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ORIGINAL ARTICLE

Hemodynamics in pig-to-baboon heterotopic thoracic cardiac xenotransplantation: Recovery from perioperative cardiac xenograft dysfunction and impairment by cardiac overgrowth

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Abstract

Introduction: Orthotopic cardiac xenotransplantation has seen notable improvement, leading to the first compassionate use in 2022. However, it remains challenging to define the clinical application of cardiac xenotransplantation, including the back-up strategy in case of xenograft failure. In this regard, the heterotopic thoracic technique could be an alternative to the orthotopic procedure. We present hemodynamic data of heterotopic thoracic pig-to-baboon transplantation experiments, focusing on perioperative xenograft dysfunction and xenograft overgrowth.

Methods: We used 17 genetically modified piglets as donors for heterotopic thoracic xenogeneic cardiac transplantation into captive-bred baboons. In all animals, pressure probes were implanted in the graft's left ventricle and the recipient's ascending aorta and hemodynamic data (graft pressure, aortic pressure and recipient's heart rate) were recorded continuously.

Results: Aortic pressures and heart rates of the recipients' hearts were postoperatively stable in all experiments. After reperfusion, three grafts presented with low left ventricular pressure indicating perioperative cardiac dysfunction (PCXD). These animals recovered from PCXD within 48 h under support of the recipient's heart and there was no difference in survival compared to the other 14 ones. After 48 h, graft pressure increased up to 200 mmHg in all 17 animals with two different time-patterns. This led to a progressive gradient between graft and aortic pressure. With increasing gradient, the grafts stopped contributing to cardiac output. Grafts showed a marked weight increase from implantation to explantation.

Conclusion: The heterotopic thoracic cardiac xenotransplantation technique is a possible method to overcome PCXD in early clinical trials and an experimental tool to get a better understanding of PCXD. The peculiar hemodynamic situation of increasing graft pressure but missing graft's output indicates outflow tract obstruction due to cardiac overgrowth. The heterotopic thoracic technique should be successful when using current strategies of immunosuppression, organ preservation and donor pigs with smaller body and organ size.

KEYWORDS
heart, heterotopic thoracic cardiac xenotransplantation, organ growth, organ overgrowth, PCXD, perioperative xenograft dysfunction, primary graft failure

1 | INTRODUCTION

A clinical application of cardiac xenotransplantation is now more realistic than ever – in 2022, the first human cardiac xenotransplantation of a genetically modified pig heart was performed as a compassionate use and the patient lived for 2 months.[1] This success was made possible by recent findings in pig-to-baboon experiments: a suitable combination of genetic modifications of the donor pigs,[2,3] the establishment of an immunosuppressive regimen with co-stimulation blockade of the CD40/CD40L pathway,[1,3–6] the use of a cold non-ischemic heart preservation with continuous perfusion to overcome perioperative cardiac xenograft dysfunction (PCXD)[2,7–11] and the importance of growth control to overcome xenograft overgrowth.[2,7,12,13]

However, it remains challenging to define the clinical application of cardiac xenotransplantation; the exact indication as well as the back-up strategy in case of xenograft failure are discused.[14] One option to solve this challenge could be the heterotopic thoracic cardiac transplantation technique. This procedure was clinically introduced by Christiaan Barnard and his team to overcome primary (human) graft failure.[15,16] The xenograft is connected to the recipient's heart, which is left in situ. So, the xenograft can partly or fully support the recipient's organ perfusion requirements.[17–19] Although the heterotopic technique is a clinically accepted method in human allotransplantation,[19–22] only the orthotopic procedure is currently performed, in part because of the complexity of the heterotopic procedure.

We also used the heterotopic technique in pig-to-baboon experiments.[23,24] At that time co-stimulation blockade of the CD40/CD40L pathway was not available and the significance of non-ischemic heart preservation[2,7,8] as well as growth inhibition of the donor hearts[2,7] was unknown. With these experiments, we were able to show that the heterotopic intrathoracic technique may be an option for clinical xenotransplantation. However, the immunosuppressive regimens proved insufficient and extensive cardiac overgrowth led to impingement of the recipient's own heart and compression of the lungs.[23,24]

Here, we present so far unpublished data of these experiments, especially in the light of recent findings regarding PCXD, xenograft overgrowth and the first clinical application of cardiac xenotransplantation as compassionate use. Based on this analysis, we discuss the role of these phenomena in our heterotopic experiments as well as possible implications for the clinical application of cardiac xenotransplantation.

2 | MATERIALS AND METHODS

2.1 | Animals

We used 17 genetically modified piglets as donors for heterotopic thoracic xenogeneic cardiac transplantation (Table 1) – cross-bred genetic background (German Landrace and Large White), genotype: α1,3-galactosyltransferase (GGTA1) homozygous knockout/hCD46 ($n = 9$), GGTA1 homozygous knockout/heterozygous hCD46/heterozygous thrombomodulin (hTM) ($n = 7$) or GGTA1 homozygous knockout/heterozygous hCD46/heterozygous HLA-E ($n = 1$), blood group: 0/0 ($n = 13$), 0/A ($n = 1$) or not available ($n = 3$)

TABLE 1 Pre- and post-operative data of donors and recipients.

Experiment	Donor genetics	Donor bloodgroup	Recipient bloodgroup	Survival [days]	Graft weight - implantation [g]	Graft weight - explantation [g]
1	GT-KO/hCD46	0/0	A/B	1	78	108
2	GT-KO/hCD46/hTM	0/0	B	17	74	106
3	GT-KO/hCD46/hTM	0/0	A/B	18	84	124
4	GT-KO/hCD46/hTM	0/0	B	12	105	182
5	GT-KO/hCD46/hTM	0/0	B	19	100	162
6	GT-KO/hCD46/hTM	0/0	B	37	118	n/a
7	GT-KO/hCD46	0/0	B	14	135	152
8	GT-KO/hCD46	n/a	A/B	0	115	n/a
9	GT-KO/hCD46	n/a	A/B	17	83	n/a
10	GT-KO/hCD46	n/a	A/B	7	57	67
11	GT-KO/hCD46	0/0	A/B	15	64	84
12	GT-KO/hCD46/HLA-E	0/A	A/B	35	76	115
13	GT-KO/hCD46	0/0	A/B	16	82	140
14	GT-KO/hCD46	0/0	A/B	3	146	125
15	GT-KO/hCD46	0/0	B	18	74	113
16	GT-KO/hCD46/hTM	0/0	A/B	13	60	104
17	GT-KO/hCD46/hTM	0/0	A/B	35	46	143

Abbreviation: n/a, not available.

(Revivicor, Blacksburg, VA, USA and Institute for Molecular Animal Breeding and Biotechnology, Gene Center, Faculty of Veterinary Medicine, LMU, Munich, Germany).

Captive-bred baboons ($n = 17$) served as recipients (*Papio anubis* and *Papio hamadryas*), blood group: B ($n = 6$) or A/B ($n = 11$) (German Primate Center, Göttingen, Germany).

The study was approved by the local authorities and the government of Upper Bavaria. All animals received treatment in accordance to the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institute of Health (NIH publication No. 85-23, 1985) and the German Law for the Care of Experimental Animals (German Legislation for the Welfare of Laboratory Animals, Section 5, §7-§9a).

2.2 | Anesthesia

Donor anesthesia was conducted with fentanyl (Fentanyl-Janssen, Janssen-Cilag GmbH, Neuss, Germany) and propofol (Propofol-Lipuro 2%, B. Braun Melsungen AG, Melsungen, Germany), as described in detail elsewhere.[23]

Baboons were pre-medicated with intramuscular applications of midazolam (Midazolam-ratiopharm, ratiopharm GmbH, Ulm, Germany) and ketamine hydrochloride (Ketavet, Pfizer Deutschland GmbH, Berlin, Germany). Anesthesia was conducted with fentanyl (Fentanyl-Janssen, Janssen-Cilag GmbH, Neuss, Germany) and propofol (Propofol-Lipuro 2%, B. Braun Melsungen AG, Melsungen, Germany), also described in detail elsewhere.[23]

2.3 | Surgical technique

The surgical technique has been published in detail elsewhere.[23–25] Briefly, after median sterno-/pericardiotomy of the donor animal heparin was given, the ascending aorta cross-clamped and the heart perfused with a single dose (20 ml/kg) of 4°C histidine-tryptophan-ketoglutarate cardioplegia (HTK, Bretschneider solution, Custodial, Dr. F. Köhler, Bensheim, Germany).[23,24,26,27] Then the donor heart was removed and secured in plastic bags filled with cold Bretschneider solution and stored until implantation in a box filled with crushed ice (following a clinical routine).

Only then, the baboon recipient's thorax was opened at midline, after administration of heparin cardiopulmonary bypass was established and the recipient's heart also cardiopleged using Bretschneider solution. The donor heart was placed into the right chest. Subsequently, both left and right atria were conjoined and aortic end-to-side anastomosis was done. The two main pulmonary arteries were connected end-to-side by interposition of a vascular graft (Figure 1). Reperfusion of both hearts started with opening of the aortic clamp. Both hearts were defibrillated separately and warming commenced. A reperfusion period of 60 min was deemed necessary. Thereafter gradual weaning from the heart-lung machine started.

Following surgery, the baboons were weaned from ventilation and put into their cages when adequately awakened from anesthesia. Postoperative analgesia was ensured by a continuous infusion of fentanyl, ketamine hydrochloride and metamizole (Novaminsulfon-ratiopharm, ratiopharm GmbH, Ulm, Germany). All animals were housed individually and a jacket with a tethering system connected to

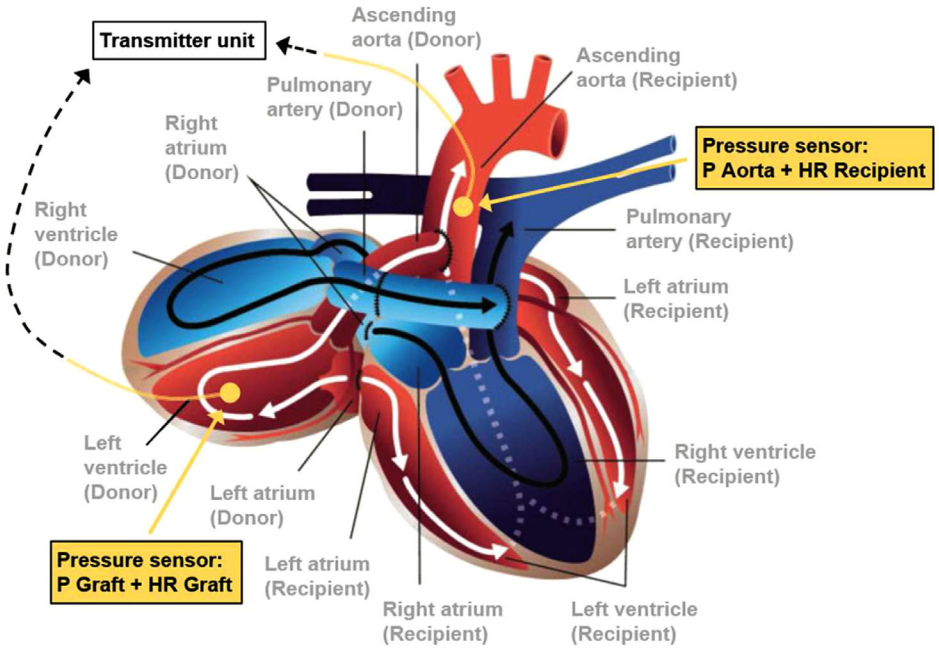


FIGURE 1 Illustration of the heterotopic thoracic surgical technique and of the localization of the two pressure probes in the graft's left ventricle and the recipient's ascending aorta.

a central venous catheter was used to apply immunosuppressive and other drugs as published elsewhere in detail.[23]

2.4 | Telemetric monitoring system

A wireless transmitter DSI PhysioTel Multiplus D70-PCTP (Data Sciences International, St. Paul, MN, USA) was used for hemodynamic monitoring. For pressure monitoring of the donor heart (graft), one pressure probe was placed through the apex in its left ventricle before declamping of the aorta. The second pressure sensor was placed in the recipient's ascending aorta through the punch hole used to administer cardioplegic solution during cardiopulmonary bypass. Both pressure lines were secured with two purse-string sutures with pledgets (Figure 1). Temperature was measured at the transmitter housing in a subcutaneous pouch on the right medioclavicular line. Measurements started immediately after implantation and were transmitted continuously for the duration of each experiment. All channels were displayed in real time on a computer screen in the operating room and recorded for off-line analysis (Dataquest A.R.T. system, Data Sciences International/DSI, St. Paul, MN, USA). For adequate signal quality, the animal cages were equipped with two receivers, during surgery an additional mobile receiver was used (RMC-1, DSI, St. Paul, MN, USA).

2.5 | Data analysis

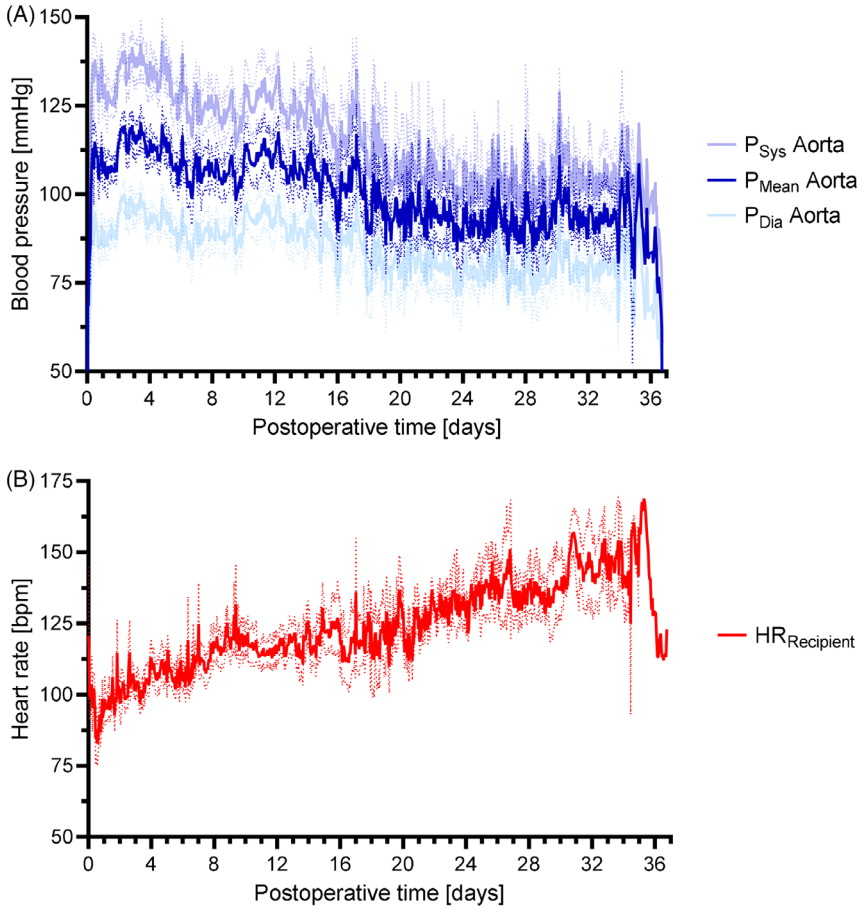
Telemetric data acquisition started after weaning from cardiopulmonary bypass and lasted until the end of the experiment. Data was analyzed with the Ponemah Physiology Platform (DSI, St. Paul, MN, USA). Heart rates were automatically derived from the telemetric arterial pressure curves by Ponemah. Data were reduced by calculating median values of pressure and heart rate measurements for each postoperative hour. Data were processed and analyzed with Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA) and GraphPad Prism 8.01 (GraphPad Software Inc., San Diego, California, USA). Data are presented as mean values +/- SD if not indicated otherwise.

3 | RESULTS

We present previously unpublished hemodynamic data. Other data, for example, pre- and postoperative immunologic parameters, causes of death and myocardial histological findings have not been subject of this data analysis and have been published elsewhere.[23,24] Only pig-to-baboon heterotopic thoracic cardiac xenotransplantation experiments with complete hemodynamic monitoring have been included in this analysis, which led to a total of 17 experiments.

3.1 | Survival data and overall systemic pressure and heart rate measurements

Mean survival of all 17 animals was 16 days with a range from 0 up to 37 days (Table 1). Aortic pressure showed a similar course in all 17 experiments. Mean aortic pressure (P_{Mean} Aorta) was 109 +/- 6 mmHg on average in the first 12 postoperative days (POD), 99 +/- 7 mmHg from POD 13 to POD 24 and declined to 92 +/- 7 mmHg from POD 25 to POD 37 (Figure 2A,C). With decreasing systemic pressure, pulse pressure ($PP = P_{systolic} - P_{diastolic}$) also declined from 38 +/- 4 mmHg (POD 0 to 12), to 30 +/- 5 mmHg (POD 13 to 24) and to 25 +/- 5 mmHg



(C)

Postoperative days	P_{Sys} Aorta [mmHg]	P_{Mean} Aorta [mmHg]	P_{Dia} Aorta [mmHg]	Pulse Pressure [mmHg]	$HR_{Recipient}$ [bpm]
0-12	128 +/- 7	109 +/- 6	91 +/- 6	38 +/- 4	109 +/- 9
13-24	114 +/- 9	99 +/- 7	84 +/- 6	30 +/- 5	122 +/- 7
25-37	104 +/- 7	92 +/- 7	78 +/- 6	25 +/-4	140 +/- 10

FIGURE 2 Systolic (P_{Sys} Aorta), mean (P_{Mean} Aorta) and diastolic (P_{Dia} Aorta) pressure in the proximal aorta (A) and heart rate of the recipient's heart ($HR_{Recipient}$) (B). Mean values +/- SEM, $n = 17$. In the table (C) mean values +/- SD for three periods of the experiments are listed.

(POD 25 to 37) (Figure 2A,C). By contrast, the recipients' heart rates ($HR_{Recipient}$) increased from 109 +/- 9 bpm (POD 0 to 12), to 122 +/- 7 bpm (POD 13 to 24) and to 140 +/- 10 bpm (POD 25 to 37) (Figure 2B,C).

3.2 | Graft pressure in the early postoperative phase

During the first 48 postoperative hours we identified two fundamentally different patterns of graft pressure (P_{Sys} Graft). In three experiments, P_{Sys} Graft was markedly lower than P_{Mean} Aorta after reperfusion. After 10 postoperative hours, for example, mean P_{Sys} Graft was 31 mmHg, while mean P_{Mean} Aorta was 126 mmHg. Over the course of the initial 48 hours, P_{Sys} Graft steadily recovered, reaching approximately same levels as P_{Mean} Aorta (Figure 3A). After recovery of P_{Sys} Graft, there was no marked decrease in survival in these three experiments. The three animals survived up to 19 days, mean survival was 12 days (mean survival in all 17 experiments: 16 days). In the other 14 experiments, P_{Sys} Graft was similar to P_{Mean} Aorta during the first 48 postoperative hours (Figure 3B), no initial decrease in P_{Sys} Graft was observed.

3.3 | Graft pressure after the early postoperative phase

Analysis of P_{Sys} Graft after the first 48 postoperative hours revealed similar courses in all experiments, but with two different time-patterns in the group of shorter (group I, survival up to 19 days, $n = 14$,

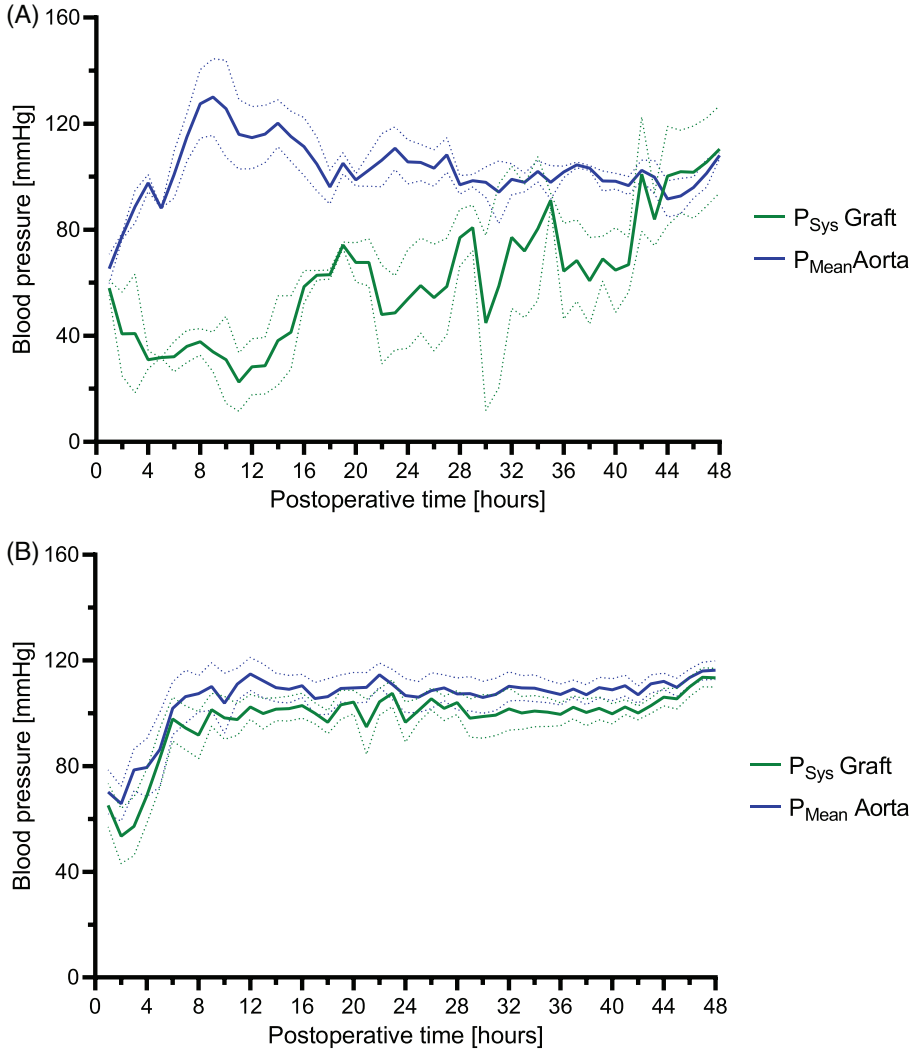


FIGURE 3 Mean pressure in the aorta (P_{Mean} Aorta) and systolic pressure in the graft (P_{Sys} Graft) in the experiments, in which P_{Sys} was markedly lower than P_{Mean} Aorta after reperfusion (A, $n = 3$) and in the other experiments of the study group (B, $n = 14$). Mean values +/- SEM.

Figure 4A) and longer survivors (group II, survival up to 37 days, $n = 3$, Figure 4B). In both groups four different phases of P_{Sys} Graft could be discriminated:

1. concurrent courses of P_{Sys} Graft and P_{Mean} Aorta,
2. constantly increasing P_{Sys} Graft,
3. "plateau" phase of P_{Sys} Graft > 160 mmHg with a maximum of about 200 mmHg,
4. constantly decreasing P_{Sys} Graft.

Each phase was markedly longer in group II compared to group I, for example 11 versus 6 days for the first phase.

3.4 | Contribution of the graft to overall cardiac output and changes in graft weight

During phase one, in all experiments contraction of the graft led to pressure pulses which were transmitted into the aortic pressure curve as visible "bumps", indicating cardiac output of the graft (Figure 5A). In the following phases there was no transmission of graft's pressure pulses in the aortic pressure curve, although P_{Sys} Graft was consistently higher than P_{Mean} Aorta (Figure 5B,C). Grafts showed a marked weight increase from a mean of 84 g at implantation to a mean of 123 g at explantation (Figure 6).

4 | DISCUSSION

4.1 | PCXD is a reversible phenomenon in heterotopic pig-to-baboon cardiac xenotransplantation

Our hemodynamic data of heterotopic thoracic experiments demonstrate that PCXD is a reversible phenomenon.

Graft pressure was markedly lower than aortic pressure in three experiments after reperfusion. When ventricular graft pressure is

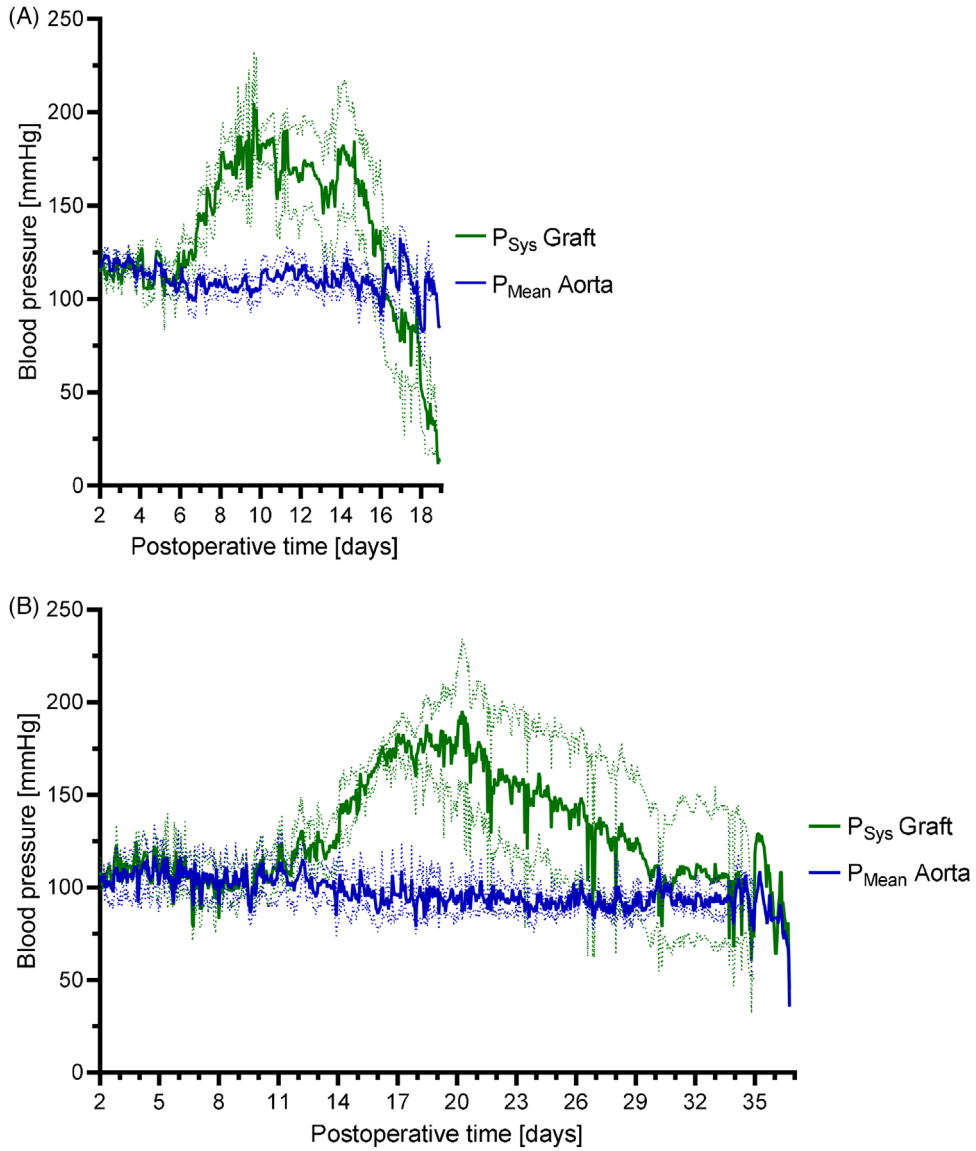


FIGURE 4 Systolic pressure in the graft (P_{Sys} Graft) and mean pressure in the aorta (P_{Mean} Aorta) in group I (A, animals surviving up to 19 days, $n = 14$) and in group II (B, animals surviving up to 37 days, $n = 3$). Values are given as mean +/- SEM.

lower than aortic pressure generated by the recipient's heart, the graft does not eject and thus does not contribute to cardiac output. We interpret this as a sign of severely impaired graft function/systolic graft failure. The three animals showed no signs of rejection.[23] This is in accordance with the definition of PCXD — graft failure within 24–48 h of transplantation that is independent of immune organ rejection.[8,10,11,28] As published elsewhere, there was no difference in the clinical appearance of these animals compared to the other ones, because systemic circulation was maintained by the healthy recipient's heart.[23] Ventricular function of all three grafts suffering from PCXD improved within 48 h under support of the recipient's heart and remained similar to experiments not affected by PCXD. This proves that PCXD is a reversible phenomenon. There is only one reported orthotopic experiment attempting to overcome PCXD with prophylactic initiation of extracorporeal membrane oxygenation (ECMO) support, but this was found to be insufficient.[5] Although the occurrence of PCXD has been bluntly reduced by the use of non-ischemic heart preservation,[2,8] it remains a threat to xenograft survival in the orthotopic position and occurs independently of mechanisms of rejection.[5]

Our findings support the thesis that the heterotopic model could be a possible method to overcome PCXD (or other forms of xenograft failure) in early clinical trials.[14,17] In our current study, PCXD was reversible with the support of hearts from healthy baboons. In the case of a future patient with terminal heart failure we cannot be sure whether the severely impaired organ function will be able to generate sufficient support to reverse PCXD as well. However, data from heterotopic cardiac allotransplantations show that even the residual myocardial function of the remaining native heart was able to hold up sufficient hemodynamics in cases of allograft failure.[22,29–31] Moreover,

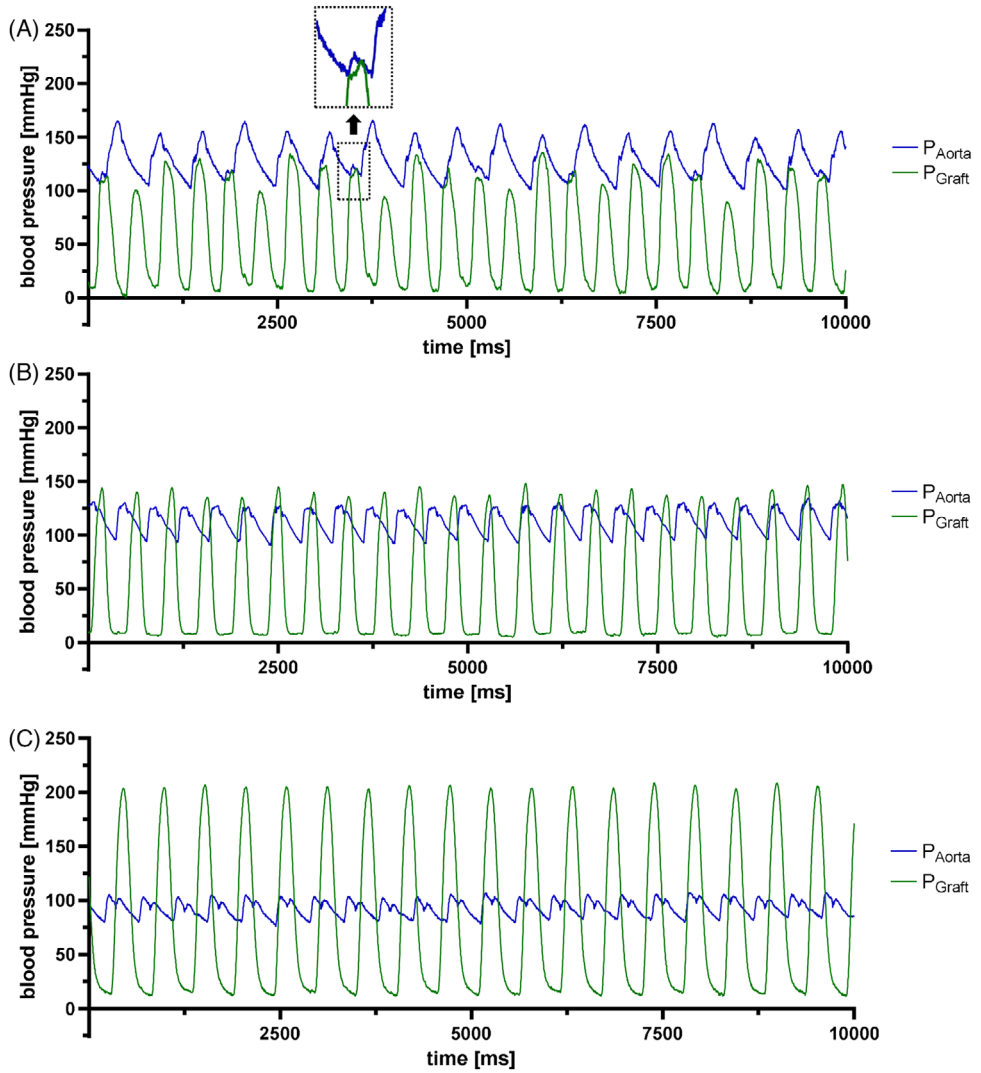


FIGURE 5 Aortic (P_{Aorta}) and graft's left ventricular pressure (P_{Graft}) in phase 1 (A), phase 2 (B) and phase 3 (C) of one representative experiment.

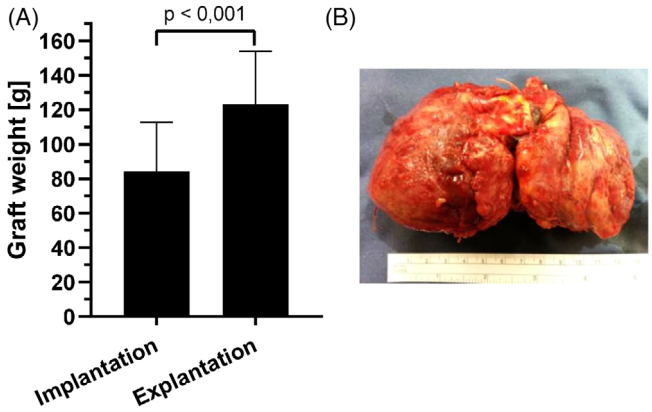


FIGURE 6 Graft weight at implantation and explantation (A, $n = 14$, 3 explantation weights not available, see also Table 1, paired t test). Macroscopic view of the graft (right) and the recipient's heart of one representative experiment at POD 19 (B). The graft was initially smaller than the recipient's heart (picture already published[23]).

the heterotopic thoracic technique could be used as an experimental tool to gain a better understanding of PCXD and develop further treatment options.

4.2 | Graft overgrowth leads to increased graft pressure and impaired cardiac output of the graft

After the initial phase of concurrent pressure in the graft and aorta, left ventricular graft pressure gradually increased to values up to 200 mmHg, whereas aortic pressure did not increase. This led to a progressive gradient between left ventricular graft and aortic (systemic) pressure. As long as there was no significant gradient, both hearts contributed to cardiac output. With increasing gradient, the graft stopped contributing to cardiac output. As reported previously,[23,24] the grafts in our heterotopic thoracic model showed extensive myocardial growth. Before we applied the current growth inhibition strategies in orthotopic xenotransplantation experiments,[2,7] donor hearts also

showed extensive growth in the orthotopic model. In these orthotopic experiments the hearts showed signs of dynamic left ventricular outflow tract obstruction, similar to hypertrophic obstructive cardiomyopathy (HCM) in humans.[32] In HCM, cardiac thickening leads to a high-pressure left ventricular apical chamber, while the outflow tract remains free of obstruction and therefore presents with lower pressure.[33,34] We assume, that cardiac overgrowth led to a similar situation in our heterotopic experiments – increasing ventricular pressure in the apical chamber (where the pressure probe is placed) and lower pressure (than aortic pressure) in the graft's outflow tract. This could explain the combination of increasing graft pressure but missing contribution to cardiac output.

However, one cannot directly compare the heterotopic and orthotopic thoracic transplantation model and obstructive HCM. Therefore, our current analysis provides only indications of this phenomenon and further studies in the heterotopic (and orthotopic model) would be needed to clarify our hypothesis in more detail.

4.3 | Graft overgrowth is also reflected in systemic pressure and recipient's heart rate

When we planned the heterotopic experiments, we did not expect any deleterious graft effect on systemic circulation, as the healthy recipients' hearts remain fully functional. However, based on our current analysis, we assume a progressive hemodynamic impairment of the recipient's heart and systemic circulation by graft overgrowth. Assuming stable vascular properties, the lowering of PP from 38 to 25 mmHg indicates a significant reduction of stroke volume (SV).[35–38]

As already published, transthoracic echocardiography and autopsy results are pointing towards a reduced ventricular filling of the recipient's heart, caused by a compression of the overgrowing xenograft.[23] To maintain cardiac output of the recipient's heart, $HR_{Recipient}$ had to increase, thus continuously compensating for the decreasing SV. The declining course of P_{Mean} Aorta indicates, however, that this compensation was insufficient over time. To summarize our findings, the courses of aortic pressure, PP and $HR_{Recipient}$ indicate mechanical impairment of the recipient's heart by graft overgrowth and so highlight the importance of growth inhibition in cardiac xenotransplantation. Similar to preclinical orthotopic cardiac xenotransplantation experiments, the ubiquitous growth hormone inhibitor rapamycin may be given.[2,7] Ideally, organs from smaller donor animals would also be used in the heterotopic thoracic setting such as pigs with growth hormone receptor deficiency[12,13] or Auckland Island pigs.[17]

5 | CONCLUSION

PCXD is a reversible phenomenon in the heterotopic thoracic cardiac xenotransplantation model. These findings qualify this technique as a possible method to overcome PCXD in early clinical trials and as an experimental tool to get a better understanding of PCXD and possible prevention and treatment options.

Furthermore, our current analysis highlights the significance of cardiac overgrowth. In synopsis with the current knowledge of cardiac xenotransplantation, the heterotopic thoracic technique should be successful using current strategies of immunosuppression, organ preservation and donor pigs with smaller body and organ size.

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CONFLICT OF INTEREST STATEMENT

Bruno Reichart, Eckhard Wolf, Paolo Brenner, Matthias Längin and Jan-Michael Abicht are founding members of XTransplant GmbH. David Ayares is chief executive officer and chief scientific officer of Revivicor Inc. The other authors have no conflicts of interest to disclose.

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