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COMMENTARY

Clinical cardiac xenotransplantation first in the clinical arena

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1 | INTRODUCTION

In the July/August 2021 issue of this journal, Thompson et al.[1] discussed the "potential detrimental role of inflammation in pig orthotopic heart xenotransplantation." After dealing with common reactions to porcine tissues, the authors focused on severe pathological responses due to the cardiopulmonary bypass that is necessary to keep the baboon recipients alive during heart replacement, emphasizing the susceptibility of the lungs to injury in this setting. In the concluding section of the commentary, various treatment options were suggested, including anti-inflammatory therapy and genetic engineering of the donor pig.

We are of the opinion, however, that minimizing or even avoiding possible detrimental processes associated with the use of a heart-lung machine must be preferable. Consequently, the following comment will focus on improved cardio-surgical techniques. It is written not only for experts in our field, but also those (including possible investors) who are not necessarily familiar with these surgical issues.

1.1 | Past and present of heart-lung machine technologies in humans

Experimental work on whole body perfusion was conducted in the late 1930s by John and Mary Gibbon at the Massachusetts General Hospital, Boston. In their device, which was a huge apparatus twice the size of a refrigerator, a roller-pump replaced the heart and a grid/screen oxygenator the lungs. The latter part was placed within an oxygen chamber in which a thin layer of blood flowed down the large surface of a metal grid or screen.[2]

Interrupted by World War II, the Gibbons applied their technique in humans in 1954, unfortunately without success.[3]

A year later, John Kirklin, then at the Mayo Clinic in Rochester, USA, reported on the first successful cases using his modified apparatus, the Mayo-Gibbon heart-lung machine[4] (it should be mentioned in this context, that Kirklin left the Mayo Clinic in 1967 to join the University of Alabama at Birmingham, the place where Thompson and colleagues[1] are now working).

It needed the genius of DeWall and Lillehei of the University of Minneapolis, just 80 kilometers away from Rochester, to simplify the oxygenator by introducing a disposable bubble technique in which oxygen was added to a patientt's blood in the form of small bubbles.[5,6]

Today, the simple principles of a heart-lung machine are still more or less the same. There is one major difference: the blood is now oxygenated in a less damaging hollow-fiber membrane device, consisting of many tiny polymer tubes separating the surrounding patient's blood.[7] The tubing is semipermeable for oxygen and carbon dioxide, but not for blood components. The two gases exchange in between the two compartments according to their partial pressure gradients:

oxygen into the desaturated blood, carbon dioxide away into the gaseous part.

Since its very first application, the pathophysiologic effects of whole-body perfusions were recognized and studied intensively.[8–11] Whenever a patient's blood enters the nonendothelialized surfaces of the tubings and the oxygenator, damaging reactions are immediately initiated on all cellular components:

- erythrocytes hemolyse due to abnormal sheer stress,
- platelets are activated and adhere to foreign plastic materials especially within the oxygenator,
- leukocytes and their cytokines cause whole-body inflammation now called systemic inflammatory response syndrome.

There are also damaging processes involving noncellular blood components, such as complement and kallikrein activation, stimulation of the coagulation cascade and hyperfibrinolysis. Concomitantly, the endothelial cells of the organs, especially of the lungs, are also affected.

Although this brief summary of possible detrimental reactions triggered by the use of a heart-lung machine might indeed sound frightening to a noncardiac surgeon (as is the description of Thompson and colleagues[1]), it is a fact that most sick patients, some of them in end-stage heart disease, survive the operations and improve after a relatively short course of post-operative care.

One must however consider that there exist important differences if one compares clinical allogeneic and preclinical xenogeneic heart transplantation procedures: porcine donor organs originate from healthy animals with no previous brain damage, including the necrosis of the pituitary gland and the corresponding hormonal deficits.[12]

1.2 | Preconditions and immediate preclinical results after successful porcine orthotopic cardiac xenotransplantation

So, why are the results after orthotopic porcine heart-xenotransplantation "mixed and largely poor" as Thompson and colleagues[1] write? Theoretically, one would expect rather the opposite. The question may be tackled by remembering John Kirklin's old axiom: "Cardiosurgical interventions fail due to errors or/and lack of knowledge."[10]

Due to genetic differences between pigs and primates/humans, there are not only immunologic but also physiologic barriers to overcome: sure, stable genetic modifications are a prerequisite, but so are adjustments to address certain porcine pathophysiologic responses. Porcine hearts are obviously more vulnerable to prolonged ischemia times, even if cooling is added.

Consequently, our group adopted several new strategies for orthotopic cardiac xenotransplantation experiments to avoid perioperative cardiac xenograft dysfunction,[13] as reported in greater detail elsewhere.[14-16] One very important step was the implementation of a nonischemic heart preservation technique, which was introduced in cooperation with S. Steen's group from Lund University.[17] In brief, the explanted porcine hearts were immediately perfused in a small heart-lung machine (now Xvivo, Gothenburg, Sweden) using low pressure and flow-controlled 8°C oxygenated, hyperoncotic cardioplegic nutrition solution with hormones, and erythrocytes of porcine or primate origin (Steen solution).

For heart replacement, the preclinical/clinical use of a heart-lung machine must cause as little damage as possible: We found that normal roller pumps were sufficient and used the Terumo Capiox FX05 oxygenator (Terumo Europe) made of microporous polypropylene hollow fibers coated with an anti-thrombotic polymer. The priming volume consisted of 190 mL 5% albumin; the flow rate for a 15 kg baboon was 1.5 L per minute.

The donor organ perfusion was continued intermittently during surgery. Since the heart remained at 8°C, the recipient's body was only lowered to 34°C. After finishing the implantation, Shumway's and Lower's orthotopic technique[18] was applied, only a short rewarming (no reperfusion, since there is no ischemic damage!) to normal body temperatures was therefore necessary.

Care was taken to keep the recipient's hemoglobin levels always above 10 g/dl. If below, primate blood was transfused. Urinary output was maintained above 1–2 mL/kg/h. Its color remained clear without signs of hemolysis.

During weaning from cardio-pulmonary bypass, and shortly thereafter, catecholamine support (Noradrenalin, Adrenalin) was low and decreasing till it was completely stopped.[13,15] The choice of catecholamines was secondary if the need was reduced to a minimum. Echocardiographically, right and left ventricles performed normally,[15] and there were no fluids within the pericardial or pleural cavities. When compared to the baboon's own heart function, the porcine cardiac index after surgery was slightly inferior (3.88 +/− 0.65 vs. 4.97 +/− 0.75 L/min/m^2) but improved after closing the chest.[16]

Central venous oxygen saturation decreases when tissue (oxygen-) contents are insufficient and are compensated with an increased arterial extraction. When compared to preoperative data, the baboon measurements were slightly inferior postoperatively but remained within normal ranges. Serum lactate levels always remained normal within the 12 postoperative hours.[15] According to these measurements, graft ischemia was avoided at all times.

Consequently, all animals were successfully weaned from ventilation and the time between closure of the skin and extubation measured on average 296 min. Shortly thereafter, the baboons were transferred to their cages, breathing normally on continuous pain medication. Three intensivists/anesthetists and three primatologists/veterinarians, all experts in their field, shared the responsibility of the postoperative care.

The following three graphs summarize our serum measurements during and after surgery:

- Figure 1 depicts heart (troponin), kidney (creatinine), and liver (bilirubin) function.
- Figure 2 shows hemoglobin levels, serum lactate dehydrogenase, a measure of hemolysis (LDH) and platelet counts.
- Figure 3 shows markers of whole-body inflammation (leukocyte counts, C-reactive protein [CRP], and interleukin [IL] 6).

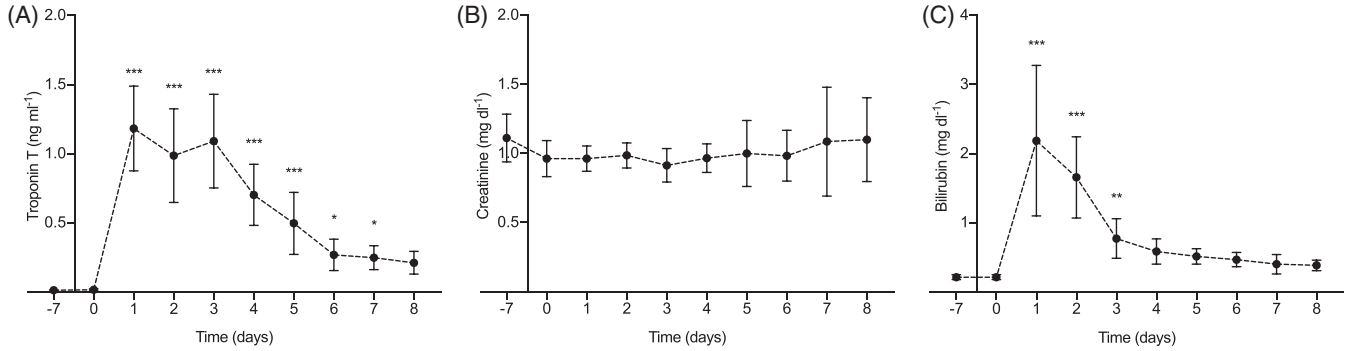

FIGURE 1 Serum parameters of various organ functions during the first 8 days after pig-to-baboon orthotopic heart transplantations. (A) Troponin levels as indicator of cardiac damage due to surgery rose significantly after transplantation but dropped quickly within the first postoperative week. (B) Creatinine levels were never elevated after transplantation, indicating no kidney damage due to cardiopulmonary bypass (CPB). (C) Bilirubin levels dropped quickly to normal after an initial perioperative rise, possibly caused by indirect bilirubin and hemolysis during CPB. Data are presented as means and standard deviations (SD). Statistically significant differences to control measurements 1 week prior to surgery were determined using paired two-sided Student's *t*-tests ($n = 8$), corrected for multiple testing using the Bonferroni method ($^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$)

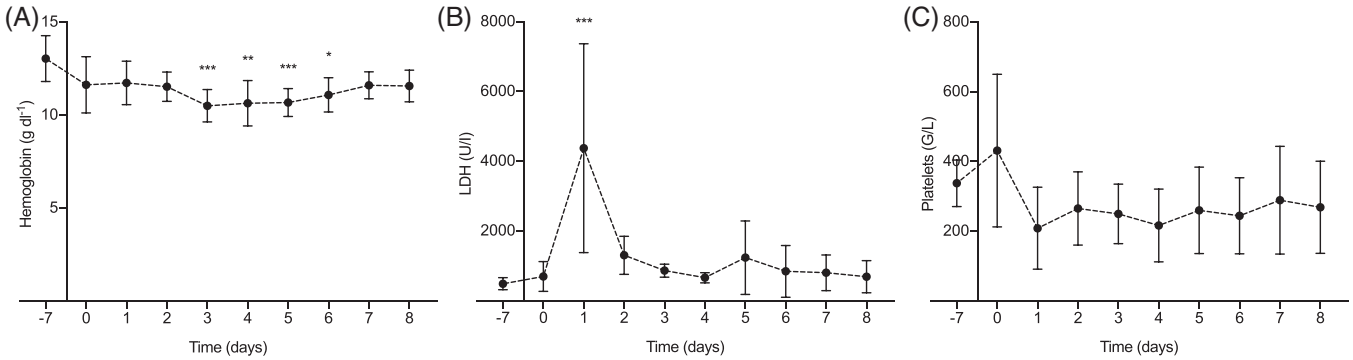

FIGURE 2 Parameters of hemolysis and coagulation during the first 8 days after pig-to-baboon orthotopic heart transplantations. (A) Hemoglobin levels did not decrease significantly after surgery. This was achieved by meticulous surgical hemostasis, and, if necessary, transfusion of primate blood within the first 24 h; target Hemoglobin levels >10 g dL^{-1} were aimed at. (B) Serum LDH increased after surgery, indicating hemolysis during cardiopulmonary bypass (CPB), but returned to normal within the first week. (C) Platelet counts did not significantly change, indicating only minor consumption during CPB and cardiac surgery. Data are presented as means and SD. Statistically significant differences to control measurements 1 week prior to surgery were determined using paired two-sided Student's *t*-tests ($n = 8$), corrected for multiple testing using the Bonferroni method ($^{*}p < .05$, $^{**}p < .01$, $^{***}p < 0.001$)

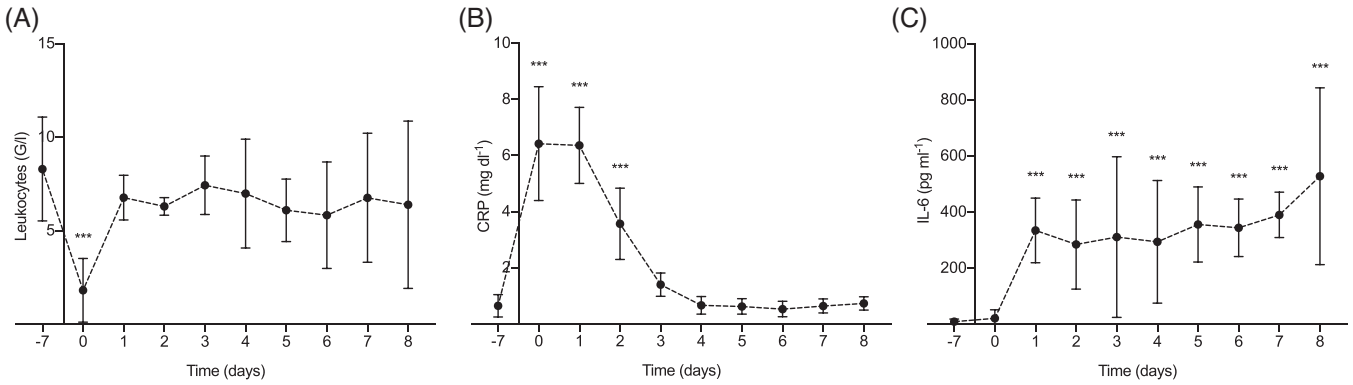

FIGURE 3 Serum parameters of inflammation during the first 8 days after pig-to-baboon orthotopic heart transplantations. (A) Leukocyte counts decreased prior to transplantation possibly due to immunosuppressive therapy; overall, there was no significant increase in leukocyte counts in the following week, although there were great differences between the individual animals. (B) C-reactive protein (CRP) levels were significantly elevated perioperatively as an acute phase reaction to surgery but normalized very quickly within 1 week. (C) Interleukin (IL)-6 levels were significantly elevated postoperatively and did not normalize within the first postoperative week. Data are presented as means and SD. Statistically significant differences to control measurements 1 week prior to surgery were determined using paired two-sided Student's *t*-tests ($n = 8$), corrected for multiple testing using the Bonferroni method ($^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$)

Remarkably, serum IL-6 levels did not return to baseline within the first postoperative week, which was probably caused by the treatment with the anti-IL-6 receptor antibody tocilizumab.[19] In contrast to Thompson and colleagues,[1] however, we did not observe any signs of severe organ dysfunction despite these elevated levels of IL-6, and the slightly abnormal changes disappeared within the first postoperative week; creatinine levels stayed normal throughout the full observation time.

In summary, positive short- and long-term results were and will be possible after orthotopic cardiac pig-to-baboon transplantation. The unavoidable use of the heart-lung machine had no overt detrimental influence: there were no signs of lasting end-organ failures, and especially remarkable was that lung function was normal and that cardiac function recovered rapidly, thus minimizing the potentially harmful catecholamine therapy and providing optimal graft oxygenation. When compared to allogeneic procedures, however, several preconditions had to be met: stable genetic modifications were of course a necessity, but also the nonischemic organ preservation and the handling of the recipient during and immediately after the use of the heart-lung machine.

Hearts are simple organs, much simpler than kidneys. Genetically modified hearts are easy to handle in the postoperative course, obviously easier when compared to kidneys[20] (even if one considers the better, but less consistent, long-term results after xenogeneic renal replacements[21–23]). Therefore, it could be argued that porcine hearts should be first in the clinical arena.

After receiving "compassionate use authorization" from the U.S. Food and Drug Administration, the Baltimore cardio-surgical team led by B. Griffith and MM Mohiuddin of the Maryland Medical University Center transplanted for the first time a genetically modified porcine heart (developed by D. Ayares and colleagues, Revivicor/United Therapeutics) into a 57-year-old American, who was ineligible for a human organ.[24,25] The patient was in biventricular failure with irregular rhythm, presumable atrial fibrillation. Under these circumstances, inotropic substances did not work effectively, also the possible support of one (or two) mechanical pump(s). An extracorporeal membrane oxygenation (ECMO, see above Section 2 on heart-lung-machine technologies) system seemed to be the last resort, maintaining the patientt's life for more than a month preoperatively. The long venous cannula was placed within the right atrium, the oxygenated blood returned with the help of a small pump into the femoral artery.

The donor animal weighed 240 pounds, approximately 110 kg. The *GGTA1*, *CMAH*, and *B4GALNT2* genes were deleted, and six human genes were included to reduce humoral and cellular rejection reactions, unspecific inflammation, and thrombotic microangiopathy; a knock-out of the growth hormone receptor[26] was also included with the goal of preventing overgrowth of the donor organ. The explanted porcine heart was perfused (Xvivo, Gothenburg, Sweden, see above Chapter 3) at low pressures with the 8°C oxygenated, hyperoncotic Steen solution[14–17]; Mohiuddint's immunosuppression was applied.[27]

The patient was extubated after surgery without any problems and the ECMO removed on POD 3. He is well and orientated.

The Munich team and all authors whole-heartedly congratulate the Baltimore team on their achievement: very well done.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

REFERENCES

1. Thompson CP, Jagdale A, Walcott G, et al. A perspective on the potential detrimental role of inflammation in pig orthotopic heart xenotransplantation. *Xenotransplantation*. 2021;28(4):e12687.
2. Gibbon JH Jr. The maintenance of life during experimental occlusion of the pulmonary artery followed by survival. *Surg Gynec Obst*. 1939;69:602-614.
3. Gibbon JH Jr. Application of a mechanical heart and lung apparatus to cardiac surgery. *Minn Med*. 1954;37(3):171-185.
4. Kirklin JW, Dushane JW, Patrick RT, et al. Intracardiac surgery with the aid of a mechanical pump-oxygenator system (gibbon type): report of eight cases. *Proc Staff Meet Mayo Clin*. 1955;30(10):201-206.
5. Dewall RA, Gott VL, Lillehei CW, et al. A simple, expendable, artificial oxygenator for open heart surgery. *Surg Clin North Am*. 1956;36:1025-1034.
6. Lillehei CW, Dewall RA, Read RC, Warden HE, Varco RL. Direct vision intracardiac surgery in man using a simple, disposable artificial oxygenator. *Dis Chest*. 1956;29(1):1-8.
7. Iwahashi H, Yuri K, Nosé Y. Development of the oxygenator: past, present, and future. *J Artif Organs*. 2004;7(3):111-120.
8. Kirklin JW, Barrat-Boyes G. *Cardiac Surgery*. 1st ed. John Wiley and Sons Ltd; 1986.
9. Kirklin JW, Barrat-Boyes G. *Cardiac Surgery*. 2nd ed. Churchill Livingstone; 1993.
10. Kouchoukos NT, Kirklin JW, Barrat-Boyes G, et al. *Kirklin/Barrat-Boyes - Cardiac surgery*. 4th ed. Churchill Livingstone; 2003.
11. Mavroudis C, Backer CL. *Pediatric Cardiay Surgery*. 3rd ed. Mosby; 2003.
12. Novitzky D, Cooper DK, Human PA, Reichart B, Zuhdi N. Triiodothyronine therapy for heart donor and recipient. *J Heart Transplant*. 1988;7(5):370-376.
13. Mohiuddin MM, Reichart B, Byrne GW, McGregor CGA. Current status of pig heart xenotransplantation. *Int J Surg*. 2015;23(Pt B):234-239.
14. Längin M, Mayr T, Reichart B, et al. Consistent success in life-supporting porcine cardiac xenotransplantation. *Nature*. 2018;564(7736):430-433.
15. Reichart B, Längin M, Radan J, et al. Pig-to-non-human primate heart transplantation: the final step toward clinical xenotransplantation? *J Heart Lung Transplant*. 2020;39(8):751-757.
16. Längin M, Reichart B, Steen S, et al. Cold non-ischemic heart preservation with continuous perfusion prevents early graft failure in orthotopic pig-to-baboon xenotransplantation. *Xenotransplantation*. 2021;28(1):e12636.
17. Steen S, Paskevicius A, Liao Q, Sjöberg T. Safe orthotopic transplantation of hearts harvested 24 hours after brain death and preserved for 24 hours. *Scand Cardiovasc J*. 2016;50(3):193-200.
18. Lower RR, Shumway NE. Studies on orthotopic homotransplantation of the canine heart. *Surg Forum*. 1960;11:18-19.
19. Nishimoto N, Terao K, Mima T, Nakahara H, Takagi N, Kakehi T. Mechanisms and pathologic significances in increase in serum interleukin-6 (IL-6) and soluble IL-6 receptor after administration of an anti-IL-6 receptor antibody, tocilizumab, in patients with rheumatoid arthritis and Castleman disease. *Blood*. 2008;112(10):3959-3964.

20. Iwase H, Yamamoto T, Cooper DKC. Episodes of hypovolemia/dehydration in baboons with pig kidney transplants: a new syndrome of clinical importance? *Xenotransplantation*. 2019;26(2):e12472.
21. Adams AB, Kim SC, Martens GR, et al. Xenoantigen deletion and chemical immunosuppression can prolong renal xenograft survival. *Ann Surg*. 2018;268(4):564-573.
22. Kim SC, Mathews DV, Breeden CP, et al. Long-term survival of pig-to-rhesus macaque renal xenografts is dependent on CD4 T cell depletion. *Am J Transplant*. 2019;19(8):2174-2185.
23. Ma D, Hirose T, Lassiter G, et al. Kidney transplantation from triple-knockout pigs expressing multiple human proteins in cynomolgus macaques. *Am J Transplant*. 2022;11:46-57.
24. Rabin RC. In a first, man receives a heart from a genetically altered pig. The New York Times. Jan. 10, 2022, 2022.
25. Weintraub K. First-ever pig-to-human heart transplant offers hope for thousands in need of organs. USA TODAY. Jan. 10, 2022, 2022.
26. Hinrichs A, Kessler B, Kurome M, et al. Growth hormone receptor-deficient pigs resemble the pathophysiology of human Laron syndrome and reveal altered activation of signaling cascadesin the liver. *Mol Metab*. 2018;11:113-128.
27. Mohiuddin MM, Singh AK, Corcoran PC, et al. Chimeric 2C10R4 anti-CD40 antibody theraoy is critical for long-term survival of GTKO.hCD46.hTBM pig-to-primate cardiac xenograft. *Nature Comm*. 2016;7:11138.

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