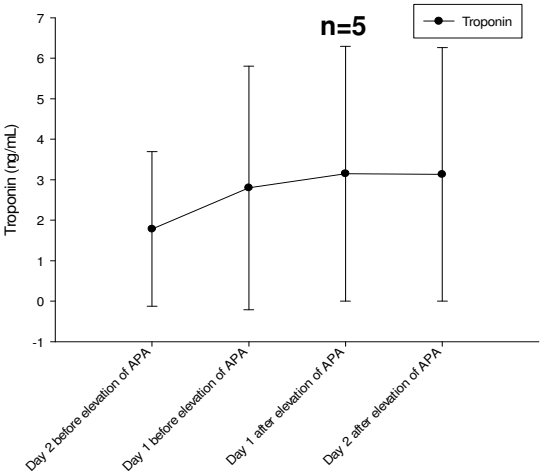


Figure 3.10e

Figure 3.10(a-e): Comparison of hemodynamic and ECG parameters before and after elevation of the serum level of APA.

4 Discussion

In recent years, the heterotopic thoracic cardiac transplantation technique has been established as a preclinical pig-to-baboon cardiac xenotransplant model. As the circulation of the recipient is not fully dependent on xenograft function, the systemic circulation of the recipient can still be maintained by the native heart in case of graft failure. In this xenotransplant setting, the implemented technique enables recipients to survive the critical early posttransplant period associated with higher risk of acute xenograft rejection. Thus, this model, which is thought to combine the advantage of a working heart model and the safety of heterotopic cardiac transplantation, could open a new path for addressing relevant immunological issues.

As mentioned above, xenograft function only contributes partly to the recipient's circulation, which still can be maintained by its native heart in the event of graft failure. Therefore, it could not be accurately and timely reflected by the recipient's hemodynamic status, necessitating continuous monitoring to identify cessation of graft function. Moreover, due to the unconventional placement of xenograft in the thoracic cavity, palpation of the graft is not possible. Thus, to overcome these problems, we proposed telemetry as means to achieve continuous monitoring of xenograft function in this study.

4.1 Telemetric detection of rejection episodes

In this study, due to successful avoidance of hyperacute rejection by αGalT-knockout genetical modification of pig donors, cardiac xenograft function was well preserved in the seven baboons during the first week post transplantation, as indicated by maintenance of normal graft hemodynamics during the period. However, from the second postoperative week onward, acute humoral rejection remains an insurmountable barrier to these recipients' long survival in this study and graft function ceased mostly in the second or third week post transplantation. In these recipients xenoreactive antibody response or myocardial damage occurred as evidenced by elevation of anti-pig antibody or troponin serum level, resulting in impaired graft function as reflected by deterioration of a series of hemodynamic and ECG variables including LVPSP, ±dP/dtmax, QRSA and HR. There is no doubt that

multiple parameter telemetric monitoring enables more comprehensive and detailed evaluation of cardiac graft function as compared to traditional manual palpation or auscultation aimed at heart rate count, allowing prompt intervention and reversal of possible rejection at an early stage. However, not all telemetric parameters have predictive values in detecting deterioration of cardiac graft function in case of acute cardiac rejection in the setting of telemetry monitoring. Thus, to identify the reliable hemodynamic and ECG indicators of acute cardiac rejection, which can be readily analysed and used as evidences to justify the following therapeutical intervention in combination with other relevant parameters during the critical early postoperative period, is of particular significance. To this end, the major hemodynamic and ECG variables mentioned above were retrospectively analysed and compared between recipients with and without postoperative elevation of troponin serum level, and within the group of recipients with elevated troponin level.

It was found LVPSP, ±dP/dtmax, HR and QRSA were significantly lower in the group with elevation of troponin level compared to the other group on the last two days prior to cessation of graft function, indicating that LVPSP, ±dP/dtmax, HR and QRSA are closely relevant to troponin serum level and reflect the alteration of graft function resulting from myocardial damage accurately. However, there was no evidence that LVEDP was correlated with troponin level in this study, as opposed to the finding of a previous similar study which suggested LVEDP was a parameter as valuable as LVPSP for evaluation of cardiac graft function during the cardiac posttransplant period [79]. Furthermore, LVEDP was maintained relatively constant throughout the entire postoperative period in recipients either with or without elevated troponin level except extremely higher LVEDP of baboon 5's cardiac graft was observed on POD 7, POD 8 and POD 9, for which we still have no reasonable explanation so far. This finding could be explained by the fact that LVEDP is determined by left ventricular compliance which is a continuously changing variable during the first few hours or days after acute myocardial infarction and due to an interaction of normal and infarcted cardiac muscle, LV compliance is increased rather than decreased in this short period. Thus, LVEDP may be lower for any given end diastolic volume immediately after acute myocardial infarction [80]. However, as a result of fibrocellular infiltration, ventricular compliance is diminished during the healing phase of acute myocardial infarction and LVEDP is elevated accordingly in

the meantime. In the present study, considerable myocardial damage was found to occur mostly two or three days prior to graft failure, as evidenced by marked elevation of troponin serum level. Thus, it is not difficult to understand why LVEDP remained unchanged shortly after troponin serum levels were elevated. Nevertheless, it is assumed that LVEDP will be elevated dramatically as a result of further myocardial damage in the long term. Unfortunately, due to immediate failure of xenografts after acute rejection, we failed to conduct further investigation to validate this viewpoint.

To assess the graft's overall left ventricular function, evaluation of contraction and relaxation of the left ventricle (LV) should be involved. With respect to the contractile phase, many investigators have used cardiac contractility as a factor to gauge LV systolic function [81–84]. Myocardial contractility is the intrinsic ability of myocytes to exert force at a certain length and it increases upon sympathetic stimulation. Normally, cardiac contractility is expressed as the maximum changing pressure divided by the change of time and it denotes the maximal rate of elevation of intracardiac pressure during isovolumic contraction expressed as +dP/dtmax [85–89]. Ventricular relaxation is utilized to evaluate left ventricular diastolic function and it is often expressed either as the maximum (negative) change in pressure divided by the change of time [90–94], which denotes the maximal rate of decline of left ventricular pressure during isovolumic relaxation expressed as -dP/dtmax. As a result of contraction and relaxation activity, intracardiac pressure of different phases reflects cardiac function directly. As for the left ventricular function, LVPSP and LVEDP are two of the most commonly used parameters for its evaluation, which are conventionally utilized as indicators of left ventricular systolic and diastolic function respectively and constitute an essential part of hemodynamic analysis in either clinical or experimental setting. Therefore, in this study, to accomplish a systemic evaluation of xenograft function, we proposed the use of the aforementioned hemodynamic parameters. Notably, based on a comparative analysis (Section 3.3), LVPSP seemed to be the most sensitive parameter for evaluation of overall cardiac xenograft function among these four parameters thanks to its more significant and prompt change compared to other parameters at the time of changes of graft hemodynamics.

During episodes of rejection, the electrophysiological state of the transplanted heart is altered. These changes may be monitored by use of the electrocardiogram. The surface and intramyocardial electrocardiogram have been used as non-invasive technique for the diagnosis of cardiac allograft rejection for many years [95–98]. Alteration in conduction, amplitude of QRS complex and rhythm was thought to be associated with episodes of rejection. In one study, intramyocardial ECG analysis indicated QRS amplitude was a reliable indicator of acute cardiac rejection with a sensitivity of 95% and specificity of 78% [99]. Müller, et al validated the role of QRS amplitude in diagnosing rejection in heart transplant children, which was monitored continuously using a telemetric technique and suggested diagnosis of rejection could be established when QRS amplitude fell more than 8% below average baseline levels for three consecutive days [100]. Thus, determination of amplitude of QRS is an accurate method for the early detection of cardiac rejection. As expected in this study, a continuous QRS amplitude loss was observed as troponin serum level began to rise dramatically indicating a correlation between QRS amplitude and rejection-related myocardial damage.

QRS duration is a reliable predictor of underlying myocardial injury even in the normally functioning left ventricle and its prolongation, which results from left bundle branch block, is well associated with transmural myocardial damage [101,102]. As for its application in predicting acute cardiac rejection, little is known to date except a couple of surface ECG analysis indicating QRS duration didn't show any characteristic change during rejection episodes in orthotopic heart transplant patients [103,104]. In the present study, QRS duration of cardiac xenograft did not show any significant change after elevation of troponin level despite a continuous slight increase observed during the following two days. However, due to limited number of subjects and possible statistical bias, the results should be interpreted with caution before further study involving large number of subjects is available to validate this finding.

In this study, S-T segment deviation, as a conventional marker of myocardial ischemia and infarction in ECG analysis, was also documented and analysed, which occurs in the form of either elevation or depression. In order to enable comparison between negative and positive voltages representative of depression and elevation of S-T segment respectively, absolute values of the measurements were adopted for

computing and statistical analysis. Deviation of S-T segment was commonly observed in ECG of all the recipients postoperatively either in the form of elevation or depression. However, this ECG parameter appeared to show no significant changes associated with alteration of troponin and APA level. Though elevation and depression of S-T segment are associated with both acute ischemia and infarction, it may also have links to non-ischemic events, such as left bundle branch block, left ventricular hypertrophy [105,106], which are likely to be involved in the postoperative course, complicating identification of the exact cause of S-T segment deviation observed. In this study, though we did not observe any significant change in this ECG variable associated with elevation of troponin serum level, it might be assertive to exclude this variable as a possible predictor of acute rejection just based on the finding of this study. Furthermore, investigation of S-T segment deviation in cardiac transplant setting has been poorly described before. Consequently, with regard to the predictive value of this variable for acute rejection further studies are warranted. In addition, there is still no standard technique for placement of the ECG leads into the epicardium in the setting of intramyocardial ECG and variation in epicardial location of the leads may affect determination of ST-segment positive and negative vectors, leading to possible artefacts in the signal data acquisition. Therefore, standard placement procedures also need to be established in order to achieve accurate acquisition of myocardial bioelectric signals with fewer artefacts.

Other electrocardiographic parameters such as R wave amplitude, the QT and QTc interval have been investigated as predictors of acute cardiac rejection [104, 107,108]. Based on our experience in monitoring and analysing these variables, it is preferable to apply amplitude analysis rather than interval analysis as means for early detection of acute cardiac rejection in the setting of telemetric monitoring as analysis of intervals is much more difficult to perform than that of amplitudes in some cases where atypical electrocardiography patterns make it hard to characterize the waves and define the interval parameters accurately. Furthermore, it was reported that amplitude analysis has a sensitivity of 100% and a specificity of 86% for the diagnosis of rejection while interval analysis is not predictive of rejection even when conduction is prolonged with intervention in a similar intramyocardial ECG analysis [108]. In general, alteration of these electrocardiographic variables occurs early in the time course of acute xenograft rejection and analysis of their measurements may

lead to earlier detection of episodes of rejection. This would allow treatment to be instituted more expeditiously and minimize damage to the graft.

4.2 The role of troponin in detecting acute rejection-related myocardial damage

Cardiac troponin I (cTnI) serves as a useful and sensitive marker of myocardial damage because of its cardiospecificity [109]. Based on its role in diagnosing myocardial damage, cTnI was also proposed as a marker of acute rejection following cardiac transplantation by some studies. For instance, Dengler, et al [110] reported increasing troponin concentration paralleled with the severity of graft rejection. Despite some researchers arguing that troponin T and troponin I may not be reliable markers of cardiac transplant rejection [111-113], some studies demonstrated that cTnI is still a specific marker of acute cardiac rejection despite its limitations of sensitivity [114]. Undoubtedly, endomyocardial biopsy (EMB) is still deemed the “gold standard” for diagnosing cellular rejection after cardiac transplantation due to its high sensitivity and effectiveness. However, biopsies are not a practical screening tool and cannot be done repeatedly to detect rejection before myocardial damage [115]. Moreover, due to complexity of anatomical location of the graft relative to the native heart, EMB is actually impractical technically for heterotopic thoracic models involved in this study. Additionally, the potential lethal injury and infection EMB procedures might cause to recipients are also concerns investigators should take into consideration before performing it. Thus, we still propose determination of serum cardiac troponin I level as an effective method to detect possible acute cardiac rejection.

In this study, we demonstrated that in all baboons in which we suspected an episode of acute rejection based on elevation of troponin serum level, the functional telemetric parameters underwent some characteristic changes. A significant decrease could be observed in LVPSP, ±dP/dtmax, HR and QRSA after obvious elevation of troponin serum level in all of these baboons, suggesting close correlation between these telemetric variables and troponin level. Normally, elevation of troponin level occurred two or three days prior to cessation of graft function in these recipients. A comparative analysis (Section 3.3) also indicated significant difference in these telemetric parameters between recipients with and without

elevation of troponin level during the last two days post operation, further validating the correlation of troponin level with these parameters from another perspective. Additionally, an extraordinarily large amount of troponin was released right after operation in all the recipients, leading to extremely high level of troponin concentration in the peripheral circulation, which was associated with intraoperative surgical procedure-related myocardial damage of both the native heart and graft rather than any episode of rejection. The extraordinarily high postoperative troponin level was not thought to be associated with any deterioration of the investigated telemetric variables. Thereafter, the serum concentration of troponin decreased gradually and normally took about one week to reach a low level, which was maintained until occurrence of myocardial damage associated with acute rejection.

4.3 The role of Anti-pig antibody in detecting acute xenogeneic rejection

Despite the use of intensive immunosuppressive regimen and GalT-KO transgenic organ-source pigs, acute humoral xenograft rejection remains a major barrier to long term survival of cardiac xenografts in pig-to-primate xenotransplant model, which is characterized by thrombotic microangiopathy and associated with the presence of preformed and/or elicited anti-pig antibodies (anti-Gal and anti-non-Gal). In the present study, although anti-pig antibody could be diminished by immunoadsorption over the period of a few days after transplantation, anti-pig antibody reemerged as a result of a T-cell dependent antibody response, inevitably leading to AXHR. An anti-pig antibody hemolytic assay was employed to monitor the peripheral anti-pig antibody response of recipient baboons on a daily basis post transplantation, which represents a cytolytic activity assay. In this study, APA serum concentration was suppressed at a low level during the first postoperative week due to perioperative intensive immunosuppression. Afterwards, considerable elevation of APA was observed in the second week in five recipients. Notably, only three of them were shown to have dramatic elevation of troponin levels subsequently. Not all the elevation of anti-pig antibody levels inevitably led to the elevation of troponin levels in these recipients during the postoperative period. On the other hand, the elevation of troponin level is not always associated with the elevation of anti-pig antibody level, as in one of four recipients with elevation of troponin level, no marked increase of anti-pig antibody level was recorded at all. Furthermore, no significant change of any

hemodynamic parameters or ECG variables was observed following the elevation of anti-pig antibody level. All these findings might suggest that anti-pig antibody serum level maybe is not a perfect indicator of acute humoral xenograft rejection, which is invariably followed by various degrees of myocardial injury and impairment of xenograft function. This is in agreement with the findings of Richards, et al [116], which indicated no individual assay for serum anti-pig antibody measurement was consistently predictive for AHXR and suggested multiple assays be employed in detection of elicited anti-pig antibody response associated with AHXR.

That is why we proposed to perform hemagglutination assay, anti-Gal assay and binding assays of IgG and IgM simultaneously as means to detect episodes of acute cardiac rejection in another study.

Furthermore, Richards, et al argued that the mere presence of anti-pig antibodies in the periphery does not necessarily imply that they are destructive to the graft unless it is certain that these antibodies are definitely bound to the endothelial cells of the graft and the bound antibodies are involved in damaging the graft. Previous studies indicated that anti-Gal antibodies may exert a non-destructive effect on the xenografts by either inducing accommodation of the target cells or inhibiting the binding of cytotoxic antibodies to the grafts [117, 118]. Moreover, there were also several examples of relevant antibody levels in the circulation without an apparent decline in graft function. Additionally, it is also postulated that in case of APA-mediated acute rejection, there is a “time window” between occurrence of rejection episodes and onset of myocardial damage, ranging from a few hours to a few days and featuring a series of pathological events including cellular infiltration, interstitial edema and haemorrhage before myocytes are damaged and cardiac graft function is impaired, leading to a drop in QRSA and compromised systolic and diastolic function eventually. This point also needs to be considered when explaining dyssynchronous changes in APA and troponin serum level and other investigated parameters. Thus, further work is needed to ascertain not only the effects of individual components of anti-pig antibodies on the xenografts but also their specific roles in AHXR and their relationship with other predictors.

4.4 Advantages and disadvantages of telemetric monitoring technique in experimental cardiac xenotransplantation

In this study, a telemetric technique was applied and assessed with a focus on analysis of the alteration of a variety of telemetric variables following pig-to-non-human primate cardiac xenotransplantation in an attempt to explore the possibility of using this technique for detection of possible acute xenograft rejection in combination with other surveillance techniques. To sum up, in the context of cardiac xenotransplant, the advantages of the system are as follows: Telemetry allows monitoring of the graft without restraint stress to the animal. Consequently, stress-induced artefact is significantly reduced. It permits long-term, long-range and wireless monitoring of conscious, freely moving laboratory animals. Furthermore, it enables continuous and simultaneous monitoring of multiple parameters in more than one animal. Telemetric measurements are free from the effects of anesthesia and experimental data can be recorded and stored in a real-time manner by the system, and retrospective analysis of the data is also made possible. Data obtained by telemetry contains no cable or commutator artefacts commonly seen in the wiring monitoring systems. Despite these aforementioned benefits brought by this novel technique, a couple of disadvantages still remain, including the complex surgical procedures for implantation of the transmitter may result in prolonged intraoperative cardiac arrest, which can be associated with worse graft function postoperatively. Additionally, the possible infection posed by subcutaneous implantation of devices and ECG electrodes may lead to an increased mortality rate of recipients maintained on long-term immunosuppressive regimen following transplantation. Data loss or distortion may be caused by technical failures of the transmitter unit such as catheter and electrode displacement, electronic component failures and battery problem, which are unsolvable due to their inaccessible positions. Radio frequency transmission of signals between the transmitter and receiver tends to be disrupted by radio frequency interference and electromagnetic interference by other laboratory instruments in the surroundings. Furthermore, the complexity of the initial equipment installation added to the difficulty and challenge of implementation of this technique.

4.5 Limitations and outlook

The heterotopic thoracic xenotransplant model applied in this study is perceived to be a complex preclinical experimental model involving complicated surgical techniques, sophisticated medical instruments, intensive surveillance, individualized diagnostic and therapeutic scheme, and concerted efforts by research staff. Moreover, there may be many either known or unknown, controllable or uncontrollable factors involved in any part of the experimental process, complicating the interpretation of the experimental data. Even though these baboon experiments were subjected to a completely identical experimental condition, changes of those parameters in them might be totally different too. For this consideration, we observed and documented those parameters' changes in individual baboons continuously, which gave us the first hand information in the future retrospective analysis. From this perspective, findings from an individual-based study may be more persuasive than those from any small group study. That is why we investigated these baboons individually and described their changes on a daily basis.

This study demonstrated the usefulness of a telemetry system in assessing xenograft function in a pig-to-non-human primate cardiac xenotransplant model, and indicated that LVPSP, HR, ±dP/dtmax and QRSA might be useful telemetric parameters for accurate prediction of acute rejection involved in cardiac xenotransplantation and LVPSP and QRSA may be the most sensitive predictors among them, due to their effectiveness in assessing graft function and closer correlationship with myocardial damage marker troponin. Despite the proper design and implementation of this study, it still has a couple of limitations. The first one is the limited number of recipient animals due to high financial costs and technical complexity of the pig-to-non-human primate cardiac xenotransplant model, which might lead to increased statistical biases and even erroneous conclusions on some occasions. Thus, further studies involving a larger number of recipients are needed to validate the findings obtained from the present study. Secondly, due to huge amount of data acquired by the telemetric system during the experiment, it is difficult to make detailed and complete description of all data and only data collected at 6 o'clock of each postoperative day was selected for analysis instead. Therefore, we inevitably missed changes of those parameters occurring at other timepoints, which might make a difference in overall data analysis and were very likely to obtain biased

findings on some occasions. Thirdly, though troponin serum level is deemed a reliable indicator of rejection episodes in the setting of cardiac transplant, histological and immunohistochemical findings remain an irreplaceable evidence for making a accurate diagnosis of a rejection episode. As mentioned above, due to the complexity of anatomical location of the graft relative to the native heart, EMB is impractical technically for these heterotopic thoracic models.

ISHLT proposed that clinical trials on human could be initiated only after routine survivals of a minimum of three months have been achieved for pig hearts which are transplanted into non-human primates in a life-supporting manner or a minimum of 6 month survival is achievable for these cardiac xenografts transplanted in a non-life-supporting manner [73].

However, survivals for cardiac xenotransplants achieved so far that vary from a maximum of 57 days in life-supporting model [119] to 236 days in non-life-supporting model [120] is still far from this goal. We believe that the application of this telemetric technique will definitely contribute to acceleration of the progress towards clinical application of this heterotopic thoracic technique.

5 Summary

Xenotransplantation is thought to have the potential to solve the critical shortage of donor's hearts with the development of genetically modified organ-source pigs and improvement of immunosuppressive strategies. As an appropriate preclinical animal model, the pig-to-baboon cardiac xenotransplantation model has been widely adopted. Traditionally, the evaluation of cardiac xenograft function in a pig-to-baboon model has been accomplished by means of palpation, ultrasound and biopsy. However, those methods pose increased risk of complications such as bleeding, infection, and apnea, leading to increased morbidity and mortality. Telemetric monitoring systems have been widely applied in the life science research involving a wide variety of animal models. These systems enable non-invasive, reliable and continuous measurements of cardiac xenograft function and provide information valuable for the improved understanding of transplantation pathophysiology. Importantly, these systems do not only avoid repeated anesthesia of the animals but also allow measurements independent from the effects of anesthetics.

In preclinical pig-to-baboon xenotransplantations, telemetry systems have only been used in the heterotopic abdominal experiments. The aim of this study was the evaluation of a telemetry system for the monitoring of cardiac xenograft function after heterotopic thoracic cardiac xenotransplantation.

Seven baboons underwent heterotopic thoracic cardiac xenotransplantation, for which double or triple transgenic pigs were used as donors. Hemodynamic parameters such as left ventricular peak systolic pressure (LVPSP), left ventricular end diastolic pressure (LVEDP), heart rate (HR), maximal rate of rise or decline of left ventricular pressure (±dP/dtmax), deviation of S-T segment, and the duration and amplitude of QRS complex in electrocardiogram (QRSA) were continuously monitored, using a telemetry system (DSI, St. Paul, MN, USA). Postoperatively, the serum levels of anti-pig antibodies (APA) were analyzed using a hemolytic assay with pig erythrocytes and the serum level of troponin was determined by the clinical laboratory on a daily basis. Based on the changes of troponin levels and APA levels, the baboons were grouped into elevated and unelevated. All parameters were compared between groups and between days.

During the last two days of their survival, LVPSP, HR, +dP/dtmax, -dP/dtmax and QRSA were significantly lower in the baboons with elevated troponin compared to the group with unelevated troponin. Regarding specifically the group of baboons with elevation of troponin, the following observation was made: LVPSP, HR, -dP/dtmax, and QRSA decreased significantly on the day elevation of troponin was observed and the next day. In addition, The decrease of the parameter +dP/dtmax was significant one day after troponin increased. In contrast, the analysis of the group of baboons with elevated anti-pig antibodies revealed no significant changes in hemodynamic or electrocardiographic parameters before and after the rise of antibodies.

In conclusion, this study demonstrates the ability of the telemetry system to assess changes in xenograft function in the heterotopic thoracic pig-to-baboon cardiac xenotransplant model. In particular, the parameters LVPSP, HR, ±dP/dtmax and QRSA are perceived to be reliable indicators of myocardial damage associated with graft rejection at an early stage following cardiac xenotransplantation. The use of the telemetry system might help to guide immunosuppressive therapy and further improve graft survival in future experiments.

6 Zusammenfassung

Die Xenotransplantation stellt eine vielversprechende Möglichkeit dar, durch die Entwicklung genetisch modifizierter Schweine und einer Verbesserung immunsuppressiver Strategien, den zunehmenden Bedarf an Spenderorganen in der Transplantationsmedizin zu decken und das Problem der Knappheit menschlicher Spenderorgane zu lösen. Als einziges angemessenes präklinisches Tiermodell wird die Transplantation transgener Schweineherzen in Primaten angesehen. Ursprünglich wurde in diesen Versuchen die Funktion des Transplantats durch Palpation, Ultraschall und Biopsien überprüft. Diese Methoden bergen jedoch ein erhöhtes Risiko an Komplikationen, wie Blutungen, Infektionen oder Aspiration infolge der notwendigen Narkose, welche wiederum eine erhöhte Morbidität beziehungsweise Mortalität zur Folge haben können. Telemetrische Messsysteme sind in der Biowissenschaft weit verbreitet und werden dabei in verschiedensten Tiermodellen verwendet. Diese Systeme ermöglichen verlässliche und kontinuierliche Messungen der kardialen Funktion und liefern darüber hinaus wertvolle Informationen, um pathophysiologische Vorgänge verstehen zu können. Die Telemetrie bietet die Möglichkeit wiederholte Narkosen zu vermeiden und erlaubt dadurch Messungen unabhängig von etwaigen Effekten verwendeter Betäubungsmittel.

Bisher wurden bei der xenogenen Herztransplantation Telemetriesysteme ausschließlich in heterotop abdominellen Experimenten verwendet. Ziel dieser Arbeit war die Evaluierung eines solchen Systems für die Überwachung der Transplantatfunktion nach heterotop thorakaler xenogener Herztransplantation.

In der vorliegenden Arbeit erfolgte bei sieben Pavianen eine heterotop thorakale xenogene Herztransplantation mit doppel- beziehungsweise tripeltransgenen Schweineherzen. Durch die Verwendung eines telemetrischen Systems (DSI, St. Paul, MN, USA) konnten hämodynamische Parameter wie maximaler linksventrikulärer systolischer Druck (LVPSP), linksventrikulärer enddiastolischer Druck (LVEDP), Herzfrequenz (HR), maximaler Druckanstieg/Druckabfall pro Zeiteinheit im linken Ventrikel (±dP/dtmax) und Amplitude des QRS-Komplexes (QRSA) kontinuierlich überwacht werden. Postoperativ wurde die Serumkonzentration sogenannter anti-pig-Antikörper (APA) mittels hämolytischem

Schweineerythrozyten Assay bestimmt. Außerdem wurde täglich die Troponin Serumkonzentration als laborchemischer Marker für eine Myokardischämie gemessen. Basierend auf den Veränderungen der Troponin- beziehungsweise APA-Konzentration im Laufe eines Versuches, wurden diese in zwei verschiedene Gruppen (mit Anstieg / ohne Anstieg) eingeteilt. Alle Parameter wurden jeweils zwischen den Gruppen und innerhalb einer Gruppe an verschiedenen Tagen miteinander verglichen.

Die Parameter LVPSP, HR, +dP/dtmax, -dP/dtmax and QRSA waren an den letzten beiden Lebenstagen der Paviane mit erhöhtem Troponin signifikant niedriger. Innerhalb der Gruppe der Paviane mit erhöhtem Troponin-Serum-Spiegel zeigte sich, dass sowohl LVPSP, HR, -dP/dtmax als auch QRSA am selben Tag und am nächsten Tag nach Troponinanstieg signifikant abfielen. Der Abfall des Parameters +dp/dtmax war erst einen Tag nach Troponinanstieg signifikant abgefallen. Innerhalb der Gruppe mit Anstieg der APA Konzentration konnten keine signifikanten Unterschiede bezüglich der hämodynamischen und elektrokardiographischen Parameter vor und nach dem Zeitpunkt des Antikörper Anstiegs gemessen werden.

Zusammenfassend geht aus dieser Dissertationsarbeit hervor, dass sich die Telemetrie als geeignetes System erweist, um Veränderungen der Transplantatfunktion im beschriebenen heterotop thorakalen xenogenen Herztransplantationsmodell festzustellen. Insbesondere die Parameter des LVPSP, der Herzfrequenz, des ±dp/dtmax und der Amplitude des QRS-Komplexes ermöglichen es Myokardschäden, die mit einer Abstoßungsreaktion des Transplantats assoziiert sind, frühzeitig zu erkennen. Möglicherweise kann das telemetrische Monitoring in zukünftigen Versuchen beitragen die immunsuppressive Therapie zu steuern und so das Transplantatüberleben weiter verbessern.

7 Reference

1. Shankarkumar U. Xenotransplantation Ethics and Immunological Hurdles. *Indian Journal of Medical Sciences* 2003;57(7):311-318.

2. Roux FA, Saï P, Deschamps JY. Xenotransfusions, past and present. *Xenotransplantation* 2007;14(3):208–216.

3. Cooper DKC, A brief history of cross-species organ transplantation. *Proc (Bayl Univ Med Cent)* 2012;25(1):49–57.

4. Cooper DK, Dorling A, Pierson RN III, et al. Alpha1, 3-galactosyltransferase gene knockout pigs for xenotransplantation: where do we go from here? *Transplantation* 2007;84(1):1–7.

5. Allan JS. The risk of using baboons as transplant donors: exogenous and endogenous viruses. *Ann N Y Acad Sci* 1998;862:87–99. .

6. Goodall J. Ethical concerns in the use of animals as donors. In: Hardy MA, editor. Xenograft 25. *Excerpta Medica International Congress Series, Elsevier Science; Amsterdam, Netherlands*: 1989. p. 335-349.

7. Rose AG, Cooper DK, Human PA, et al. Histopathology of hyperacute rejection of the heart: experimental and clinical observations in allografts and xenografts. *J Heart Lung Transplant 1991*;10(2):223–234. .

8. Rose AG, Cooper DKC. Venular thrombosis is the key event in the pathogenesis of antibody-mediated cardiac rejection. *Xenotransplantation* 2000;7(1):31–41.

9. Shimizu A, Yamada K. Pathology of renal xenograft rejection in pig to non-human primate transplantation. *Clin Transplant* 2006;20 Suppl 15:46–52.

10. Good AH, Cooper DK, Malcolm AJ, et al. Identification of carbohydrate structures that bind human antiporcine antibodies: implications for discordant xenografting in man. *Transplant Proc* 1992;24(2):559–62.

11. Cooper DKC, Good AH, Koren E, et al. Identification of α-galactosyl and other carbohydrate epitopes that are bound by human anti-pig antibodies: relevance to discordant xenografting in man. *Transpl Immunol* 1993;1(3):198–205.

12. Galili U, Shohet SB, Kobrin E, Stults CL, Macher BA. Man, apes, and Old World monkeys differ from other mammals in the expression of alpha-galactosyl epitopes on nucleated cells. *J Biol Chem* 1988;263 (33):17755-62.

13. Auchincloss H Jr, Sachs DH. Xenogeneic transplantation. *Annu Rev Immunol* 1998;16:433-70.

14. Oriol R, Ye Y, Koren E, Cooper DK. Carbohydrate antigens of pig tissues reacting with human natural antibodies as potential targets for hyperacute vascular rejection in pig-to-man organ xenotransplantation. *Transplantation* 1993;56(6):1433-42.

15. Holzknecht ZE, Platt JL. Identification of porcine endothelial cell membrane antigens recognized by human xenoreactive natural antibodies. *J Immunol* 1995; 154(9):4565-75.

16. Parker W, Bruno D, Holzknecht ZE, Platt JL. Characterization and affinity isolation of xenoreactive human natural antibodies. *J immunol* 1994;153(8): 3971-803.

17. La Vecchio JA. Dunne AD, Edge AS. Enzymatic removal of alpha-galactosyl epitopes from porcine endothelial cells diminishes the cytotoxic effect of natural antibodies. *Transplantation* 1995;60(8):841-7.

18. Galili U, Anaraki F, Thall A, Hill-Black C, Radic M. One percent of human circulating B lymphocytes are capable of producing the natural anti-Gal antibody. *Blood* 1993;82(8):2485.

19. Kroshus TJ, Bolman RM, Dalmasso AP. Selective IgM depletion prolongs organ survival in an ex vivo model of pig-to-human xenotransplantation. *Transplantation* 1996;62(1):5-12.

20. Soares M, Lu X, Havaux X, et al. In vivo IgM depletion by anti-mu monoclonal antibody therapy. The role of IgM in hyperacute vascular rejection of discordant xenografts. *Transplantation* 1994;57(7):1003-9.

21. Gollackner B, Goh S-K, Qawi I, et al. Acute vascular rejection of xenografts: roles of natural and elicited xenoreactive antibodies in activation of vascular endothelial cells and induction of procoagulant activity. *Transplantation* 2004;77(11):1735–1741.

22. Inverardi L, Clissi B, Stolzer AL, et al. Human natural killer lymphocytes directly recognize evolutionarily conserved oligosaccharide ligands expressed by xenogeneic tissues. *Transplantation* 1997;63(9):1318–1330.

23. Baumann BC, Forte P, Hawley RJ, Rieben R, Schneider MK, Seebach JD. Lack of galactose-α1,3-galactose expression on porcine endothelial cells prevents complement-induced lysis but not direct xenogeneic NK cytotoxicity. *J Immunol* 2004;172(10):6460–6467.

24. Rieben R, Seebach JD. Xenograft rejection: IgG1, complement and NK cells team up to activate and destroy the endothelium. *Trends Immunol* 2005;26(1):2–5.

25. Fox A, Mountford J, Braakhuis A, et al. Innate and adaptive immune responses to nonvascular xenografts: evidence that macrophages are direct effectors of xenograft rejection. *J Immunol* 2001;166(3):2133–2140.

26. Sachs DH, Sykes M, Robson SC, Cooper DK. Xenotransplantation. *Adv Immunol* 2001;79:129-223.

27. Cascalho M, Ogle BM, Platt JL. Xenotransplantation and the future of renal replacement. *J Am Soc Nephrol* 2004;15(5):1106-12.

28. Platt JL, Lin SS. The future promises of xenotransplantation. *Ann NY Acad Sci* 1998;862:5-18.

29. Sandrin MS, McKenzie IF. Recent advances in xenotransplantation. *Curr Opin Immunol* 1999;11(5):527-31.

30. Logan JS. Prospects for xenotransplantation. *Curr Opin Immunol* 2000;12(5): 563-8.

31. Yamada K, Sachs DH, DerSimonian H. Human anti-porcine xenogeneic T cell response. Evidence for allelic specificity of mixed leukocyte reaction and for both direct and indirect pathways of recognition. *J Immunol* 1995;155(11):5249–5256.

32. Dorling A, Lechler RI. T cell-mediated xenograft rejection: specific tolerance is probably required for long term xenograft survival. *Xenotransplantation* 1998;5(4):234–245.

33. Mirenda V, Golshayan D, Read J, et al. Achieving permanent survival of islet xenografts by independent manipulation of direct and indirect T-cell responses. *Diabetes* 2005;54(4):1048–1055.

34. Buhler LH, Cooper DKC. How strong is the T cell response in the pig-to-primate model? *Xenotransplantation* 2005;12(2):85–87.

35. Lin YJ, Hara H, Tai H-C, et al. Suppressive efficacy and proliferative capacity of human regulatory T cells in allogeneic and xenogeneic responses. *Transplantation* 2008;86(10):1452–1462.

36. McCurry KR, Parker W, Cotterell AH, et al. Humoral responses to pig-to-baboon cardiac transplantation: implications for the pathogenesis and treatment of acute vascular rejection and for accommodation. *Hum Immunol* 1997;58(2):91–105.

37. Cozzi E, Bhatti F, Schmoeckel M, et al. Long-term survival of non-human primates receiving life supporting transgenic porcine kidney xenografts. *Transplantation* 2000;70(1):15–21.

38. Cozzi E, Vial C, Ostlie D, et al. Maintenance triple immunosuppression with cyclosporine A, mycophenolate sodium and steroids allows prolonged survival of primate recipients of hDAF porcine renal xenografts. *Xenotransplantation* 2003;10(4):300–310.

39. Houser SL, Kuwaki K, Knosalla C, et al. Thrombotic microangiopathy and graft arteriopathy in pig hearts following transplantation into baboons. *Xenotransplantation* 2004;11(5):416–425.

40. Chen G, Qian H, Starzl T, et al. Induced anti-non-Gal antibodies lead to acute humoral xenograft rejection in baboons using α1,3-galactosyltransferase gene-knockout pigs as kidney donors. *Nat Med* 2005;11:1295–1298.

41. Byrne GW, Davies WR, Oi K, et al. Increased immunosuppression, not anticoagulation, extends cardiac xenograft survival. *Transplantation* 2006;82(12):1787–1791.

42. Ekser B, Cooper DKC. Overcoming the barriers to xenotransplantation: prospects for the future. *Expert Rev Clin Immunol* 2010;6(2):219–230.

43. Siegel JB, Grey ST, Lesnikoski BA, et al. Xenogeneic endothelial cells activate human prothrombin. *Transplantation* 1997;64(6):888–896.

44. Lawson JH, Daniels LJ, Platt JL. The evaluation of thrombomodulin activity in porcine to human xenotransplantation. *Transplant Pro.* 1997;29(1-2):884–885.

45. Roussel JC, Moran CJ, Salvaris EJ, et al. Pig thrombomodulin binds human thrombin but is a poor cofactor for activation of human protein C and TAFI. *Am J Transplant* 2008;8(6):1101–1112.

46. Miwa Y, Yamamoto K, Onishi A, et al. Potential value of human thrombomodulin and DAF expression for coagulation control in pig-to-human xenotransplantation. Xenotransplantation 2010;17(1):26–37.

47. Schulte Am Esch J, Robson SC, Knoefel WT, et al. O-linked glycosylation and functional incompatibility of porcine vonWillebrand factor for human platelet GPIb receptors. *Xenotransplantation* 2005;12(1):30–37.

48. Kopp CW, Siegel JB, Hancock WW, et al. Effect of porcine endothelial tissue factor pathway inhibitor on human coagulation factors. *Transplantation* 1997; 63(5):749–758.

49. Kopp CW, Robson SC, Siegel JB, et al. Regulation of monocyte tissue factor activity by allogeneic and xenogeneic endothelial cells. *Thromb Haemost* 1998; 79(3):529–538.

50. Kobayashi T, Taniguchi S, Neethling FA, Rose AG, Hancock WW, Ye Y, Niekrasz M, Kosanke S, Wright LJ, White DJ, Cooper DK. Delayed xenograft rejection of pig-to-baboon cardiac transplants after cobra venom factor therapy. *Transplantation* 1997;64(9):1255-61.

51. Azimzadeh A, Meyer C, Ravanat C, Cazenave JP. Wolf P. Xenograft rejection: molecular mechanisms and therapeutic prospects. *Hematol Cell Ther* 1996;38(4):331-43.

52. Leventhal JR, John R, Fryer JP, et al. Removal of baboon and human antiporcine IgG and IgM natural antibodies by immunoadsorption, *Transplantation* 1995;59(2):294-300.

53. Yang YG, Sykes M. Xenotransplantation: current status and a perspective on the future. *Nat Rev Immunol* 2007;7(7):519-31.

54. Wilmut I, Schnieke AE, McWhir J, et al. Viable offspring derived from fetal and adult mammalian cells. *Nature* 1997;385(6619):810–3.

55. Polejaeva I, Chen S, Vaught T, et al. Cloned pigs produced by nuclear transfer from adult somatic cells. *Nature* 2000;407(6800):86–90.

56. Phelps CJ, Koike C, Vaught TD, et al. Production of α1,3-galactosyltransferase-deficient pigs. *Science* 2003;299(5605):411–14.

57. Kuwaki K, Tseng YL, Dor FJ, Shimizu A, Houser SL, Sanderson TM, et al. Heart transplantation in baboons using α1,3-galactosyltransferase gene-knockout pigs as donors: initial experience. *Nat Med* 2005;11(1):29–31.

58. Tseng YL, Kuwaki K, Dor FJ, Shimizu A, Houser S, Hisashi Y, et al. α1,3 -Galactosyltransferase gene-knockout pig heart transplantation in baboons with survival approaching 6 months. *Transplantation* 2005;80(10):1493–500.

59. Yamada K, Yazawa K, Shimizu A, Iwanaga T, Hisashi Y, Nuhn M, et al. Marked prolongation of porcine renal xenograft survival in baboon through the use of

alpha 1,3-galactosyltransferase gene-knockout donors and the cotransplantation of thymic tissue. *Nat Med* 2005;11(1):32–4.

60. Baumann BC, Stussi G, Huggel K, et al. Reactivity of human natural antibodies to endothelial cells from Galα(1,3)Gal-deficient pigs. *Transplantation* 2007;83(2):193–201. .

61. Hara H, Ezzelarab M, Rood PPM, et al. Allosensitized humans are at no greater risk of humoral rejection of GT-KO pig organs than other humans. *Xenotransplantation* 2005;13(4):357–365.

62. Ezzelarab M, Hara H, Busch J, et al. Antibodies directed to pig nonGal antigens in naïve and sensitized baboons. *Xenotransplantation* 2006;13:400–407.

63. Seebach JD, Comrack C, Germana S, LeGuern C, Sachs DH, DerSimonian H. HLA-Cw3 expression on porcine endothelial cells protects against xenogeneic cytotoxicity mediated by a subset of human NK cells. *J Immunol* 1997;159(7):3655–3661.

64. Byrne GW, McGregor CG. Cardiac xenotransplantation: progress and challenges. *Curr Opin Organ Transplant*. 2012;17(2):148-54.

65. Cozzi E, White DJG. The generation of transgenic pigs as potential organ donors for humans. *Nat Med* 1995;1(1):964–69.

66. Diamond LE, Quinn CM, Martin MJ, et al. A human CD46 transgenic pig model system for the study of discordant xenotransplantation. *Transplantation* 2001; 71(1):132–42.

67. Cowan PJ, Aminian A, Barlow H, et al. Renal xenografts from triple-transgenic pigs are not hyperacutely rejected but cause coagulopathy in non-immunosuppressed baboons. *Transplantation* 2000; 69(12):2504–15.

68. Morgan BP, Berg CW, Harris CL. “Homologous restriction” in complement lysis: roles of membrane complement regulators. *Xenotransplantation* 2005;12(4):258–65.

69. Miyagawa S, Yamamoto A, Matsunami K, et al. Complement regulation in the GalT KO era. *Xenotransplantation*. 2010;17(1):11–25.

70. Azimzadeh AM, Kelishadi S, Ezzelarab M, et al. Early graft failure of GTKO pig organs in baboons is reduced by hCPRP expression. *Xenotransplantation* 2009;16(5):356.

71. McGregor CG, Davies WR, Oi K, et al. Cardiac xenotransplantation: recent preclinical progress with 3-month median survival. *J Thorac Cardiovasc Surg* 2005;130(3):844–851.

72. Mohiuddin MM, Corcoran PC, Singh AK, et al. Pig to baboon cardiac xenotransplantation: essential role of B cell depletion in prolonging cardiac xenograft survival. *Am J Transplant* 2010; 10 (Supplement):186.

73. Cooper DKC, Keogh AM, Brink J, et al. Report of the Xenotransplantation Advisory Committee of the International Society for Heart and Lung Transplantation: the present status of xenotransplantation and its potential role in the treatment of end-stage cardiac and pulmonary diseases. *J Heart Lung Transplant* 2000;19(12):1125–1165.

74. Bauer A, Postrach J, Thormann M, et al. First experience with heterotopic thoracic pig-to-baboon cardiac xenotransplantation. *Xenotransplantation* 2010; 17:243–249.

75. Chen RH, Kadner A, Adams DH. Monitoring pig-to-primate cardiac xenografts with live internet images of recipients and xenograft telemetric signals: histologic and immunohistochemical correlations. *J Heart Lung Transplant* 2000;19(6):591-7.

76. Pirolo JS, Shuman TS, Brunt EM, et al. Non-invasive detection of cardiac allograft rejection by prospective telemetric monitoring. *J Thorac Cardiovasc Surg* 1992;103(5):969-79.

77. Groeneveld WH, Kort WJ. Endpoint detection of heterotopic grafted rat hearts using an implanted transmitter. *J Surg Res* 1981;31(1):82-86.

78. Koike K, Hesslein PS, Dasmahapatra HK, et al. Telemetric detection of cardiac allograft rejection. Correlation of electrophysiological, histological, and biochemical changes during unmodified rejection. *Circulation* 1988;78 (3 Pt 2):I106-12.

79. Horvath KA, Corcoran PC, Singh AK, Hoyt RF, Carrier C, Thomas ML, Mohiuddin MM. Left ventricular pressure measurement by telemetry is an effective means to evaluate transplanted heart function in experimental heterotopic cardiac xenotransplantation. *Transplant Proc* 2010;42(6):2152-5.

80. J. S. Forrester, G. Diamond, W. W. Parmley, H. J. C. Swan. Early Increase in Left Ventricular Compliance after Myocardial Infarction. *J Clin Invest* 1972;51(3):598-603.

81. Brothers RM, Bhella PS, Shibata S, Wingo JE, Levine BD, Crandall CG. Cardiac systolic and diastolic function during whole body heat stress. *Am J Physiol Heart Circ Physiol* 2009;296(4):H1150-1156.

82. Mullner M, Domanovits H, Sterz F, Herkner H, Gamper G, Kurkciyan I, Laggner AN. Measurement of myocardial contractility following successful resuscitation: quantitated left ventricular systolic function utilising non-invasive wall stress analysis. *Resuscitation* 1998;39(1-2):51-59.

83. Royse CF, Connelly KA, MacLaren G, Royse AG. Evaluation of echocardiography indices of systolic function: a comparative study using pressure-volume loops in patients undergoing coronary artery bypass surgery. *Anaesthesia* 2007;62(2):109-116.

84. Tomson CR. Echocardiographic assessment of systolic function in dialysis patients. *Nephrol Dial Transplant* 1990;5(5):325-331.

85. Royse CF, Connelly KA, MacLaren G, Royse AG. Evaluation of echocardiography indices of systolic function: a comparative study using pressure-volume loops in patients undergoing coronary artery bypass surgery. *Anaesthesia* 2007;62(2):109-116.

86. Dhainaut JF, Bricard C, Monsallier FJ, Salmon O, Bons J, Fourestie V, Schlemmer B, Carli A. Left ventricular contractility using isovolumic phase indices during PEEP in ARDS patients. *Crit Care Med* 1982;10(10):631-635.

87. Dowell RT, Houdi AA. Aortic peak flow velocity as an index of myocardial contractility in the conscious rat. *Methods Find Exp Clin Pharmacol* 1997;19(8):533-539.

88. Mason DT, Braunwald E, Covell JW, Sonnenblick EH, Ross J Jr. Assessment of cardiac contractility. The relation between the rate of pressure rise and ventricular pressure during isovolumic systole. *Circulation* 1971;44(1):47-58.

89. Schmidt HD, Hoppe H. Influence of the contractile state of the heart of the preload dependence of the maximal rate of intraventricular pressure rise dP/dt max. *Cardiology* 1978;63(2):112-125.

90. Ahmed SS, Regan TJ. Assessment of left ventricular contractile performance from isovolumic relaxation phase in man. *Cardiology* 1981;68(1):1-18.

91. Hirota Y. A clinical study of left ventricular relaxation. *Circulation* 1980;62(4):756-763.

92. Ohte N, Narita H, Hashimoto T, Kobayashi K, Akita S, Fujinami T. Left ventricular isovolumic relaxation flow and left ventricular systolic performance*. J Am Soc Echocardiogr* 1995;8(5 Pt 1):690-695.

93. Voon WC, Su HM, Yen HW, Lin TH, Lai WT, Sheu SH. Validation of isovolumic relaxation flow propagation velocity as an index of ventricular relaxation. *Ultrasound Med Biol* 2007;33(7):1098-1103.

94. Yellin EL, Nikolic S, Frater RW. Left ventricular filling dynamics and diastolic function. *Prog Cardiovasc Dis* 1990;32(4):247-271.

95. Rosenbloom M, Laschinger JC, Saffitz JE, Cox JL, Bolman RM. Noninvasive detection of cardiac allograft rejection by analysis of the unipolar peak-to-peak amplitude of intramyocardial electrograms. *Ann Thorac Surg* 1989;47(3):407-411.

96. Castejon R, Gamallo C, Cabo J, Diez PJ, Ruiz MR, Cordovilla G. Electrophysiologic changes during acute rejection of heterotopically transplanted rat hearts. *J Heart Lung Transplant* 1991;10 (1 Pt 1):100-105.

97. Castejon R, Cabo J, Gamallo C , Diez PJ, Cordrvilla G. Electrophysiological and anatomical findings in heart transplantation: experimental study. *Pace Pacing Clin Electrophysiol* 1990;13(7):845-851.

98. Grauhan O, Warnecke H, Muller J, et al. Intramyocardial electrogram recordings for diagnosis and therapy monitoring of cardiac allograft rejection. *Eur Cardiorhoracic Surg* 1993;7(9):489-494 .

99. Wang M, Wu S. M, Tang H, et al. Intramyocardial electrocardiogram measurements for diagnosis of acute cardiac allograft rejection in rats. *Journal of Shandong University (Health Sciences)* 2009;47(10):32-34

100. Müller J, Warnecke H, Spiegelsberger S, Hummel M, Cohnert T, Hetzer R. Reliable noninvasive rejection diagnosis after heart transplantation in childhood. *J Heart Lung Transplant* 1993;12(2):189-98.

101. Sata N, Kawano T, Hamada N, Horinouchi T, Amitani S, Moriyama Y, Miyahara K. Predictor of underlying myocardial damage in normally functioning left ventricle with narrow QRS complex: relationship between QRS duration at right ventricle pacing and iodine-123 metaiodobenzylguanidine myocardial scintigraphy. *Ann Nucl Med* 2009;23(7):639-41.

102. Wiegerinck RF, Gálvez-Monton C, Jorge E, Martínez R, Ricart E, Cinca J. Changes in QRS duration and R-wave amplitude in electrocardiogram leads with ST segment elevation differentiate epicardial and transmural myocardial injury. *Heart Rhythm* 2010;7(11):1667-73.

103. Haberl R, Weber M, Reichenspurner H, Kemkes BM, Osterholzer G, Anthuber M, Steinbeck G. Frequency analysis of the surface electrocardiogram for recognition of acute rejection after orthotopic cardiac transplantation in man. *Circulation* 1987;76(1):101-8.

104. Everett JE, Irwin E, Jesserun J, Slovut D, Shumway SJ. Noninvasive diagnosis of cardiac allograft rejection: the effect of procainamide. *J Invest Surg* 1995;8(3):195-201.

105. Kyuhyun Wang, Richard W Asinger, Henry JL Marriott. ST-Segment Elevation in Conditions Other Than Acute Myocardial Infarction. *N Engl J Med* 2003; 349(22):2128-2135.

106. Pollehn T, Brady WJ, Perron AD, Morris F. The electrocardiographic differential diagnosis of ST segment depression. *Emerg Med J* 2002;19(2):129-135 .

107. Tenderich G, Jahanyar J, Zittermann A, Schleithoff SS, Wlost S, Körfer R. Predictive value of ECG changes for acute cardiac rejections in heart transplant recipients. *Med Klin (Munich)* 2006;101(2):99-106.

108. Jia YX, Meng X, Sun LB, Han J, Chen YT. Using intramyocardial electrograms combined with other noninvasive methods for monitoring acute rejection following human heart transplantation. *Chin Med J (Engl)* 2009;122(2):136-9.

109. Adams JE III. Utility of cardiac troponins in patients with suspected cardiac trauma or after cardiac surgery. *Clin Lab Med* 1997;17(4):613–623.

110. Dengler TJ, Zimmermann R, Braun K, et al. Elevated serum concentrations of cardiac troponin T in acute allograft rejection after human heart transplantation. *J Am Coll Cardiol* 1998;32(2):405–412.

111. Walpoth TH, Celik B, Printzen G, et al. Assessment of troponin-T for detection of clinical cardiac rejection. *Transpl Int* 1998;11(Suppl 1):502–507.

112. Forni A, Faggian G, Luciani GB, et al. Correlation between troponin I serum level and acute cardiac allograft rejection: a preliminary report. *Transpl Proc* 2000;32(1):167–168.

113. Alexis JD, Lao LD, Selter JG, et al. Cardiac troponin T: a noninvasive marker for heart transplant rejection*? J Heart Lung Transplant* 1998;17(4):395–398.

114. Siaplaouras J, Thul J, Krämer U, Bauer J, Schranz D. Cardiac troponin I: a marker of acute heart rejection in infant and child heart recipients? *Pediatr Transplantation* 2003;7(1):43–45.

115. Irwin ED, Bianco RW, Clack R, et al. Use of epicardial electrocardiograms for detecting cardiac allograft rejection. *Ann Thorac Surg* 1992;54(4):669–74.

116. Richards AC, Davies HF, McLaughlin ML, et al. Serum anti-pig antibodies as potential indicators of acute humoral xenograft rejection in pig-to-cynomolgus monkey kidney transplantation. *Transplantation* 2002;73(6):881-9.

117. Dalmasso AP, He T, Benson BA. Human IgM xenoreactive natural antibodies can induce resistance of porcine endothelial cells to complement mediated injury. *Xenotransplantation* 1996;3(1):54-62.

118. Yu PB, Holzknecht ZE, Bruno D, Parker W, Platt JL. Modulation of natural IgM binding and complement activation by natural IgG antibodies: a role for IgG anti-Gal alpha1-3Gal antibodies. *J Immunol* 1996;157(11):5163-8.

119. McGregor CGA, Davies WR, Oi K, Tazelaar HD,Walker RC, Chandrasekaran K, et al. Recovery of cardiac function after pig-to-primate orthotopic heart transplant. *Am J Transplant* 2008;8(suppl2):205.

120. Mohiuddin MM, Corcoran PC, Singh AK, et al. Over six month survival of cardiac xenograft is achievable but heterotopic placement of the graft may limit consistent prolonged survival. *Transplantation*. 2010;90(suppl):325.

8 Appendix

Appendix A: Protocol of Anti-Pig Antibody Assay

1. Human serum and baboon serum are heated at temperature of 56 ºC for 30 min and then centrifuged for 5 min at full speed to get rid of small residual particles.

2. 1×CFD-buffer (Complement fixation test diluent) is prepared by adding 50 ml CFD to 200 ml distilled water. This 1×CFD-buffer is used for all following steps.

3. 3 ml pig blood is diluted in 30 ml CFD buffer.

4. The pig blood suspension is then centrifuged at 750 g for 6 min. Supernatant is discarded and the centrifuge tube is filled with 30 ml CFD buffer again. This washing step is performed 4 times. It should be noted that it is important to wash the pig blood cells as thoroughly as possible in order to get rid of endogenous complement factor proteins from the blood. After the last round of washing the supernatant is discarded and the pellet is then centrifuged at the maximal speed in the ultracentrifuge for 5 min.

5. 100 ul of the pellet are then transferred to a tube with 9.9 ml CFD buffer. This cell suspension is called PRBC (Purified Red Blood Cell).

6. 400 ul baboon serum plus 600 CFD-buffer and 400 ul human serum plus 600 ul CFD-buffer are mixed in 1.5 ml centrifuge tubes. 100 ul baboon serum-dilution is transferred to cells of A1 and B1 of the 96 well plate with cone-shaped bottom (figure 11), respectively. If more than one baboon serum is to be assayed 100 ul of the second baboon serum sample is added in C1 and D1 each and 100 ul of the third serum sample is added in E1 and F1 each and so on for more serum samples. Normally, G1 and H1 are reserved for human serum with 100 ul of it added in each of them.

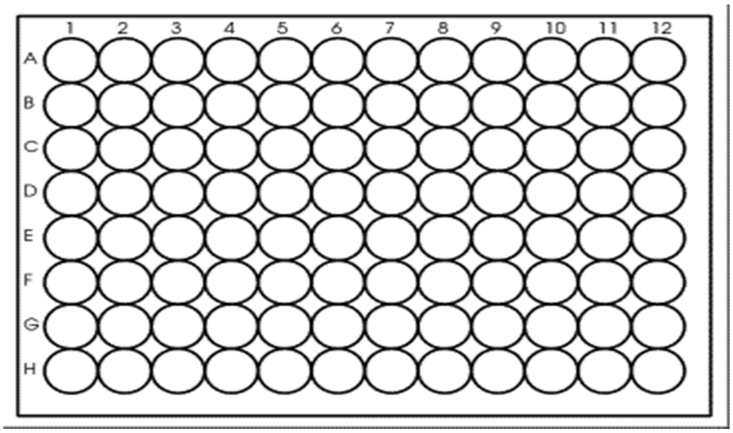


Figure 11: Diagram of a 96 well plate.

7. 50 ul CFD-buffer is added in the wells from Column 2 to Column 10 as well as in B11 and B12.

8. 50 ul of the diluted serum in the wells from A1 to H1 are transferred to the wells from A2 to H2 with a multi-channel pipette. The fluid is mixed thoroughly by pipetting 3 times and then 50 ul of it is transferred to the next row (A3-H3) and mixed thoroughly as aforementioned, followed by further serum serial dilution in the same way in wells from Column A4-H4 to Column A12-H12. Lastly, 50 ul of Column A12-H12 is discarded.

9. PRBCs are added in B11 and B12 as negative controls while A11 and A12 (positive controls) are left empty.

10. 50 ul PRBCs are then pipetted in the wells from Column 1 to Column 10. This is done by adding 50 ul PRBCs to each of the wells from A10 to H10 and then to each of the wells from A9 to H10 and so on, so as not to disrupt the serial serum dilution on the plate. This can be done with the same tips.

All wells which are not used for assay in the plate are left empty in steps 8 and 10.

11. The plate is covered with a lid and incubated at the temperature of 37 ºC, being shaken gently for 1 hour.

12. After the incubation 100 ul CFD buffer is added to all serum samples on the plate. The fluid in each well is mixed well by repeated pipetting. Care is taken not to use the same tips for serum dilution and negative controls and start from A10-H10 to

A1-H1 so as not to change the dilution of sera on the plate. Then, the plate is centrifuged at 20 ºC at 500 g for 5 min.

13. After centrifugation pellets are checked and if no pellets are observed, centrifugation is repeated. As soon as pellets are detected the supernatant is discarded by flinging out forcibly and care is taken not to shake the plate.

14. 200 ul CFD buffer is added to each of the wells used for the assay and mixed well. This step starts with the negative controls and then is performed from the highest dilution of serum (A10-H10) to A1-H1. It should be noted that the fresh tips are used for the negative controls.

15. The plate is then centrifuged at 500 g for 10 min at 20 ºC. Afterwards the pellets are checked before the supernatant is discarded in the same way as that in step 13.

16. To prepare Baby-rabbit-complement preparation, a pack of Baby-rabbit-complement is dissolved by adding 1 ml distilled water as well as 9 ml CFD-buffer.

17. 150 ul of Baby-rabbit-complement preparation is added to each well with the sample except A11 and A12.

18. 100 ul distilled water and 50 ul PRBCs are added in A11 and A12 (positive controls).

19. The plate is then covered with a lid and incubated at 37 ºC, being shaken for 1 hour.

20. Multichannel-Photometer is switched on and warmed up for at least ½ hour before use.

21. The plate is taken out of the incubator and then centrifuged at 500 g for 10 min at 20 ºC.

22. After centrifugation 100 ul supernatant is taken out from all the wells and transferred to the corresponding wells of another new plate with flat bottom for measurement.

23. Lastly, the samples are assayed for their optical absorption values at the wave length of 420 nm using Multichannel-Photometer.

Appendix B: Score Sheet

Score Sheet Herz-Xenotransplantation

Pavian Nr.	Datum	Uhrzeit	Versuchstag	KG präop

Flüssigkeits- und Nahrungsaufnahme	**absolutes Abbruchkriterium**	**Score**	**0**	**1**	**2**
Flüssigkeit (Wasser, Flüssignahrung) ml			400 -600	200-400	<200
Futter (Obst, Gemüse, Nüsse, Gräser)			normal	wenig	nichts

Verhalten/Psyche/Erscheinungsbild

Aufmerksamkeit			ja	mäßig	nein
Neugierde			ja	mäßig	nein
Aktivität			aktiv	mäßig aktiv	inaktiv
Rückzug			nein	mäßig	ja
abnorme Körperhaltung			nein	mäßig	ja
bedrücktes Erscheinungsbild			nein	mäßig	ja
Haarkleid			gepflegt	ungepflegt	struppig
abnorme Reaktionen auf einen Stimulus			nein	gelegentlich	immer
Automutilation	auftretende Automutilation				

allgemeine Krankheitsanzeichen

Körpertemperatur (°C)			< 39,5	39,5 40,5	> 40,5
Herzfrequenz (Eigenherz) (Schläge/min)			120-160	160-200	> 200
Atemfrequenz (Normo-, Brady-, Tachy-, Dyspnoe)			30-40	40-60	> 60
Dehydratationsgrad			< 6%	6-8%	> 8%
Augen (blass / eingefallen) und Augenlider (geschlossen)			nein	ggr.	hrg.
sichtbares Schmerzempfinden			nein	ggr.	hgr.
Geschwollenes / vorgewölbtes Abdomen			nein	ggr.	hgr.
Durchfall	anhaltend, therapieresistent		nein	Form erhalten	flüssig
Vomitus	anhaltend, therapieresistent		nein	Appetitlosigkeit	Erbrechen
Gewichtsverlust	> 20 %		0-5 %	5-10%	10-20%

versuchsspezifische Krankheitsanzeichen

Neurologische Auffälligkeiten (Tremor, Dyskinesien)			nein	ggr.	hgr.
Wundheilungsstörungen	anhaltend, therapieresistent		geringe Rötung	starke Rötung	Dehiszenz
Hinweise auf Lungenödem	schaumigem Auswurf		nein	ggr. RG	deutliche RG
Blutungen aus Körperöffnungen	anhaltend, therapieresistent		nein	mikroskopisch	makroskopisch

Abbruch bei Scoresumme ≥24		
absolutes Abbruchkriterium ja/nein		

Unterschrift

9 Acknowledgement

It would not have been possible to write this doctoral thesis without the help and support of the kind people around me, to only some of whom I would here like to express my thanks.

First of all, I would like to thank chairman of the Transregio Group (Xenotransplantation, FOR 535), Professor Bruno Reichart for providing all necessary facilities and allowing me to pursue my research work at the institute.

I am heartily thankful to my supervisor Professor Paolo Brenner for his full support and providing all facilities for conducting this work. I wish to express my thanks to Dr Eckart Thein for his kind support and valuable guidance in my study as well.

This thesis would not have been possible without the help, support and patience of Dr. Jan-Michael Abicht from whom I have learned so much, from figuring out what research topic is, to choosing a research agenda, to learning how to present my work and I owe my deepest gratitude to him. Many thanks specially go to Dr. Tanja Mayr for her persistent encouragement and unselfish help with correction and refinement of this work. The good advice, support and friendship of Dr. Michael Thormann has been invaluable at both an academic and a personal level, to whom I am extremely grateful.

I would like to acknowledge the CSC (China Scholarship Council) that provided the necessary financial support for my doctoral program.

I wish to thank Ari Dienel, Dirk Rosentreter, Shuai Lv, Song Shi, Jia Liu, Jie Pan and Peng Fu for helping me get through the difficult times, and for all the emotional support, entertainment, and care they provided.

I also would like to thank Walter Brendel Center of Experimental Medicine for their support and assistance since the start of my work, especially the head of department, Professor Ulrich Pohl for providing all necessary facilities and allowing me to carry out my research at the institute.

Specials thanks go to Dr. Stephan Buchholz and Dr. Sonja Güthoff of our research group. I learned a lot from them scientifically and they made my stay valuable and also pleasant.

Lastly, and most importantly, I wish to thank my parents for their never-ending support and encouragement. To them I dedicate this thesis.